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(54) Titre : TRAITEMENT D'UN TROUBLE DE STRESS POST-TRAUMATIQUE
(54) Title: TREATMENT OF POST-TRAUMATIC STRESS DISORDER

(57) **Abrégé/Abstract:**

Provided are methods of treating a patient diagnosed with post-traumatic stress disorder, by administering to the patient a therapeutically effective amount of Compound A. Also provided are methods of improving resilience in a patient by administering a therapeutically effective amount of Compound A. Also provided are methods of diagnosing post-traumatic stress disorder in a patient by administering to the patient a therapeutically effective amount of Compound A and assessing at least one of sign, symptom, or symptom cluster of post-traumatic stress disorder; and diagnosing post-traumatic stress disorder in the patient if the Compound A reduces at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder.

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(54) Title: TREATMENT OF POST-TRAUMATIC STRESS DISORDER

(57) Abstract: Provided are methods of treating a patient diagnosed with post-traumatic stress disorder, by administering to the patient a therapeutically effective amount of Compound A. Also provided are methods of improving resilience in a patient by administering a therapeutically effective amount of Compound A. Also provided are methods of diagnosing post-traumatic stress disorder in a patient by administering to the patient a therapeutically effective amount of Compound A and assessing at least one of sign, symptom, or symptom cluster of post-traumatic stress disorder; and diagnosing post-traumatic stress disorder in the patient if the Compound A reduces at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder.

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TREATMENT OF POST-TRAUMATIC STRESS DISORDER

Cross-Reference to Related Applications

[001] This application claims the benefit of and priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application Serial No. 60/935,036, "TREATMENT OF POST-TRAUMATIC STRESS DISORDER" filed July 23, 2007, which is incorporated herein by reference in its entirety.

Field of Invention

[002] This relates generally to methods for treating post-traumatic stress disorder and more particularly methods of treating post-traumatic stress disorder using compound A, an inhibiting dopamine β -hydroxylase. Also provided are methods of improving resilience in a patient by administering a therapeutically effective amount of Compound A. Also provided are methods of diagnosing post-traumatic stress disorder in a patient by administering to the patient a therapeutically effective amount of Compound A and assessing at least one of sign, symptom, or symptom cluster of post-traumatic stress disorder; and diagnosing post-traumatic stress disorder in the patient if the Compound A reduces at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder.

Background of the Invention

[003] Anxiety disorders are the most commonly occurring disorders of the psychiatric illnesses with an immense economic burden. In addition to generalized anxiety disorder, they encompass post-traumatic stress disorder, panic disorder, obsessive compulsive disorder and social as well as other phobias.

[004] Post-traumatic stress disorder can be severe and chronic, with some studies suggesting a lifetime prevalence of 1.3% to 7.8% in the general population. Post-traumatic stress disorder typically follows a psychologically distressing traumatic event. These events may include military combat, terrorist incidents, physical assault, sexual assault, motor vehicle accidents, and natural disasters, for example. The response to the event can involve intense fear, helplessness, or horror. Most people recover from the traumatic event with time and return to normal life. In contrast, in post-traumatic stress disorder victims, symptoms persist and may worsen with time, preventing a return to normal life.

[005] Psychotherapy is currently the backbone of post-traumatic disorder treatment. Methods include cognitive-behavioral therapy, exposure therapy, and eye movement desensitization and reprocessing. Medication can enhance the effectiveness of psychotherapy. Selective serotonin reuptake inhibitors (SSRIs), such as sertraline (Zoloft®) and paroxetine (Paxil®), are the only medications approved for treating PTSD by the Food and Drug Administration. Many unwanted side effects and characteristics are associated with SSRI usage. These include concerns about drug interactions, gastrointestinal side effects, sexual side effects, suicidal ideation, acute anxiogenic effects, and slow onset of action. Some tricyclic antidepressants (TCAs) and monamine oxidase inhibitors (MAOIs) appear to have some efficacy but patient tolerance is low due to the high incidence of side effects. MAOIs have dietary restriction requirements and are linked to hypertensive events. TCAs have anticholinergic and cardiovascular side effects. Lamotrigine, a sodium channel blocker, has had some efficacy in treating post-traumatic stress disorder in a small scale placebo controlled study. Difficulty in the use of lamotrigine due to the necessity for titration and the risk of developing Steven Johnson Syndrome, a life threatening rash, render it a poor candidate for therapeutic use.

[006] There is a need for the development of treatments for post-traumatic stress disorder that are safe and effective.

[007] Dopamine is a catecholamine neurotransmitter found predominately, along with specific dopaminergic receptors, in the central nervous system. Norepinephrine is a circulating catecholamine, which acts at adrenergic receptors in central and peripheral systems. Dopamine β -hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine and is found in both central and peripheral sympathetic neurons. Inhibition of DBH concurrently elevates dopamine levels by blocking its metabolism and reduces norepinephrine levels by blocking its synthesis. Nepicastat ((S)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride), is a DBH inhibitor.

Summary of the Invention

[008] Provided herein are methods of treating a patient diagnosed with post-traumatic stress disorder, by administering to the patient a therapeutically effective amount of Compound A.

[009] Also provided are methods of improving resilience in a patient by administering a therapeutically effective amount of Compound A.

[010] Also provided are methods of diagnosing post-traumatic stress disorder in a patient by administering to the patient a therapeutically effective amount of Compound A and assessing at least one of sign, symptom, or symptom cluster of post-traumatic stress disorder; and diagnosing post-traumatic stress disorder in the patient if the Compound A reduces at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder.

Description of Drawings

[011] **Figure 1** Shows Details of the individual enzymatic assays.

[012] **Figure 2** Shows the Effects of Nepicastat on Tissue Noradrenaline and Dopamine Content in the mesenteric artery (a), left ventricle (b) and cerebral cortex (c) of SHR.

[013] **Figure 3** Shows the Effects of Nepicastat Tissue Dopamine/Noradrenaline ratio in the mesenteric artery (a), left ventricle (b), and cerebral cortex (c) of SHR.

[014] **Figure 4** Shows the Effects of Nepicastat on Tissue Noradrenaline and Dopamine Content in renal artery, left ventricle, and cerebral cortex of beagle dogs.

[015] **Figure 5** Shows the Effects of Nepicastat on Tissue Dopamine/Noradrenaline ratio in the renal artery, left ventricle, and cerebral cortex of beagle dogs.

[016] **Figure 6** Shows the Effects of Nepicastat on Plasma Concentrations of Noradrenaline (a), Dopamine (b), and Dopamine/Noradrenaline ratio (c) in beagle dogs.

[017] **Figure 7** Shows the Effect of Nepicastat and (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, at 30 mg.kg⁻¹; po, on noradrenaline content, dopamine content and dopamine/noradrenaline ratio in mesenteric artery, left ventricle and cerebral cortex of SHR.

[018] **Figure 8** Shows Structures of 1, 2a (nepicastat), and 2b ((*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride).

[019] **Figure 9** Shows Chemical Scheme.

[020] **Figure 10** Shows a Table Describing the Nepicastat Interaction of Nepicastat at DBH and a range of selected enzymes and receptors.

[021] **Figure 11** Shows Effects of Nepicastat on tissue DA/NE Ratio in SHR (A) and normal beagle dogs (B).

[022] **Figure 12** Shows Effects of Chronic Administration of Nepicastat on plasma DA/NE ratio in normal beagle dogs.

[023] **Figure 13** Shows Effects of Orally administered Nepicastat on mean arterial pressure in SHR.

[024] **Figure 14** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine and Dopamine in Samples of Plasma Collected from a Peripheral Vein of Supine CHF Patients During Daily Oral Administration of Placebo for 3 Months.

[025] **Figure 15** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine and Dopamine in Samples of Plasma Collected from a Peripheral Vein of Supine CHF Patients During Daily Oral Administration of 20mg of Nepicastat Free Base for 3 Months.

[026] **Figure 16** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine and Dopamine in Samples of Plasma Collected from a Peripheral Vein of Supine CHF Patients During Daily Oral Administration of 40mg of Nepicastat Free Base for 3 Months.

[027] **Figure 17** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine and Dopamine in Samples of Plasma Collected from a Peripheral Vein of Supine CHF Patients During Daily Oral Administration of 60mg of Nepicastat Free Base for 3 Months.

[028] **Figure 18** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine and Dopamine in Samples of Plasma Collected from a Peripheral Vein of Supine CHF Patients During Daily Oral Administration of 80mg of Nepicastat Free Base for 3 Months.

[029] **Figure 19** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine and Dopamine in Samples of Plasma Collected from a Peripheral Vein of Supine CHF Patients During Daily Oral Administration of 120mg of Nepicastat Free Base for 3 Months.

[030] **Figure 20** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine in Samples of Plasma Collected from the Arterial Vein and Coronary Sinus of Catheterized CHF Patients During Daily Oral Administration of Placebo for 3 Months.

[031] **Figure 21** Shows the Concentrations (pg/ml) of the Free Base of Dopamine in Samples of Plasma Collected from the Arterial Vein and Coronary Sinus of Catheterized CHF Patients During Daily Oral Administration of Placebo for 3 Months.

[032] **Figure 22** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine in Samples of Plasma Collected from the Arterial Vein and Coronary Sinus of

Catheterized CHF Patients During Daily Oral Administration of 20mg of Nepicastat Free Base for 3 Months

[033] **Figure 23** Shows the Concentrations (pg/ml) of the Free Base of Dopamine in Samples of Plasma Collected from the Arterial Vein and Coronary Sinus of Catheterized CHF Patients During Daily Oral Administration of 20mg of Nepicastat Free Base for 3 Months.

[034] **Figure 24** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine in Samples of Plasma Collected from the Arterial Vein and Coronary Sinus of Catheterized CHF Patients During Daily Oral Administration of 40mg of Nepicastat Free Base for 3 Months.

[035] **Figure 25** Shows the Concentrations (pg/ml) of the Free Base of Dopamine in Samples of Plasma Collected from the Arterial Vein and Coronary Sinus of Catheterized CHF Patients During Daily Oral Administration of 40mg of Nepicastat Free Base for 3 Months

[036] **Figure 26** Shows the Concentrations (pg/ml) of the Free Base of Norepinephrine in Samples of Plasma Collected from the Arterial Vein and Coronary Sinus of Catheterized CHF Patients During Daily Oral Administration of 60mg of Nepicastat Free Base for 3 Months.

[037] **Figure 27** Shows the Concentrations (pg/ml) of the Free Base of Dopamine in Samples of Plasma Collected from the Arterial Vein and Coronary Sinus of Catheterized CHF Patients During Daily Oral Administration of 60mg of Nepicastat Free Base for 3 Months.

[038] **Figure 28** denotes data that should be discounted from further statistical analysis together with the reason for such an action.

[039] **Figure 29** Shows Pharmacokinetics Parameters of Nepicastat in Rats.

[040] **Figure 30** Shows the Concentration of Nepicastat in Plasma of Male Rats Following a Single 10 mg/kg Oral Dose of Nepicastat.

[041] **Figure 31** Shows the Concentration of Nepicastat in Plasma of Male Rats Following a Single 30 mg/kg Oral Dose of Nepicastat.

[042] **Figure 32** Shows the Concentration of Nepicastat in Plasma of Male Rats Following a Single 100 mg/kg Oral Dose of Nepicastat.

[043] **Figure 33** Shows the Concentration of Mean Concentration of Nepicastat in Plasma of Rats Following a Single 10, 30, or 100 mg/kg oral dose of Nepicastat. Values are the means of three rats per time point.

[044] **Figure 34** Shows the Linear Relationship Between Dose and Values of AUC for Nepicastat in Plasma.

[045] **Figure 35** Shows the Concentration of Nepicastat in Plasma of Female Rats Following a Single 30 mg/kg Oral Dose of Nepicastat.

[046] **Figure 36** Shows the Mean Concentrations of Nepicastat in Plasma and Brain of Male Rats Following a Single 10 mg/kg oral dose of Nepicastat.

[047] **Figure 37** Shows the Concentration of Nepicastat in Brain of Male Rats Following a Single 10 mg/kg Oral Dose of Nepicastat.

[048] **Figure 38** Shows the Norepinephrine Concentration in the Mesenteric Artery.

[049] **Figure 39** Shows the Dopamine Concentration in the Mesenteric Artery.

[050] **Figure 40** Shows the Dopamine/Norepinephrine Concentration in the Mesenteric Artery.

[051] **Figure 41** Shows the Norepinephrine Levels in the Rat Left Ventricle.

[052] **Figure 42** Shows the Dopamine Levels in the Rat Left Ventricle.

[053] **Figure 43** Shows the Dopamine/Norepinephrine Levels in the Rat Left Ventricle.

[054] **Figure 44** Shows the Dopamine Concentration ($\mu\text{g/g}$ wet weight) in the cerebral cortex of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[055] **Figure 45** Shows the Norepinephrine Concentration ($\mu\text{g/g}$ wet weight) in the cerebral cortex of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[056] **Figure 46** Shows the Dopamine/Norepinephrine Concentration ($\mu\text{g}/\mu\text{g}$ wet weight) in the cerebral cortex of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[057] **Figure 47** Shows the Dopamine Concentration ($\mu\text{g/g}$ wet weight) in the left ventricle of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[058] **Figure 48** Shows the Norepinephrine Concentration ($\mu\text{g/g}$ wet weight) in the left ventricle of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[059] **Figure 49** Shows the Dopamine/Norepinephrine Concentration ($\mu\text{g}/\mu\text{g}$ wet weight) in the left ventricle of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[060] **Figure 50** Shows the Dopamine Concentration ($\mu\text{g/g}$ wet weight) in the Mesenteric artery of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[061] **Figure 51** Shows the Norepinephrine Concentration ($\mu\text{g/g}$ wet weight) in the Mesenteric artery of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[062] **Figure 52** Shows the Dopamine/Norepinephrine Concentration ($\mu\text{g}/\mu\text{g}$ wet weight) in the Mesenteric artery of SHR plotted as a function of dose. Tissue was harvested six hours after the third of three oral doses administered 12 hours part. (n=9)

[063] **Figure 53** Shows Dopamine concentration ($\mu\text{g/g}$ wet weight) in the cerebral cortex of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9)

[064] **Figure 54** Shows Norepinephrine ($\mu\text{g/g}$ wet weight) in the cerebral cortex of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9)

[065] **Figure 55** Shows the Dopamine/Norepinephrine Concentration ($\mu\text{g}/\mu\text{g}$ wet weight) in the cerebral cortex of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9).

[066] **Figure 56** Shows the Dopamine Concentration ($\mu\text{g/g}$ wet weight) in the left ventricle of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9)

[067] **Figure 57** Shows the Norepinephrine Concentration ($\mu\text{g/g}$ wet weight) in the left ventricle of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-

difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9)

[068] Figure 58 Shows the Dopamine/Norepinephrine Concentration ($\mu\text{g}/\mu\text{g}$ wet weight) in the left ventricle of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9).

[069] Figure 59 Shows Dopamine Concentration ($\mu\text{g}/\text{g}$ wet weight) in the Mesenteric Artery of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9).

[070] Figure 60 Shows Norepinephrine Concentration ($\mu\text{g}/\text{g}$ wet weight) in the Mesenteric Artery of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9).

[071] Figure 61 Shows Dopamine/Norepinephrine Concentration ($\mu\text{g}/\mu\text{g}$ wet weight) in the Mesenteric Artery of SHR following administration of Nepicastat, (*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, or dH2O vehicle, or SKF102698 or PEG 400:dH2O (1:1) vehicle. Tissue was harvested six hours after the third of three oral doses administered 12 hours apart. (n=9).

[072] Figure 62 Shows the Catecholamine levels in the cortex, striatum, and mesenteric artery.

[073] Figure 63 Shows the Triiodothyronine levels in serum.

[074] Figure 64 Shows the Thyroxine levels in serum.

[075] Figure 65 Shows the Concentrations of Dopamine and Norepinephrine in Dog Kidney Medulla in Response to Nepicastat.

[076] **Figure 66** Shows the Concentration of Dopamine and Norepinephrine in Dog Kidney Cortex in Response to Nepicastat.

[077] **Figure 67** Shows the Effect of placebo or Nepicastat on plasma DA levels (pg/ml) in normal beagle dogs.

[078] **Figure 68** Shows the Effect of placebo or Nepicastat on plasma NE levels (pg/ml) in normal beagle dogs.

[079] **Figure 69** Shows the Effect of placebo or Nepicastat on plasma DA/NE ratio in normal beagle dogs.

[080] **Figure 70** Shows the Effect of placebo or Nepicastat on plasma EPI levels (pg/ml) in normal beagle dogs.

[081] **Figure 71** Shows the Effects of chronic administration (14.5 days) of Nepicastat on plasma levels of NE, DA and EPI in normal beagle dogs. N=8 per group. * <0.05 vs. placebo

[082] **Figure 72** Shows the Effect Concentrations (ng/ml) of the free base of Nepicastat and RS 47831 in samples of plasma collected following oral administration of RS 25560-197 (2 mg/kg; bid) to beagle dogs for 14.5 days.

[083] **Figure 73** Shows the Dopamine Levels in the Renal Artery in Dogs.

[084] **Figure 74** Shows the Norepinephrine Levels in the Renal Artery in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p<0.01$ vs. placebo (mean $\pm$ SD).

[085] **Figure 75** Shows the Dopamine Levels in the Renal Artery in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p<0.01$ vs. placebo (mean $\pm$ SD).

[086] **Figure 76** Shows the Dopamine Levels in the Cerebral Cortex in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p<0.01$; M $0.05<p<0.10$ vs. placebo (mean $\pm$ SD).

[087] **Figure 77** Shows the Norepinephrine Levels in the Cerebral Cortex in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p<0.01$ vs. placebo (mean $\pm$ SD).

[088] **Figure 78** Shows the Dopamine/Norepi ratio Levels in the Cerebral Cortex in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p<0.01$ vs. placebo (mean $\pm$ SD).

[089] **Figure 79** Shows the Dopamine Levels in the Left Ventricle in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$ vs. placebo (mean $\pm$ SD).

[090] **Figure 80** Shows the Norepinephrine Levels in the Left Ventricle in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$ vs. placebo (mean $\pm$ SD).

[091] **Figure 81** Shows the Dopamine/Norepi Levels in the Left Ventricle in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$ vs. placebo (mean $\pm$ SD).

[092] **Figure 82** Shows the Dopamine Levels in the Renal Cortex in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$ vs. placebo (mean $\pm$ SD).

[093] **Figure 83** Shows the Norepinephrine Levels in the Renal Cortex in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$ vs. placebo (mean $\pm$ SD).

[094] **Figure 84** Shows the Dopamine/Norepi Levels in the Renal Cortex in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$ vs. placebo (mean $\pm$ SD).

[095] **Figure 85** Shows the Dopamine Levels in the Renal Medulla in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$; M, $0.05 < p < 0.10$ vs. placebo (mean $\pm$ SD).

[096] **Figure 86** Shows the Norepinephrine Levels in the Renal Medulla in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$; M, $0.05 < p < 0.10$ vs. placebo (mean $\pm$ SD).

[097] **Figure 87** Shows the Dopamine/Norepi Levels in the Renal Medulla in Dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, * $p < 0.01$ vs. placebo (mean $\pm$ SD).

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[0193] **Figure 183** shows heart rates of the four groups of rats on day 2 of the drug treatment.

[0194] **Figure 184** shows motor activities (in arbitrary units) of the four groups of rats on day 3 of the drug treatment.

[0195] **Figure 185** shows changes in body weights of the four groups of rats during the first 6 day treatment.

[0196] **Figure 186** shows the significance levels for each time point on mean blood pressure.

[0197] **Figure 187** shows the significance levels for each time point on mean blood pressure.

Detailed Description

[0198] As used herein, the following words and phrases are generally intended to have the meanings as set forth below, except to the extent that the context in which they are used indicates otherwise.

[0199] As used herein “Compound A” includes nepicastat (((*S*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride)), ((*R*)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride), and mixtures thereof, as well as pharmaceutically acceptable salts thereof.

[0200] “Pharmaceutically acceptable salts” include, but are not limited to salts with inorganic acids, such as hydrochlorate, phosphate, diphosphate, hydrobromate, sulfate, sulfinate, nitrate, and like salts; as well as salts with an organic acid, such as malate, maleate, fumarate, tartrate, succinate, citrate, acetate, lactate, methanesulfonate, p-toluenesulfonate, 2-hydroxyethylsulfonate, benzoate, salicylate, stearate, and alkanoate such as acetate, $\text{HOOC}-(\text{CH}_2)_n\text{-COOH}$ where n is 0-4, and like salts.

[0201] In addition, if a compound is obtained as an acid addition salt, the free base can be obtained by basifying a solution of the acid salt. Conversely, if the product is a free base, an addition salt, particularly a pharmaceutically acceptable addition salt, may be produced by dissolving the free base in a suitable organic solvent and treating the solution with an acid, in accordance with conventional procedures for preparing acid addition salts from base compounds. Those skilled in the art will recognize various synthetic methodologies that may be used to prepare non-toxic pharmaceutically acceptable addition salts.

[0202] As used herein, the term “treating” refers to any manner in which at least one sign, symptom, or symptom cluster of a disease or disorder is beneficially altered so as to prevent or delay the onset, reduce the incidence or frequency, reduce the severity or intensity, retard the progression, prevent relapse, or ameliorate the symptoms or associated symptoms of the disease or disorder. For example, in post-traumatic stress disorder, treating the disorder can, in certain embodiments, cause a reduction in at least one of the frequency and intensity of at least one of a sign, symptom, and symptom cluster of post-traumatic stress disorder.

[0203] As used herein the phrase “diagnosed with post-traumatic stress disorder (PTSD)” refers to having a sign, symptom, or symptom cluster indicative of post-traumatic stress disorder, a psychiatric disorder triggered by a traumatic event. Non-limiting examples of such traumatic events include military combat, terrorist incidents, physical assault, sexual assault, motor vehicle accidents, and natural disasters.

[0204] The Diagnostic and Statistical Manual of Mental Disorders-IV-Text revised (DSM-IV-TR), a handbook for mental health professionals that lists categories of mental

disorders and the criteria, classifies post-traumatic stress disorder as an anxiety disorder. According to the DSM-IV-TR, a PTSD diagnosis can be made if:

[0205] 1. the patient experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others and the response involved intense fear, helplessness, or horror;

[0206] 2. as a consequence of the traumatic event, the patient experiences at least 1 re-experiencing/intrusion symptom, 3 avoidance/numbing symptoms, and 2 hyperarousal symptoms, and the duration of the symptoms is for more than 1 month; and

[0207] 3. the symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

[0208] In certain embodiments, if the patient's disorder fulfills DSM-IV-TR criteria, the patient is diagnosed with post-traumatic stress disorder. In certain embodiments, if the patient has at least one sign, symptom, or symptom cluster of post-traumatic stress disorder, the patient is diagnosed with post-traumatic stress disorder. In certain embodiments, a scale is used to measure a sign, symptom, or symptom cluster of post-traumatic stress disorder, and post-traumatic stress disorder is diagnosed on the basis of the measurement using that scale. In certain embodiments, a "score" on a scale is used to diagnose or assess a sign, symptom, or symptom cluster of post-traumatic stress disorder. In certain embodiments, a "score" can measure at least one of the frequency, intensity, or severity of a sign, symptom, or symptom cluster of post-traumatic stress disorder.

[0209] As used herein, the term "scale" refers to a method to measure at least one sign, symptom, or symptom cluster of post-traumatic stress disorder in a patient. In certain embodiments, a scale may be an interview or a questionnaire. Non-limiting examples of scales are Clinician-Administered PTSD Scale (CAPS), Clinician-Administered PTSD Scale Part 2 (CAPS-2), Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA), Impact of Event Scale (IES), Impact of Event Scale-Revised (IES-R), Clinical Global Impression Scale (CGI), Clinical Global Impression Severity of Illness (CGI-S), Clinical Global Impression Improvement (CGI-I), Duke Global Rating for PTSD scale (DGRP), Duke Global Rating for PTSD scale Improvement (DGRP-I), Hamilton Anxiety Scale (HAM-A), Structured Interview for PTSD (SI-PTSD), PTSD Interview (PTSD-I), PTSD Symptom Scale (PSS-I), Mini International Neuropsychiatric Interview (MINI), Montgomery-Åsberg Depression Rating Scale (MADRS), Beck Depression Inventory (BDI), Hamilton Depression Scale (HAM-D), Revised Hamilton

Rating Scale for Depression (RHRSD), Major Depressive Inventory (MDI), Geriatric Depression Scale (GDS-30), and Children's Depression Index (CDI).

[0210] As used herein, the terms "sign" and "signs" refer to objective findings of a disorder. In certain embodiments, a sign can be a physiological manifestation or reaction of a disorder. In certain embodiments, a sign may refer to heart rate and rhythm, body temperature, pattern and rate of respiration, blood pressure. In certain embodiments, signs can be associated with symptoms. In certain embodiments, signs can be indicative of symptoms.

[0211] As used herein, the term "symptom" and "symptoms" refer to subjective indications that characterize a disorder. Symptoms of post-traumatic stress disorder may refer to, for example, but not limited to recurrent and intrusive trauma recollections, recurrent and distressing dreams of the traumatic event, acting or feeling as if the traumatic event were recurring, distress when exposed to trauma reminders, physiological reactivity when exposed to trauma reminders, efforts to avoid thoughts or feelings associated with the trauma, efforts to avoid activities or situations, inability to recall trauma or trauma aspects, markedly diminished interest in significant activities, feelings of detachment or estrangement from others, restricted range of affect, sense of a foreshortened future, social anxiety, anxiety with unfamiliar surroundings, difficulty falling or staying asleep, irritability or outbursts of anger, difficulty concentrating, hypervigilance, and exaggerated startle response. In certain embodiments, potentially threatening stimuli can cause hyperarousal or anxiety. In certain embodiments, the physiological reactivity manifests in at least one of abnormal respiration, abnormal cardiac rate of rhythm, abnormal blood pressure, abnormal function of a special sense, and abnormal function of sensory organ. In certain embodiments, restricted range of effect characterized by diminished or restricted range or intensity of feelings or display of feelings can occur and a sense of a foreshortened future can manifest in thinking that one will not have a career, marriage, children, or a normal life span. In certain embodiments, children and adolescents may have symptoms of post-traumatic stress disorder such as, for example and without limitation, disorganized or agitated behavior, repetitive play that expresses aspects of the trauma, frightening dreams which lack recognizable content, and trauma-specific reenactment. In certain embodiments, a symptom can be stress associated with memory recall.

[0212] As used herein, the term "symptom cluster" refers to a set of signs, symptoms, or a set of signs and symptoms, that are grouped together because of their relationship to

each other or their simultaneous occurrence. For example, in certain embodiments post-traumatic stress disorder is characterized by three symptom clusters: re-experiencing/intrusion, avoidance/numbing, and hyperarousal.

[0213] As used herein, the term “re-experiencing/intrusion” refers to at least one of recurrent and intrusive trauma recollections, recurrent and distressing dreams of the traumatic event, acting or feeling as if the traumatic event were recurring, distress when exposed to trauma reminders, and physiological reactivity when exposed to trauma reminders. In certain embodiments, the physiological reactivity manifests in at least one of abnormal respiration, abnormal cardiac rate of rhythm, abnormal blood pressure, abnormal function of a special sense, and abnormal function of sensory organ.

[0214] As used herein, the term “avoidance/numbing” refers to at least one of efforts to avoid thoughts or feelings associated with the trauma, efforts to avoid activities or situations, inability to recall trauma or trauma aspects, markedly diminished interest in significant activities, feelings of detachment or estrangement from others, restricted range of affect, and sense of a foreshortened future. Restricted range of effect characterized by diminished or restricted range or intensity of feelings or display of feelings can occur. A sense of a foreshortened future can manifest in thinking that one will not have a career, marriage, children, or a normal life span. Avoidance/numbing can also manifest in social anxiety and anxiety with unfamiliar surroundings.

[0215] As used herein, the term “hyperarousal” refers to at least one of difficulty falling or staying asleep, irritability or outbursts of anger, difficulty concentrating, hypervigilance, and exaggerated startle response. Potentially threatening stimuli can cause hyperarousal or anxiety.

[0216] As used herein, the term “significantly” refers to a set of observations or occurrences that are too closely correlated to be attributed to chance. For example, in certain embodiments, “significantly changes”, “significantly reduces”, and “significantly increases” refers to alterations or effects that are not likely to be attributed to chance. In certain embodiments, statistical methods can be used to determine whether an observation can be referred to as “significantly” changed, reduced, increased, or altered.

[0217] Patients diagnosed with post-traumatic stress disorder may feel “on guard”, uneasy, and intensely anxious. Depression, anxiety, panic attacks, and bipolar disorder are often associated with post-traumatic stress disorder. Alcohol and drug abuse are also common. In certain embodiments, disorders cormorbid with post-traumatic stress

disorder can include for example but without limitation depression, alcohol abuse, and drug abuse.

[0218] As used herein, the term “Clinician-Administered PTSD Scale (CAPS)” refers to a measure for diagnosing and assessing post-traumatic stress syndrome. The CAPS is a 30-item structured interview that corresponds to the DSM-IV criteria for PTSD. Different versions of this measure have been developed.

[0219] As used herein, the term “Clinician-Administered PTSD Scale-Part1 (CAPS-1)” is a version of CAPS that assesses current and lifetime PTSD and is also known as CAPS-DX (for diagnosis).

[0220] As used herein, the term “Clinician-Administered PTSD Scale-Part 2 (CAPS-2)” refers to a version of CAPS used to assess one week symptom status in patients with post-traumatic stress disorder and also refers to a CAPS-SX (for symptom),

[0221] As used herein, the term “Clinician-Administered PTSD Scale for children and adolescents (CAPS-CA)” refers to a version of CAPS developed for children and adolescents.

[0222] As used herein, the term “Impact of Event Scale (IES)” refers to a scale developed by Mardi Horowitz, Nancy Wilner, and William Alvarez to measure subjective stress related to a specific event. It is a self-reported assessment and can be used to make measurements over time to monitor a patient’s status.

[0223] As used herein, the term “Impact of Event Scale-Revised (IES-R)” refers to the revision of the IES developed by Daniel S. Weiss and Charles Marmar to assess the hyperarousal symptom cluster of PTSD.

[0224] As used herein, the term “Clinical Global Impression Scale (CGI)” refers to a scale for making psychiatric assessments. Patients are interviewed and the CGI is used to measure the severity of illness (CGI-S), global improvement (CGI-I), and efficacy index.

[0225] As used herein, the term “Clinical Global Impression Severity of Illness (CGI-S)” refers to an assessment of the patient’s current symptoms. Generally, it is rated on a seven-point scale, ranging from a score of 1 (normal) to 7 (extremely ill). The severity of the patient’s illness is compared to the severity of other patients’ illness. For example, the CGI-S score can be used to measure a patient’s condition after treatment with Compound A, and the scores before and after treatment may be compared.

[0226] As used herein, the term “Clinical Global Impression Improvement (CGI-I)” refers to a comparison of a patient’s current condition to his baseline condition.

Generally, it is rated on a seven-point scale ranging from 1 (very much improved) to 7 (very much worse). The CGI-I score can be used to measure, for example, improvement of post-traumatic stress disorder in response to Compound A treatment.

[0227] As used herein, the term “efficacy index” refers to a score taken on CGI and compares the patient’s baseline condition with a ratio of current therapeutic benefit to severity of side effects. Generally, it is rated on a four-point scale ranging from 1 (none) to 4 (outweighs therapeutic effect). In assessing post-traumatic stress disorder, the efficacy index could, for example, assess the risk-benefit of treating with a therapy such as Compound A.

[0228] As used herein, the term “Duke Global Rating for PTSD scale (DGRP)” refers to a scale that measures severity and improvement for each of the three PTSD symptom clusters: re-experiencing/intrusion, avoidance/numbing, and hyperarousal as well as overall PTSD severity.

[0229] As used herein, the term “Duke Global Rating for PTSD scale-Improvement (DGRP-I)” refers to a scale used to distinguish responders (DGRP-I of 1 (very much improved) and 2 (much improved)) from nonresponders (DGRP-I > 2) of in response to a treatment, for example, Compound A, for post-traumatic stress disorder.

[0230] As used herein, the term “Hamilton Anxiety Scale (HAM-A)” refers to a scale developed by Max Hamilton in 1959 to diagnose and quantify symptoms of anxiety and post-traumatic stress disorder. It consists of 14 items, each defined by a series of symptoms. No standardized probe questions to elicit information from patients or behaviorally specific guidelines were developed for determining item scoring. Each item is rated on a 5-point scale, ranging from 0 (not present) to 4 (severe). Items include assessing anxious mood, fears, intellectual effects, somatic complaints, e.g. on musculature, cardiovascular symptoms, tension, insomnia, depressed mood, somatic sensory complaints, respiratory symptoms, gastrointestinal symptoms, autonomic symptoms, genitourinary symptoms, and behavior at the time of assessment. For example, a reduction in the HAM-A score would indicate improvement in a disorder such as post-traumatic stress disorder.

[0231] As used herein, the terms “Structured Interview for PTSD (SI-PTSD), PTSD Interview (PTSD-I), PTSD Symptom Scale (PSS-I), Mini International Neuropsychiatric Interview (MINI), Montgomery–Åsberg Depression Rating Scale (MADRS), Beck Depression Inventory (BDI), Hamilton Depression Scale (HAM-D), Revised Hamilton Rating Scale for Depression (RHRSD), Major Depressive Inventory (MDI), Geriatric

Depression Scale (GDS-30), and Children's Depression Index (CDI)" refer to additional scales that diagnose, assess, measure a sign, symptom, symptom cluster of post-traumatic stress disorder, anxiety, or depression.

[0232] As used herein, the term "score" refers to a score of at least one item or parameter measured on a scale that measures at least one sign, symptom, or symptom cluster of psychiatric symptoms, anxiety, or post-traumatic stress disorder. In certain embodiments, a score measures the frequency, intensity, or severity of a sign, symptom, symptom cluster, associated symptom, or impact on daily life of post-traumatic stress disorder. In certain embodiments, a "score" that assesses post-traumatic stress disorder can be significantly changed, for example, by treatment for post-traumatic stress disorder.

[0233] As used herein, the term "endpoint score" refers to a score on an instrument that assesses post-traumatic stress disorder taken during or after treatment.

[0234] As used herein, the term "baseline score" refers to a score on an instrument that assesses post-traumatic stress disorder prior to initiation of a treatment.

[0235] As used herein, the term "overall score" refers to a sum of the scores on an instrument that assesses post-traumatic stress disorder. In certain embodiments, an overall score is the sum of a score of at least one of symptoms, symptoms clusters, associated symptoms, impact on daily life, efficacy, and improvement.

[0236] As used herein, the term "relapse" refers to reoccurrence or worsening of at least one symptom of a disease or disorder in a patient.

[0237] As used herein the phrase "therapeutically effective amount" refers to the amount sufficient to provide a therapeutic outcome regarding at least one sign, symptom, or associated symptom of a disease, disorder, or condition. For example, the disease, disorder, or condition is PTSD.

[0238] As used herein, the phrase "improving resilience" refers to increasing the ability of a patient to experience a traumatic event without suffering post-traumatic stress disorder or with less post-event symptomatology or disruption of normal activities of daily living. In certain embodiments, improving resilience can, in certain embodiments, reduce at one of the signs, symptoms, or symptom clusters of post-traumatic stress disorder.

[0239] As used herein, the term "coadministering" refers to a dosage regimen for a first agent that overlaps with the dosage regimen of a second agent, or to simultaneous administration of the first agent and the second agent. A dosage regimen is characterized by dosage amount, frequency, and duration. Two dosage regimens overlap if between

initiation of a first and initiation of a second administration of a first agent, the second agent is administered.

[0240] As used herein, the term “agent” refers to a substance including, but not limited to a chemical compound, such as a small molecule or a complex organic compound, a protein, such as an antibody or antibody fragment or a protein comprising an antibody fragment, or a genetic construct which acts at the DNA or mRNA level in an organism.

[0241] As used herein, the term “dopamine β -hydroxylase activity” refers to conversion of dopamine to norepinephrine mediated by dopamine β hydroxylase. Activity of dopamine β -hydroxylase can be assayed by measuring dopamine or norepinephrine levels.

[0242] As used herein, the term “modulates” refers to changing or altering an activity, function, or feature. For example, an agent may modulate levels of a factor by elevating or reducing the levels of the factor.

[0243] As used herein, the term catecholamine refers to a compound that contains an amine group attached to a catechol portion and that serves as a hormone or neurotransmitter. By way of example and without limitation, dopamine and norepinephrine are catecholamines.

[0244] Provided herein are methods of treating a patient diagnosed with post-traumatic stress disorder. The methods include administering to the patient a therapeutically effective amount of Compound A.

[0245] In certain embodiments the methods further comprise coadministering a therapeutically effective amount of at least one other agent, selected from benzodiazepine, a selective serotonin reuptake inhibitor (SSRI), a serotonin-norepinephrine reuptake inhibitor (SNRI), a norepinephrine reuptake inhibitor (NRI), a serotonin 5-hydroxytryptamine_{1A} (5HT_{1A}) antagonist, a dopamine β -hydroxylase inhibitor, an adenosine A_{2A} receptor antagonist, a monoamine oxidase inhibitor (MAOI), a sodium (Na) channel blocker, a calcium channel blocker, a central and peripheral alpha adrenergic receptor antagonist, a central alpha adrenergic agonist, a central or peripheral beta adrenergic receptor antagonist, a NK-1 receptor antagonist, a corticotropin releasing factor (CRF) antagonist, an atypical antidepressant/antipsychotic, a tricyclic, an anticonvulsant, a glutamate antagonist, a gamma-aminobutyric acid (GABA) agonist, and a partial D₂ agonist.

[0246] In certain embodiments the at least one other agent is a SSRI selected from paroxetine, sertraline, citalopram, escitalopram, and fluoxetine.

[0247] In certain embodiments the at least one other agent is a SNRI selected from duloxetine, mirtazapine, and venlafaxine.

[0248] In certain embodiments the at least one other agent is a NRI selected from bupropion and atomoxetine.

[0249] In certain embodiments the at least one other agent is disulfiram.

[0250] In certain embodiments the at least one other agent is the adenosine A2A receptor antagonist istradefylline.

[0251] In certain embodiments the at least one other agent is a sodium channel blocker selected from lamotrigine, carbamazepine, oxcarbazepine, and valproate.

[0252] In certain embodiments the at least one other agent is a calcium channel blocker selected from lamotrigine and carbamazepine.

[0253] In certain embodiments the at least one other agent is the central and peripheral alpha adrenergic receptor antagonist prazosin.

[0254] In certain embodiments the at least one other agent is the central alpha adrenergic agonist clonidine.

[0255] In certain embodiments the at least one other agent is the central or peripheral beta adrenergic receptor antagonist propranolol.

[0256] In certain embodiments the least one other agent is an atypical antidepressant/antipsychotic selected from olanzapine, risperidone, and quetiapine.

[0257] In certain embodiments the least one other agent is a tricyclic selected from amitriptyline, amoxapine, desipramine, doxepin, imipramine, nortriptyline, protriptyline, and trimipramine.

[0258] In certain embodiments the least one other agent is an anticonvulsant selected from lamotrigine, carbamazepine, oxcarbazepine, valproate, topiramate, and levetiracetam.

[0259] In certain embodiments the least one other agent is the glutamate antagonist topiramate.

[0260] In certain embodiments the least one other agent is a GABA agonist selected from valproate and topiramate.

[0261] In certain embodiments the least one other agent is the partial D2 agonist aripiprazole.

[0262] In certain embodiments the patient has abnormal brain levels of at least one catecholamine.

[0263] In certain embodiments the Compound A reduces dopamine β hydroxylase activity in the brain of the patient.

[0264] In certain embodiments the Compound A modulates brain levels of at least one catecholamine in the patient.

[0265] In certain embodiments the at least one catecholamine is norepinephrine and the Compound A reduces brain levels of the norepinephrine in the patient.

[0266] In certain embodiments the at least one catecholamine is dopamine and the Compound A elevates brain levels of the dopamine in the patient.

[0267] In certain embodiments the Compound A reduces stress associated with memory recall in the patient.

[0268] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one sign of the post-traumatic stress disorder in the patient.

[0269] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one symptom of the post-traumatic stress disorder in the patient.

[0270] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one symptom cluster of the post-traumatic stress disorder in the patient, wherein the symptom cluster is selected from re-experiencing/intrusion, avoidance/numbing, and hyperarousal.

[0271] In certain embodiments the re-experiencing/intrusion comprises at least one of recurrent and intrusive trauma recollections, recurrent and distressing dreams of the traumatic event, acting or feeling as if the traumatic event were recurring, distress when exposed to trauma reminders, and physiological reactivity when exposed to trauma reminders.

[0272] In certain embodiments the physiological reactivity comprises at least one of abnormal respiration, abnormal cardiac rate of rhythm, abnormal blood pressure, abnormal function of at least one special sense, and abnormal function of at least one sensory organ.

[0273] In certain embodiments the at least one special sense is selected from sight, hearing, touch, smell, taste, and sense.

[0274] In certain embodiments the at least one sensory organ is selected from eye, ear, skin, nose, tongue, and pharynx.

[0275] In certain embodiments the avoidance/numbing comprises at least one of efforts to avoid thoughts or feelings associated with the trauma, efforts to avoid activities or situations, inability to recall trauma or trauma aspects, markedly diminished interest in

significant activities, feelings of detachment or estrangement from others, restricted range of affect, sense of a foreshortened future, social anxiety, and anxiety associated with unfamiliar surroundings.

[0276] In certain embodiments the hyperarousal comprises at least one of difficulty falling or staying asleep, irritability or outbursts of anger, difficulty concentrating, hypervigilance, exaggerated startle response, and anxiety from potentially threatening stimuli.

[0277] In certain embodiments the Compound A does not reduce the physical ability of the patient to respond appropriately and promptly to the potentially threatening stimuli.

[0278] In certain embodiments the Compound A reduces the difficulty of staying asleep by reducing stress associated with memory recall and dreaming.

[0279] In certain embodiments the patient is a child or an adolescent.

[0280] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one sign or symptom of the post-traumatic stress disorder in the patient, wherein the sign or symptom is selected from disorganized or agitated behavior, repetitive play that expresses aspects of the trauma, frightening dreams which lack recognizable content, and trauma-specific reenactment.

[0281] In certain embodiments the Compound A reduces the incidence of at least one disorder comorbid with post-traumatic stress disorder selected from drug abuse, alcohol abuse, and depression in the patient.

[0282] In certain embodiments the Compound A is administered to the patient once or twice a day.

[0283] In certain embodiments the Compound A does not cause at least one of drowsiness, lassitude, or alteration of mental and physical capabilities.

[0284] In certain embodiments the Compound A is administered to the patient before or immediately after a traumatic event.

[0285] In certain embodiments at least one sign, symptom, or symptom cluster of post-traumatic stress syndrome is diagnosed or assessed with at least one of Clinician-Administered PTSD Scale (CAPS), Clinician-Administered PTSD Scale Part 2 (CAPS-2), Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA), Impact of Event Scale (IES), Impact of Event Scale-Revised (IES-R), Clinical Global Impression Scale (CGI), Clinical Global Impression Severity of Illness (CGI-S), Clinical Global Impression Improvement (CGI-I), Duke Global Rating for PTSD scale (DGRP), Duke Global Rating for PTSD scale Improvement (DGRP-I), Hamilton Anxiety Scale

(HAM-A), Structured Interview for PTSD (SI-PTSD), PTSD Interview (PTSD-I), PTSD Symptom Scale (PSS-I), Mini International Neuropsychiatric Interview (MINI), Montgomery-Åsberg Depression Rating Scale (MADRS), Beck Depression Inventory (BDI), Hamilton Depression Scale (HAM-D), Revised Hamilton Rating Scale for Depression (RHRSD), Major Depressive Inventory (MDI), Geriatric Depression Scale (GDS-30), and Children's Depression Index (CDI).

[0286] In certain embodiments the Compound A significantly changes a score on at least one of CAPS, CAPS-2, CAPS-CA, IES, IES-R, CGI, CGI-S, CGI-I, DGRP, DGRP-I, HAM-A, SI-PTSD, PTSD-I, PSS-I, MADRS, BDI, HAM-D, RHRSD, MDI, GDS-30, and CDI.

[0287] In certain embodiments the Compound A significantly reduces an endpoint score compared to a baseline score on at least one of CAPS, CAPS-2, IES, IES-R, and HAMA.

[0288] In certain embodiments the Compound A significantly increases the proportion of responders on the CGI-I having a CGI-I score of at least one of 1 (very much improved) and 2 (much improved).

[0289] In certain embodiments the Compound A increases the proportion of responders on the DGRP-I having a DGRP-I score of at least one of 1 (very much improved) and 2 (much improved).

[0290] In certain embodiments an overall score of at least 65 on at least one of the CAPS and the CAP-2 is indicative of post-traumatic stress disorder.

[0291] In certain embodiments an overall score of at least 18 on HAM-A is indicative of anxiety disorder.

[0292] In certain embodiments a score of at least 3 on at least one of the CGI-I and the DGRP-I is indicative of post-traumatic stress disorder.

[0293] Also provided are methods of treating post-traumatic stress disorder in a patient. The methods include diagnosing the patient with post-traumatic stress disorder; administering to the patient a therapeutically effective amount of Compound A; assessing at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder; and determining that the post-traumatic stress syndrome is improved if the Compound A reduces at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder.

[0294] In certain embodiments the method includes coadministering a therapeutically effective amount of at least one other agent, selected from benzodiazepine, a selective serotonin reuptake inhibitor (SSRI), a serotonin-norepinephrine reuptake inhibitor

(SNRI), a norepinephrine reuptake inhibitor (NRI), a serotonin 5-hydroxytryptamine_{1A} (5HT_{1A}) antagonist, a dopamine β -hydroxylase inhibitor, an adenosine A_{2A} receptor antagonist, a monoamine oxidase inhibitor (MAOI), a sodium (Na) channel blocker, a calcium channel blocker, a central and peripheral alpha adrenergic receptor antagonist, a central alpha adrenergic agonist, a central or peripheral beta adrenergic receptor antagonist, a NK-1 receptor antagonist, a corticotropin releasing factor (CRF) antagonist, an atypical antidepressant/antipsychotic, a tricyclic, an anticonvulsant, a glutamate antagonist, a gamma-aminobutyric acid (GABA) agonist, and a partial D₂ agonist.

[0295] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one sign of the post-traumatic stress disorder in the patient.

[0296] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one symptom of the post-traumatic stress disorder in the patient.

[0297] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one symptom cluster of the post-traumatic stress disorder in the patient, wherein the symptom cluster is selected from re-experiencing/intrusion, avoidance/numbing, and hyperarousal.

[0298] In certain embodiments at least one sign, symptom, or symptom cluster of post-traumatic stress syndrome is diagnosed or assessed with at least one of Clinician-Administered PTSD Scale (CAPS), Clinician-Administered PTSD Scale Part 2 (CAPS-2), Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA), Impact of Event Scale (IES), Impact of Event Scale-Revised (IES-R), Clinical Global Impression Scale (CGI), Clinical Global Impression Severity of Illness (CGI-S), Clinical Global Impression Improvement (CGI-I), Duke Global Rating for PTSD scale (DGRP), Duke Global Rating for PTSD scale Improvement (DGRP-I), Hamilton Anxiety Scale (HAM-A), Structured Interview for PTSD (SI-PTSD), PTSD Interview (PTSD-I), PTSD Symptom Scale (PSS-I), Mini International Neuropsychiatric Interview (MINI), Montgomery-Åsberg Depression Rating Scale (MADRS), Beck Depression Inventory (BDI), Hamilton Depression Scale (HAM-D), Revised Hamilton Rating Scale for Depression (RHRSD), Major Depressive Inventory (MDI), Geriatric Depression Scale (GDS-30), and Children's Depression Index (CDI).

[0299] Also provided are methods of improving resilience in a patient. The methods include administering a therapeutically effective amount of Compound A.

[0300] In certain embodiments the method includes coadministering a therapeutically effective amount of at least one other agent, selected from benzodiazepine, a selective

serotonin reuptake inhibitor (SSRI), a serotonin-norepinephrine reuptake inhibitor (SNRI), a norepinephrine reuptake inhibitor (NRI), a serotonin 5-hydroxytryptamine_{1A} (5HT_{1A}) antagonist, a dopamine β -hydroxylase inhibitor, an adenosine A_{2A} receptor antagonist, a monoamine oxidase inhibitor (MAOI), a sodium (Na) channel blocker, a calcium channel blocker, a central and peripheral alpha adrenergic receptor antagonist, a central alpha adrenergic agonist, a central or peripheral beta adrenergic receptor antagonist, a NK-1 receptor antagonist, a corticotropin releasing factor (CRF) antagonist, an atypical antidepressant/antipsychotic, a tricyclic, an anticonvulsant, a glutamate antagonist, a gamma-aminobutyric acid (GABA) agonist, and a partial D₂ agonist.

[0301] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one sign of the post-traumatic stress disorder in the patient.

[0302] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one symptom of the post-traumatic stress disorder in the patient.

[0303] In certain embodiments the Compound A reduces at least one of the frequency and intensity of at least one symptom cluster of the post-traumatic stress disorder in the patient, wherein the symptom cluster is selected from re-experiencing/intrusion, avoidance/numbing, and hyperarousal.

[0304] In certain embodiments at least one sign, symptom, or symptom cluster of post-traumatic stress syndrome is diagnosed or assessed with at least one of Clinician-Administered PTSD Scale (CAPS), Clinician-Administered PTSD Scale Part 2 (CAPS-2), Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA), Impact of Event Scale (IES), Impact of Event Scale-Revised (IES-R), Clinical Global Impression Scale (CGI), Clinical Global Impression Severity of Illness (CGI-S), Clinical Global Impression Improvement (CGI-I), Duke Global Rating for PTSD scale (DGRP), Duke Global Rating for PTSD scale Improvement (DGRP-I), Hamilton Anxiety Scale (HAM-A), Structured Interview for PTSD (SI-PTSD), PTSD Interview (PTSD-I), PTSD Symptom Scale (PSS-I), Mini International Neuropsychiatric Interview (MINI), Montgomery-Åsberg Depression Rating Scale (MADRS), Beck Depression Inventory (BDI), Hamilton Depression Scale (HAM-D), Revised Hamilton Rating Scale for Depression (RHRSD), Major Depressive Inventory (MDI), Geriatric Depression Scale (GDS-30), and Children's Depression Index (CDI).

[0305] Also provided are methods of diagnosing post-traumatic stress disorder in a patient. The methods include administering to the patient a therapeutically effective amount of Compound A and assessing at least one of sign, symptom, or symptom cluster

of post-traumatic stress disorder; and diagnosing post-traumatic stress disorder in the patient if the Compound A reduces at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder.

[0306] In certain embodiments the patient is a child, adolescent, or adult.

[0307] Various scales can assess post-traumatic stress disorder (PTSD) and the effect of rufinamde and other therapies on the treatment and prevention of the disorder. These are, for example and without limitation, Clinician-Administered PTSD Scale (CAPS), Clinician-Administered PTSD Scale Part 2 (CAPS-2), Clinician-Administered PTSD Scale for Children and Adolescents (CAPS-CA), Impact of Event Scale (IES), Impact of Event Scale-Revised (IES-R), Clinical Global Impression Scale (CGI), Clinical Global Impression Severity of Illness (CGI-S), Clinical Global Impression Improvement (CGI-I), Duke Global Rating for PTSD scale (DGRP), Duke Global Rating for PTSD scale Improvement (DGRP-I), Hamilton Anxiety Scale (HAM-A), Structured Interview for PTSD (SI-PTSD), PTSD Interview (PTSD-I), PTSD Symptom Scale (PSS-I), Mini International Neuropsychiatric Interview (MINI), Montgomery-Åsberg Depression Rating Scale (MADRS), Beck Depression Inventory (BDI), Hamilton Depression Scale (HAM-D), Revised Hamilton Rating Scale for Depression (RHRSD), Major Depressive Inventory (MDI), Geriatric Depression Scale (GDS-30), and Children's Depression Index (CDI). These measures generally are assessed by interviews or questionnaires. In certain embodiments, not all the parts of a scale are administered. In certain embodiments, the scales are used for diagnosing and assessing signs, symptoms, associated symptoms, or impact on daily life of PTSD. In certain embodiments, one or more scales are used to diagnose, assess, or confirm post-traumatic stress disorder in a patient. In certain embodiments, scales will measure signs, symptoms, symptom clusters by scoring at least one of the frequency and intensity of the signs, symptoms, or symptom clusters.

[0308] Examples of scales for post-traumatic stress disorder assessment are versions of CAPS, including CAPS, CAPS-1, and CAPS-2, which score 17 core PTSD symptoms with these items:

[0309] 1. Recurrent and intrusive trauma recollections

[0310] 2. Distress when exposed to trauma reminders

[0311] 3. Acting or feeling as if event were recurring

[0312] 4. Recurrent and distressing dreams of event

[0313] 5. Efforts to avoid thoughts or feelings

[0314] 6. Efforts to avoid activities or situations

- [0315] 7. Inability to recall trauma or trauma aspects
- [0316] 8. Markedly diminished interest in significant activities
- [0317] 9. Feelings of detachment or estrangement from others
- [0318] 10. Restricted range of affect
- [0319] 11. Sense of a foreshortened future
- [0320] 12. Difficulty falling or staying asleep
- [0321] 13. Irritability or outbursts of anger
- [0322] 14. Difficulty concentrating
- [0323] 15. Hypervigilance
- [0324] 16. Exaggerated startle response
- [0325] 17. Physiologic reactivity
- [0326] Questions also target the impact of symptoms on social and occupational functioning or daily life, improvement in symptoms since a previous CAPS administration, overall response validity, overall PTSD severity, and frequency and intensity of associated symptoms. These items are:
- [0327] 18. Impact on Social Functioning
- [0328] 19. Impact on Occupational Functioning
- [0329] 20. Global Improvement (since earlier measurement occasion)
- [0330] 21. Rating Validity
- [0331] 22. Global Improvement
- [0332] 23. Guilt over acts committed or omitted
- [0333] 24. Survivor Guilt
- [0334] 25. Homicidality
- [0335] 26. Disillusionment with authority
- [0336] 27. Feelings of hopelessness
- [0337] 28. Memory Impairment
- [0338] 29. Sadness and depression
- [0339] 30. Feelings of being overwhelmed
- [0340] To assess the frequency of symptoms, interviewers follow standard questions, clarifying or rephrasing as needed. Standard questions, by way of example and without limitation, are: Have you ever had unwanted memories of the traumatic event? What were they like? What did you remember? If the question requires rephrasing, the interviewer can ask a question such as: Did they ever occur while you were awake or only in dreams? or How often have you had these memories in the past month (week)? A

score of 0 indicates a frequency of never, 1 indicates once or twice, 2 indicates once or twice a week, 3 indicates several times a week, and 4 indicates daily or almost every day.

[0341] To assess the intensity of symptoms, an interviewer may ask standard questions such as by way of example and without limitation: How much distress or discomfort did these memories cause you? Were you able to put them out of your mind and think about something else? How hard did you have to try? How much did they interfere with your life? A score of 0 indicates none, 1 indicates mild, minimal distress or disruption of activities, 2 indicates moderate, distress clearly present but still manageable, some disruption of activities, 3 indicates severe, considerable distress, difficulty dismissing memories, marked disruption of activities, and 4 indicates extreme, incapacitating distress, cannot dismiss memories, unable to continue activities.

[0342] In certain embodiments the scoring rule used counts a symptom as present if it has a frequency of at least 1 and an intensity of at least 2. In certain embodiments severity scores are calculated by summing the frequency and intensity ratings for each symptom.

[0343] In certain embodiments, a total or overall score of all items on a version of CAPS is calculated. In certain embodiments, a total score for each symptom cluster is calculated. In certain embodiments, a total score for core symptoms of PTSD is calculated. In certain embodiments, an endpoint score is compared to a baseline score to determine the change in severity of post-traumatic stress disorder. In certain embodiments, a significant reduction of an endpoint score compared to a baseline score is considered improvement of PTSD. In certain embodiments, an overall score on CAPS, CAPS-1, CAPS-2, or CAPS-CA greater than 65 is indicative of PTSD.

[0344] Another example is the IES which assesses 15 items: 7 items measure intrusive symptoms and 8 items measure avoidance symptoms. The self assessed items ask how frequently each of the following comments are true: I thought about it when I didn't mean to, I avoided letting myself get upset when I thought about it or was reminded of it, I tried to remove it from memory, I had trouble falling asleep or staying asleep because of pictures or thoughts about it that came into my mind, I had waves of strong feelings about it, I had dreams about it, I stayed away from reminders of it, It felt as if it hadn't happened or wasn't real, I tried not to talk about it, Pictures about it popped into my mind, Others things kept making me think about it, I was aware that I still had a lot of feelings about it, but I didn't deal with them, I tried not to think about it, Any reminder brought back feelings about it, and My feelings were kind of numb. The items are generally rated on a four point scale: 0 (not at all), 1 (rarely), 3 (sometimes), and 5 (often). The total of the

scores provide an overall assessment of the severity of the symptoms or overall subjective stress. It has been suggested that a score from 0 to 8 is in the subclinical range, 9-25 is in the mild range, 26-43 is in the moderate range, and greater than 44 is in the severe range of stress.

[0345] In certain embodiments, a total or overall score of all items on IES is calculated. In certain embodiments, a total score for each symptom cluster is calculated. In certain embodiments, an endpoint score is compared to a baseline score to determine the change in severity of PTSD. In certain embodiments, a reduction of an endpoint score by 30% compared to a baseline score is considered improvement of PTSD.

[0346] The IES-R, a revision of the IES, changed the IES by splitting the original IES item, I had trouble falling asleep or staying asleep into two items: I had trouble falling asleep and I had trouble staying asleep and by adding six items to the IES items. These additional items are: I felt irritable and angry, I was jumpy and easily startled, I found myself acting or feeling as though I was back at that time, I had trouble concentrating, Reminders of it caused me to have physical reactions, such as sweating, trouble breathing, nausea, or a pounding heart, and I felt watchful or on guard. The scoring system also changed to 0 (not at all), 1 (a little bit), 2 (moderately), 3 (quite a bit), and 4 (extremely).

[0347] In certain embodiments, a total or overall score of all items on IES-R is calculated. In certain embodiments, a total score for each symptom cluster is calculated. In certain embodiments, an endpoint score is compared to a baseline score to determine the change in severity of post-traumatic stress disorder. In certain embodiments, a significant reduction of an endpoint score compared to a baseline score on the IES-R is considered improvement of post-traumatic stress disorder.

[0348] In the DGRP-I scale, the effectiveness of Compound A in treating post-traumatic stress disorder can be assessed by measuring the increase in the proportion of responders on the DGRP-I having a DGRP-I of 1 (very much improved) or 2 (much improved). In certain embodiments, a score of at least 3 on the DGRP-I is indicative of post-traumatic stress

[0349] In the CGI, the effectiveness of Compound A to treat post-traumatic stress disorder can be assessed by the CGI-S, CGI-I, and efficacy index. For example, in certain embodiments, an increase in the proportion of responders on the CGI-I having a CGI-I of 1 (very much improved) or 2 (much improved) after treatment indicates that the treatment is effective. In certain embodiments, a score of at least 3 on the CGI-I is

indicative of post-traumatic stress disorder. In certain embodiments, the efficacy index on the CGI can measure the efficacy of Compound A for treatment of post-traumatic stress disorder.

[0350] In HAMA-A, to assess anxiety or post-traumatic stress disorder, generally a total or overall score of all items on HAM-A is calculated. In certain embodiments, an endpoint score is compared to a baseline score on HAM-A to determine the change in severity of anxiety and post-traumatic stress disorder. In certain embodiments, a significant reduction of an endpoint score compared to a baseline score on HAM-A is considered improvement of anxiety and post-traumatic stress disorder. In certain embodiments, an overall score on HAM-A of at least 18 is indicative of anxiety and post-traumatic stress disorder.

[0351] In general, Compound A or a pharmaceutically acceptable derivative will be administered in therapeutically effective amounts, either singly or in combination with another therapeutic agent. The pharmaceutical compositions will be useful, for example, for the treatment of post-traumatic stress disorder.

[0352] Pharmaceutically acceptable derivatives include acids, bases, enol ethers, and esters, esters, hydrates, solvates, and prodrug forms. The derivative is selected such that its pharmacokinetic properties are superior with respect to at least one characteristic to the corresponding neutral agent. The Compound A may be derivatized prior to formulation.

[0353] A therapeutically effective amount of Compound A or a pharmaceutically acceptable derivative may vary widely depending on the severity of the post-traumatic stress disorder, the age and relative health of the subject, the potency of the compound used and other factors. In certain embodiments a therapeutically effective amount is from about 0.1 milligram per kg (mg/kg) body weight per day to about 50 mg/kg body weight per day. In other embodiments the amount is about 1.0 to about 10 mg/kg/day. Therefore, In certain embodiments a therapeutically effective amount for a 70 kg human is from about 7.0 to about 3500 mg/day, while in other embodiments it is about 70 to about 700 mg/day.

[0354] One of ordinary skill in the art of treating such diseases will be able to ascertain a therapeutically effective amount of Compound A for post-traumatic stress disorder without undue experimentation and in reliance upon personal knowledge and the disclosure of this application. In general, by way of example and without limitation, Compound A will be administered as pharmaceutical compositions by one of the following routes: oral, systemic (e.g., transdermal, intranasal or by suppository) or

parenteral (e.g., intramuscular, intravenous or subcutaneous). Compositions can, by way of example and without limitation, take the form of tablets, pills, capsules, semisolids, powders, sustained release formulations, solutions, suspensions, elixirs, aerosols, or any other appropriate composition and are comprised of, in general, Compound A in combination with at least one pharmaceutically acceptable excipient. Acceptable excipients are, by way of example and without limitation, non-toxic, aid administration, and do not adversely affect the therapeutic benefit of the compound. Such excipient may be, for example, any solid, liquid, semisolid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

[0355] Solid pharmaceutical excipients include by way of example and without limitation starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, and the like. Liquid and semisolid excipients may be selected from for example and without limitation water, ethanol, glycerol, propylene glycol and various oils, including those of petroleum, animal, vegetable or synthetic origin (e.g., peanut oil, soybean oil, mineral oil, sesame oil, etc.). Preferred liquid carriers, particularly for injectable solutions, include by way of example and without limitation water, saline, aqueous dextrose and glycols. Compressed gases may be used to disperse the compound in aerosol form. Inert gases suitable for this purpose are by way of example and without limitation nitrogen, carbon dioxide, nitrous oxide, etc.

[0356] The pharmaceutical preparations can by way of example and without limitation, moreover, contain preservatives, solubilizers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavorants, salts for varying the osmotic pressure, buffers, masking agents or antioxidants. In certain embodiments, they can contain still other therapeutically valuable substances. Other suitable pharmaceutical carriers and their formulations are described in A. R. Alfonso *Remington's Pharmaceutical Sciences* 1985, 17th ed. Easton, Pa.: Mack Publishing Company.

[0357] The amount of Compound A in the composition may vary widely depending for example, upon the type of formulation, size of a unit dosage, kind of excipients and other factors known to those of skill in the art of pharmaceutical sciences. In general, the final composition will comprise from 10% w to 90% w of the compound, preferably 25% w to 75% w, with the remainder being the excipient or excipients. Preferably the pharmaceutical composition is administered in a single unit dosage form for continuous

treatment or in a single unit dosage form ad libitum when relief of symptoms is specifically required.

[0358] The Compound A or a pharmaceutically acceptable derivative thereof is administered simultaneously with, prior to, or after administration of one or more of the above agents.

[0359] The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

[0360] A clinical study is performed to demonstrate the efficacy and tolerability of Compound A in the treatment of post-traumatic stress disorder (PTSD).

[0361] The research design includes an 8-week randomized, double-blind, placebo-controlled treatment trial of Compound A for the treatment of PTSD.

[0362] After signing an informed consent and meeting inclusion/exclusion criteria, patients are randomized to receive either Compound A or placebo for the 8-week duration. During the study a pharmacist maintains the randomization log and verify the order for the placebo or Compound A in look-a-like tablets. Patients' symptoms, side effects and compliance is assessed bi-weekly.

[0363] Based on symptomatology and occurrence of side effects, the investigator may increase the medication in 20-40 mg increments, as tolerated, until a maximum therapeutic benefit is achieved. The dosing is once per day unless twice per day is better tolerated. Compliance is assessed by pill count at week 4 and week 8.

[0364] Patients is given supportive clinical management during the clinic visits. An investigator is available by telephone 24 hrs a day in case of emergency. Patients may be seen more often if needed.

[0365] Efficacy is measured by the following assessment scales:

- Global Assessment of Functioning (GAF)
- Clinician Administered PTSD Scale (CAPS)
- Clinical Global Impression Severity of Illness (CGI-s)
- Clinical Global Impression of Improvement (CGI-I)
- Davidson Trauma Scale (DTS).
- Hamilton Anxiety Scale (Ham-A)
- Montgomery-Asberg Depression Rating Scale (MADRS)

- Treatment Outcome PTSD rating scale (TOP-8)

[0366] The subject inclusion criteria are:

- Diagnosis of PTSD that is confirmed by Mini International Neuropsychiatric Interview (MINI) and CAPS
- Age 13 or older
- No substance abuse or dependence for the previous 4 weeks (except for nicotine and caffeine)
- Free of psychotropic medication for 2 weeks (except 4 weeks for fluoxetine)
- Clinically normal physical and laboratory examination (Liver function tests (LFTs) up to 2.5 times the normal limit is allowed.)
- Women of childbearing potential must be using medically approved methods of birth control such as a condom, birth control pill, Depo-Provera, or diaphragm with spermicides
- Signed informed consent
- Male or female, any race or ethnic origin

[0367] The subject exclusion criteria are:

- Lifetime history of bipolar I, psychotic, or cognitive disorders
- Actively suicidal, homicidal, or psychotic
- History of sensitivity to Compound A
- Unstable general medical conditions
- Score ≥ 6 on Question #10 of MADRS regarding suicidal ideation
- Women who are pregnant, planning to become pregnant or breastfeed during the study

[0368] Fulfillment of only one exit criterion is needed to exit the study. Exit criteria are:

- Completion of the study
- Severe and intolerable side effects to Compound A or placebo treatment
- Acute development of suicidal ideation, homicidal ideation or psychotic symptoms
- Worsening of symptoms as measured by a score of 7 (very much worse) on CGI-I
- Participant's explicit request to exit the study

- The need for additional psychotropic drugs, other than the study drug or adjunctive medication as specified in the protocol, for the control of the subjects psychiatric symptoms
- The subject becomes pregnant during the course of the study
- Investigator's judgment that it is no longer in the best interest of the patient to continue in the study

Example 2

[0369] A clinical study is performed to demonstrate the efficacy and tolerability of Compound A in the prevention of PTSD.

[0370] The research design includes an open-ended randomized, double-blind, placebo-controlled treatment trial of Compound A for the prevention of PTSD. After signing an informed consent and meeting inclusion/exclusion criteria, patients are randomized to receive either Compound A versus placebo for the 8-week duration. During the study a pharmacist maintains the randomization log and verify the order for the placebo or Compound A in look-a-like tablets. Patients' symptoms, side effects and compliance are assessed bi-weekly.

[0371] Based on symptomatology and occurrence of side effects, the investigator can increase the medication in 20-40 mg increments, as tolerated, until a maximum therapeutic benefit is achieved. The dosing is once per day unless twice per day is better tolerated. Compliance is assessed by pill count at week 4 and week 8.

[0372] Patients are given supportive clinical management during the clinic visits. An investigator is available by telephone 24 hrs a day in case of emergency. Patients may be seen more often if needed.

[0373] Efficacy is measured by the following assessment scales:

- Global Assessment of Functioning (GAF)
- Clinician Administered PTSD Scale (CAPS)
- Clinical Global Impression Severity of Illness (CGI-s)
- Clinical Global Impression of Improvement (CGI-I)
- Davidson Trauma Scale (DTS).
- Hamilton Anxiety Scale (Ham-A)
- Montgomery-Asberg Depression Rating Scale (MADRS)

- Treatment Outcome PTSD rating scale (TOP-8)
- Diagnostic and Statistical Manual IV (DSM-IV)

[0374] The subject inclusion criteria are:

- Absence of PTSD, confirmed by MINI and CAPS
- Age 13 or older
- No substance abuse/dependence for the previous 4 weeks (except for nicotine and caffeine)
- Free of psychotropic medication for 2 weeks (except 4 weeks for fluoxetine)
- Clinically normal physical and laboratory examination (LFTs up to 2.5 times the normal limit is allowed.)
- Women of childbearing potential must be using medically approved methods of birth control (such as a condom, birth control pill, Depo-Provera, or diaphragm with spermicides)
- Signed informed consent
- Male or female, any race or ethnic origin

[0375] The exclusion criteria are:

- History of PTSD
- Lifetime history of bipolar I, psychotic, or cognitive disorders
- Actively suicidal, homicidal, or psychotic
- History of sensitivity to Compound A
- Unstable general medical conditions
- Score ≥ 6 on Question #10 of MADRS regarding suicidal ideation
- Women who are pregnant, planning to become pregnant or breastfeed during the study

[0376] Fulfillment of only one exit criterion is needed to exit the study. Exit Criteria are:

- Completion of the study
- Severe and intolerable side effects to Compound A or placebo treatment
- Acute development of suicidal ideation, homicidal ideation or psychotic symptoms
- Appearance of signs or symptoms compatible with a diagnosis of PTSD.
- Participant's explicit request to exit the study

- The need for additional psychotropic drugs, other than the study drug or adjunctive medication as specified in the protocol, for the control of the subjects psychiatric symptoms.
- The subject becomes pregnant during the course of the study.
- Investigator's judgment that it is no longer in the best interest of the patient to continue in the study.

Example 3

[0377] A clinical study is conducted to demonstrate the efficacy and tolerability of Compound A combination therapy in the treatment of PTSD.

[0378] The research design includes an 8-week randomized, double-blind, placebo-controlled treatment trial of Compound A for the treatment of PTSD. After signing an informed consent and meeting inclusion/exclusion criteria, the patient is randomized to receive either Compound A or placebo for 8-week duration. Patients can also receive therapeutically effective doses of prazosin, valproate, carbamazepine, or topiramate in combination with Compound A or placebo.

[0379] During the study a pharmacist maintains the randomization log and verifies the order for the placebo or Compound A in look-a-like tablets. Patients' symptoms, side effects and compliance is assessed bi-weekly. Based on symptomatology and occurrence of side effects, the investigator increases the medication in 20-40 mg increments, as tolerated, until a maximum therapeutic benefit is achieved. The dosing is once per day unless twice per day is better tolerated. Compliance is assessed by pill count at week 4 and week 8.

[0380] Patients are given supportive clinical management during the clinic visits. An investigator is available by telephone 24 hrs a day in case of emergency. Patients may be seen more often if needed.

[0381] Efficacy is measured by the following assessment scales:

- Global Assessment of Functioning (GAF)
- Clinician Administered PTSD Scale (CAPS)
- Clinical Global Impression Severity of Illness (CGI-s)
- Clinical Global Impression of Improvement (CGI-I)
- Davidson Trauma Scale (DTS).
- Hamilton Anxiety Scale (Ham-A)

- Montgomery-Asberg Depression Rating Scale (MADRS)
- Treatment Outcome PTSD rating scale (TOP-8)

[0382] The subject inclusion criteria are:

- Diagnosis of PTSD, confirmed by MINI and CAPS
- Age 13 or older
- No substance abuse/dependence for the previous 4 weeks (except for nicotine and caffeine)
- Free of psychotropic medication for 2 weeks (except 4 weeks for fluoxetine)
- Clinically normal physical and laboratory examination (LFTs up to 2.5 times the normal limit is allowed.)
- Women of childbearing potential must be using medically approved methods of birth control (such as a condom, birth control pill, Depo-Provera, or diaphragm with spermicides)
- Signed informed consent
- Male or female, any race or ethnic origin

[0383] The subject exclusion criteria are:

- Lifetime history of bipolar I, psychotic, or cognitive disorders
- Actively suicidal, homicidal, or psychotic
- History of sensitivity to Compound A
- Unstable general medical conditions
- Score ≥ 6 on Question #10 of MADRS regarding suicidal ideation
- Women who are pregnant, planning to become pregnant or breastfeed during the study

[0384] Fulfillment of only one exit criterion is needed to exit the study. Exit Criteria are:

- Completion of the study
- Severe and intolerable side effects to Compound A or placebo treatment
- Acute development of suicidal ideation, homicidal ideation or psychotic symptoms
- Symptoms worsen as measured by a Score of 7 (very much worse) on CGI-I
- Participant's explicit request to exit the study
- The need for additional psychotropic drugs, other than the study drug or adjunctive medication as specified in the protocol, for the control of the subjects psychiatric symptoms

- The subject becomes pregnant during the course of the study
- Investigator's judgment that it is no longer in the best interest of the patient to continue in the study

Example 4

[0385] A clinical study is performed to demonstrate the efficacy and tolerability of Compound A in the treatment of PTSD in children.

[0386] The research design includes an 8-week randomized, double-blind, placebo-controlled treatment trial of Compound A for the treatment of PTSD.

[0387] After signing an informed consent and meeting inclusion/exclusion criteria, patients are randomized to receive either Compound A or placebo for an 8-week duration. During the study a pharmacist maintains the randomization log and verify the order for the placebo or Compound A in look-a-like tablets. Patients' symptoms, side effects and compliance are assessed bi-weekly.

[0388] Based on symptomatology and occurrence of side effects, the investigator can increase the medication in 20-40 mg increments, as tolerated, until a maximum therapeutic benefit is achieved. The dosing is once per day unless twice per day is better tolerated. Compliance is assessed by pill count at week 4 and week 8.

[0389] Patients are given supportive clinical management during the clinic visits. An investigator is available by telephone 24 hrs a day in case of emergency. Patients may be seen more often if needed.

[0390] Efficacy is measured by the following assessment scales:

- Global Assessment of Functioning (GAF)
- Clinician Administered PTSD Scale (CAPS)
- Clinician Administered PTSD Scale (CAPS-CA)
- Clinical Global Impression Severity of Illness (CGI-s)
- Clinical Global Impression of Improvement (CGI-I)
- Davidson Trauma Scale (DTS).
- Hamilton Anxiety Scale (Ham-A)
- Montgomery-Asberg Depression Rating Scale (MADRS)
- Treatment Outcome PTSD rating scale (TOP-8)

[0391] The subject inclusion criteria are:

- Diagnosis of PTSD, confirmed by MINI and CAPS

- Age 12 or younger
- No substance abuse/dependence for the previous 4 weeks (except for nicotine and caffeine)
- Free of psychotropic medication for 2 weeks (except 4 weeks for fluoxetine)
- Clinically normal physical and laboratory examination (LFTs up to 2.5 times the normal limit is allowed.)
- Women of childbearing potential must be using medically approved methods of birth control (such as a condom, birth control pill, Depo-Provera, or diaphragm with spermicides)
- Signed informed consent
- Male or female, any race or ethnic origin

[0392] The subject exclusion criteria are:

- Lifetime history of bipolar I, psychotic, or cognitive disorders
- Actively suicidal, homicidal, or psychotic
- History of sensitivity to Compound A
- Unstable general medical conditions
- Score ≥ 6 on Question #10 of MADRS regarding suicidal ideation
- Women who are pregnant, planning to become pregnant or breastfeed during the study

[0393] Fulfillment of only one exit criterion is needed to exit the study. Exit Criteria are:

- Completion of the study
- Severe and intolerable side effects to Compound A or placebo treatment
- Acute development of suicidal ideation, homicidal ideation or psychotic symptoms
- Symptoms worsen as measured by a Score of 7 (very much worse) on CGI-I.
- Participant's explicit request to exit the study
- The need for additional psychotropic drugs, other than the study drug or adjunctive medication as specified in the protocol, for the control of the subjects psychiatric symptoms
- The subject becomes pregnant during the course of the study
- Investigator's judgment that it is no longer in the best interest of the patient to continue in the study

Example 5

[0394] Bovine and human dopamine- β -hydroxylase activity was assayed by measuring the conversion of tyramine to octopamine. Bovine adrenal dopamine- β -hydroxylase was obtained from Sigma Chemicals (St Louis, MO, USA) whereas human dopamine- β -hydroxylase was purified from the culture medium of the neuroblastoma cell line SK-N-SH. The assay was performed at pH 5.2 and 32°C in a medium containing 0.125 M NaAc, 10 mM fumarate, 0.5 - 2 μ M CuSO₄, 0.1 mg.ml⁻¹ catalase, 0.1 mM tyramine and 4 mM ascorbate. In a typical assay, 0.5 - 1 milliunits of enzyme were added to the reaction mixture and, subsequently, a substrate mixture containing catalase, tyramine and ascorbate was added to initiate the reaction (final volume of 200 μ l). Samples were incubated with or without the appropriate concentration of nepicastat (S-enantiomer) or (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride (R-enantiomer) at 37°C for 30 - 40 minutes. The reaction was quenched by the stop solution containing 25 mM EDTA and 240 μ M 3-hydroxytyramine (internal standard). The samples were analysed for octopamine by reverse phase high pressure liquid chromatography (HPLC) using ultraviolet-detection at 280 nm. The HPLC chromatography run was carried out at the flow rate of 1 ml.min⁻¹ using a LiChroCART 125-4 RP-18 column and isocratic elution with 10 mM acidic acid, 10 mM 1-heptane sulfonic acid, 12 mM tetrabutyl ammonium phosphate and 10% methanol. The remaining percent activity was calculated based on controls, corrected using internal standards and fitted to a non-linear four-parameter concentration-response curve.

[0395] The activity of nepicastat at twelve selected enzymes and receptors was determined using established assays. Details of individual receptor radioligand binding assays can be found in Wong et al (1993). A brief account of the principle underlying each of the enzymatic assays is given in Figure 1. Binding data were analyzed by iterative curve-fitting to a four parameter logistic equation. K_i values were calculated from IC₅₀ values using the Cheng-Prusoff equation. Enzyme inhibitory activity was expressed as IC₅₀ (concentration required to produce 50% inhibition of enzyme activity). Male SHR_s (15 - 16 weeks old, Charles River, Wilmington, MA, USA) were used in the study. On the day of the study, animals were weighed and randomly assigned to be dosed with either vehicle (control) or the appropriate dose of nepicastat (3, 10, 30 or 100 mg.kg⁻¹, po) or (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride (30 mg.kg⁻¹, po) three consecutive times, twelve

hours apart. At six hours after the third dose, the rats were anaesthetized with halothane, decapitated and tissues (cerebral cortex, mesenteric artery and left ventricle) were rapidly harvested, weighed, placed in iced perchloric acid (0.4 M), frozen in liquid nitrogen and stored at -70°C until subsequent analysis. To quantify noradrenaline and dopamine concentrations, tissues were homogenized by brief sonication and centrifuged at 13,000 rpm for 30 minutes at 4°C. The supernatant, spiked with 3, 4-dihydroxybenzylamine (internal standard), was assayed for noradrenaline and dopamine by HPLC using electrochemical detection.

[0396] Male beagle dogs (10 - 16 kg, Marshall Farms USA Inc, North Rose, NY, USA) were used in the study. On the day of the study, dogs were weighed and randomly assigned to be orally dosed with either empty capsules (control) or the appropriate dose of nepicastat (0.05, 0.5, 1.5 or 5 mg.kg⁻¹; po, b.i.d.) for 5 days. At six hours following the first dose on day-5, the dogs were euthanized with pentobarbital and the tissues (cerebral cortex, renal artery, left ventricle) were rapidly harvested. The tissues were subsequently processed and analysed for noradrenaline and dopamine as described above.

[0397] Male beagle dogs were randomized to be orally dosed with either empty capsules (control) or nepicastat (2 mg.kg⁻¹, po, b.i.d.) for 15 days. Daily venous blood samples were drawn, six hours after the first dose, for measurement of plasma concentrations of dopamine and noradrenaline. The samples were collected in tubes containing heparin and glutathione, centrifuged at -4°C and the separated plasma was stored at -70°C until analysis.

[0398] Nepicastat ((S)-5-aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-1,3-dihydroimidazole-2-thione hydrochloride) and the corresponding R-enantiomer ((R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride) were synthesized. In studies involving SHRs, the drugs were dissolved in distilled water and dosed orally with a gavage needle. In the dog studies, the drugs were filled in capsules and dosed orally. All doses are expressed as free base equivalents.

[0399] All data are expressed as mean ± s.e.mean. Tissue and plasma catecholamine data were analysed using a non-parametric one-way analysis of variance (ANOVA) or two-way ANOVA, respectively, followed by pairwise comparison using Fisher LSD test. P < 0.05 was considered statistically significant.

[0400] Nepicastat (S-enantiomer) and (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride (R-enantiomer)

produced concentration-dependent inhibition of bovine and human dopamine- β -hydroxylase activity. The calculated IC_{50} 's for nepicastat were 8.5 ± 0.8 nM and 9.0 ± 0.8 nM for the bovine and human enzyme, respectively. (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride was slightly less potent (IC_{50} 's of 25.1 ± 0.6 nM and 18.3 ± 0.6 nM for the bovine and human enzyme, respectively) than nepicastat.

[0401] Nepicastat had negligible affinity (IC_{50} s or K_i 's > 10 μ M) for a range of other enzymes (tyrosine hydroxylase, acetyl CoA synthetase, acyl CoA-cholesterol acyl transferase, Ca^{2+} /calmodulin protein kinase II, cyclooxygenase-I, HMG-CoA reductase, neutral endopeptidase, nitric oxide synthase, phosphodiesterase III, phospholipase A_2 , and protein kinase C) and neurotransmitter receptors (α_{1A} , α_{1B} , α_{2A} , α_{2B} , β_1 and β_2 adrenoceptors, M_1 muscarinic receptors, D_1 and D_2 dopamine receptors, μ opioid receptors, 5-HT $_{1A}$, 5-HT $_{2A}$, and 5-HT $_{2C}$ serotonin receptors).

[0402] Basal tissue catecholamine content (μ g.g $^{-1}$ wet weight) in control animals were as follows : mesenteric artery (noradrenaline, 10.40 ± 1.03 ; dopamine, 0.25 ± 0.02), left ventricle (noradrenaline, 1.30 ± 0.06 ; dopamine, 0.02 ± 0.00) and cerebral cortex (noradrenaline, 0.76 ± 0.03 ; dopamine, 0.14 ± 0.01). Nepicastat produced dose-dependent reduction in noradrenaline content and enhancement of dopamine content and dopamine/noradrenaline ratio in the three tissues which were studied (Figures 2 & 3). Figure 2 shows the effects of nepicastat on tissue noradrenaline (O) and dopamine ($\square$) content in the mesenteric artery (A), left ventricle (B) and cerebral cortex (C) of SHR. Data are expressed as mean $\pm$ s.e.mean; n = 7-9 per group. * $p < 0.05$ vs control (0). Figure 3 shows the effects of nepicastat on tissue dopamine/noradrenaline ratio in the mesenteric artery (O), left ventricle ($\square$) and cerebral cortex ($\triangle$) of SHR. Data are expressed as mean $\pm$ s.e.mean, n = 7-9 per group. * $p < 0.05$ vs control (0).

[0403] These changes attained statistical significance ($p < 0.05$) at doses of ≥ 3 mg.kg $^{-1}$ in the mesenteric artery and left ventricle but only at doses of 30 and 100 mg.kg $^{-1}$ in the cerebral cortex. At the highest dose studied (100 mg.kg $^{-1}$, po), the decreases in noradrenaline were 47%, 35%, 42% and increases in dopamine were 820%, 800% and 86% in the mesenteric artery, left ventricle and cerebral cortex, respectively. When tested at 30 mg.kg $^{-1}$, po, the S-enantiomer (nepicastat) produced significantly greater changes in catecholamine content, as compared to the R-enantiomer ((R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride), in the mesenteric artery and left ventricle (Figure 7). Figure 7 shows the

effect of nepicastat and (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride, at 30 mg.kg⁻¹; po, on noradrenaline content, dopamine content and dopamine/noradrenaline ratio in mesenteric artery, left ventricle and cerebral cortex of SHRs. Data are expressed as mean $\pm$ sem. n=9 per group. * p<0.05 vs. control, #p<0.05 vs nepicastat.

[0404] Basal tissue catecholamine content ($\mu\text{g.g}^{-1}$ wet weight) in control animals were as follows : renal artery (noradrenaline, 10.7 ± 1.05 ; dopamine, 0.22 ± 0.01), left ventricle (noradrenaline, 2.11 ± 0.18 ; dopamine, 0.07 ± 0.03) and cerebral cortex (noradrenaline, 0.26 ± 0.02 ; dopamine, 0.03 ± 0.00). When compared to control animals, nepicastat produced dose-dependent reduction in noradrenaline content and enhancement of dopamine content and dopamine/noradrenaline ratio in the three tissues which were studied (Figures 4 & 5). Figure 4 shows the effects of nepicastat on tissue noradrenaline (O) and dopamine ($\square$) content in renal artery (A), left ventricle (B) and cerebral cortex (C) of beagle dogs. Data are expressed as mean $\pm$ s.e.mean; n = 8 per group. * p< 0.05 vs control (0). Figure 5 shows the effects of nepicastat on tissue dopamine/noradrenaline ratio in the renal artery (O), left ventricle ($\square$) and cerebral cortex ($\triangle$) of beagle dogs. Data are expressed as mean $\pm$ s.e.mean, n = 8 per group. * p< 0.05 vs control (0).

[0405] These changes attained statistical significance (p < 0.05) at doses of $\geq 0.1 \text{ mg.kg}^{-1} \cdot \text{day}^{-1}$ in the three tissues. At the highest dose studied (5 mg.kg^{-1} , b.i.d., po), the decreases in noradrenaline were 88%, 91% and 96% and increases in dopamine were 627%, 700% and 166% in the renal artery, left ventricle and cerebral cortex, respectively. Baseline concentrations of catecholamines in two groups of animals were not significantly different from each other: plasma noradrenaline and dopamine concentrations were 460.3 ± 59.6 and $34.4 \pm 11.9 \text{ pg.ml}^{-1}$, respectively, in the control group and 401.9 ± 25.5 and $41.1 \pm 8.8 \text{ pg.ml}^{-1}$, respectively, in the nepicastat-treated group. When compared to the control group, nepicastat (2 mg.kg^{-1} , b.i.d, po) produced significant decreases in plasma concentrations of noradrenaline and increases in plasma concentrations of dopamine and dopamine/noradrenaline ratio (Figure 6). Figure 6 shows the Effects of nepicastat on plasma concentrations of noradrenaline (A), dopamine (B) and dopamine/noradrenaline ratio (C) in beagle dogs. Control dogs (O); nepicastat-treated dogs ($\bullet$). Data are expressed as mean $\pm$ s.e.mean; n = 8 per group. * p< 0.05 vs control. The peak reduction (52%) in plasma concentration of noradrenaline was observed on day-6 of dosing whereas the peak increase (646%) in plasma concentration of dopamine was observed on day-7 of dosing.

[0406] Inhibitory modulation of sympathetic nerve function, through pharmacological means, is an attractive therapeutic strategy for the management of congestive heart failure, inasmuch as elevated activity of this system has been implicated in the progressive worsening of the disease. The aim of this study was to pharmacologically characterize the effects of nepicastat, a compound which modulates noradrenaline synthesis in sympathetic nerves by inhibiting the enzyme dopamine- β -hydroxylase.

[0407] nepicastat was shown to be a potent inhibitor of human and bovine dopamine- β -hydroxylase *in vitro*. The inhibitory effects of the compound were stereospecific since the S-enantiomer (nepicastat) was marginally, but significantly, more potent than the R-enantiomer ((R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride). nepicastat displayed a high degree of selectivity for dopamine- β -hydroxylase as the compound possessed negligible affinity for twelve other enzymes and thirteen neurotransmitter receptors.

[0408] Inhibition of dopamine- β -hydroxylase *in vivo* would be expected to result in elevated levels of the substrate (dopamine) and diminished levels of the product (noradrenaline) in tissues which receive noradrenergic innervation. This expectation was borne out in experiments which investigated the effects of nepicastat on catecholamine levels in central and peripheral tissues *in vivo*. In both SHRs and beagle dogs, nepicastat produced dose-dependent reductions in noradrenaline content and increases in dopamine content in peripheral (mesenteric or renal artery, left ventricle) and central (cerebral cortex) tissues. In this respect, (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride was less potent than nepicastat which is consistent with the lower IC₅₀ of the former enantiomer for the enzyme. Although dopamine/noradrenaline ratio was also elevated, there did not appear to be stoichiometric replacement of noradrenaline with dopamine. The most likely explanation for this finding is that tissue levels of dopamine may have been underestimated due to intraneuronal metabolism of dopamine.

[0409] The ability of nepicastat to alter catecholamine levels in the cerebral cortex suggests that the drug does penetrate the blood brain barrier. In dogs, the magnitude of the changes in catecholamines in the cerebral cortex appeared comparable to those in peripheral tissues. In SHRs, however, nepicastat, at low doses ($\leq 10 \text{ mg.kg}^{-1}$), produced significant changes in noradrenaline and dopamine content in peripheral tissues without affecting catecholamines in the cerebral cortex. This suggests that, at least in SHRs, the drug does possess modest peripheral selectivity. We have also shown that nepicastat

attenuates sympathetically mediated cardiovascular responses and lowers blood pressure in SHRs without affecting motor activity (Hegde et al., 1996 a & b); these findings will be reported in a separate manuscript.

[0410] Plasma noradrenaline concentrations provide a useful measure of overall sympathetic nerve activity although this parameter may be influenced by alterations in neuronal uptake and metabolic clearance of the catecholamine. Baseline concentrations of noradrenaline in the plasma were surprisingly elevated in the dogs and is, perhaps, a reflection of the initial stress induced by the phlebotomy blood-sampling procedure. Nevertheless, compared to the control group, nepicastat produced significant decreases in plasma noradrenaline concentrations consistent with reduced transmitter synthesis and release although an indirect effect, secondary to facilitation of neuronal uptake or metabolic clearance, cannot be discounted. Since released noradrenaline represents a small fraction of the total neuronal noradrenaline stores, an inhibitor of noradrenaline biosynthesis would affect noradrenaline release only after existing stores of the catecholamine have been sufficiently depleted. Accordingly, the decreases in plasma noradrenaline concentrations did not attain statistical significance until 4 days of dosing with nepicastat suggesting gradual modulation of the sympathetic nervous system. It should be recognized that measurements of plasma noradrenaline concentrations alone do not account for regional differences in noradrenaline release (Esler et al., 1984), which underscores the need for making measurements of organ-specific noradrenaline spillover rates in future studies.

[0411] A growing body of evidence suggests that chronic activation of the sympathetic nervous system in congestive heart failure is a maladaptive response. This contention is supported by clinical trials which have shown a beneficial effect of carvedilol in congestive heart failure patients with respect to long-term morbidity and mortality. However, it should be noted that most patients do require some level of sympathetic drive to support cardiovascular homeostasis. Indeed, the therapeutic value of β -blockers, including carvedilol, may be limited by their propensity to cause hemodynamic deterioration especially during initiation of therapy. This unwanted effect, which results from abrupt withdrawal of sympathetic support, necessitates careful dose-titration. Inhibitors of dopamine- β -hydroxylase, such as nepicastat, may be devoid of this undesirable effect for the following reasons. First, this class of drugs would attenuate, but not abolish, noradrenaline release and, second, they produce gradual modulation of the system thereby obviating the need for dose-titration. Another advantage of nepicastat

over β -blockers is that it enhances dopamine levels which, via agonism of dopamine receptors, may have salutary effects on renal function such as renal vasodilation, diuresis and natriuresis.

[0412] In summary, nepicastat is a potent, selective and orally active inhibitor of dopamine- β -hydroxylase which may be of value in the treatment of cardiovascular disorders associated with over-activation of the sympathetic nervous system.

Example 6

[0413] Synthesis of nepicastat (2a) (Figure 8 and Figure 9). Oral administration of 2a to spontaneously hypertensive rats (SHR) and normal dogs produced potent and dose-dependent increases in tissue dopamine (DA)/norepinephrine (NE) ratios in peripheral arteries (renal or mesenteric), left ventricle and cerebral cortex. Chronic oral administration of 2a to normal dogs also produced sustained increases in the plasma DA/NE ratio. In conscious SHR, acute oral administration of 2a produced dose-dependent and long-lasting (> 4 h) antihypertensive effects and also attenuation of the pressor responses to pre-ganglionic sympathetic nerve stimulation. Serum T₃ and T₄ levels were unaffected by a dose (6.2 mg/kg, po, b.i.d. for 10 days) which elevated the dopamine/norepinephrine ratio in the mesenteric artery. On the basis of its ability to potently modulate the sympathetic drive to cardiovascular tissues, 2a is currently in clinical evaluation for the treatment of congestive heart failure.

[0414] Congestive heart failure (CHF) is a leading cause of mortality in the United States. CHF is characterized by marked activation of the sympathetic nervous system (SNS) and renin-angiotensin system (RAS). The simultaneous activation of these two neurohormonal systems has been increasingly implicated in the perpetuation and progression of CHF. Therapeutic interventions which block the effects of these neurohormonal systems are likely to favorably alter the natural history of CHF. Indeed, angiotensin-converting enzyme (ACE) inhibitors, which block formation of angiotensin II, have been shown to reduce morbidity and mortality in CHF patients. ACE inhibitors, however, have a limited indirect ability to attenuate the SNS. Inhibition of the SNS with β -adrenoceptor antagonists is a promising approach that is currently under clinical evaluation. An alternative strategy to directly modulate the SNS is inhibition of norepinephrine (NE) biosynthesis *via* inhibition of dopamine β -hydroxylase (DBH), the enzyme responsible for conversion of NE to dopamine (DA). Inhibition of DBH would be expected to reduce tissue levels of NE and elevate tissue levels of DA thereby increasing the tissue DA/NE ratio. This approach has potential advantages over β -

adrenoceptor antagonists, such as reduced stimulation of α -adrenoceptors and elevated DA levels that can produce renal vasodilation, natriuresis and diminished aldosterone release. Previous DBH inhibitors, such as fusaric acid and SKF 102698, have drawbacks such as low potency and specificity, that have precluded their clinical development in heart failure.

[0415] This example shows 2a (nepicastat) to be a potent and selective inhibitor of DBH related to SKF 10269. The preparation of 2a (Scheme I) was based upon the chiral reduction of tetralone 3 (available from the AlCl_3 -catalyzed Friedel-Crafts reaction of 3,5-difluorophenylacetyl chloride with ethylene in CH_2Cl_2 at -65°C) under the conditions described by Terashima⁷ (LAH, (-)-1*R*,2*S*-*N*-methylephedrine, 2-ethylaminopyridine) to give *R*-(+)-tetralol 4a (92-95% ee). Conversion of 4a to the *R*-(+)-mesylate 5a, followed by reaction with sodium azide afforded a mixture (9:1) of azide 6a and dihydronaphthalene 7. The azide was hydrogenated and the product treated with anhydrous HCl to give *S*-(-)-amine hydrochloride 8a, converted by a Strecker reaction (formaldehyde bisulfite complex and KCN) to *S*-(-)-aminonitrile 9a. Formation of the heterocycle 10a was accomplished by sequential diformylation of aminonitrile 9a followed by subsequent treatment with thiocyanic acid. Competing hydrolysis of the nitrile afforded comparable amounts of the primary amide 11a. Reduction of nitrile 10a to amine 2a (93-96% ee) was accomplished using LAH in THF. The enantiomer 2b (91.6% ee) was available by the same above described route using (+)-1*S*,2*R*-*N*-methylephedrine as a chiral auxiliary in the Terashima reduction of ketone 3. The absolute configuration of the chiral center in 4a,b, and thus 2a,b was based upon literature precedence of the previously described *S*-(-)-2-tetralol.

[0416] Tetralin 2a was demonstrated to be a competitive inhibitor of bovine ($\text{IC}_{50} = 8.5 \pm 0.8 \text{ nM}$) and human ($\text{IC}_{50} = 9.0 \pm 0.8 \text{ nM}$) DBH. The *R*-enantiomer 2b ($\text{IC}_{50\text{S}} = 25.1 \pm 0.6 \text{ nM}$; $18.3 \pm 0.6 \text{ nM}$) and 1 ($\text{IC}_{50\text{S}} = 67.0 \pm 4.2 \text{ nM}$; $85.0 \pm 3.7 \text{ nM}$) are less potent inhibitors of the bovine and human enzymes, respectively. Compound 2a showed weak affinity for a range of other enzymes and neurotransmitter receptors (Figure 10). These data suggest that 2a is a potent and highly selective inhibitor of DBH *in vitro*. Moreover, the *S*-enantiomer is approximately 2-3 fold more potent than the *R*-enantiomer suggesting stereoselectivity.

[0417] The *in vivo* biochemical effects of 2a, 2b and 1 were evaluated in spontaneously hypertensive rats (SHR) and normal beagle dogs. Oral administration of 2a produced dose-dependent increases in DA/NE ratios in the artery (mesenteric or renal), left

ventricle and cerebral cortex in SHR (Fig 11A) and dogs (Fig. 11B). At the highest dose tested (100 mg/kg in SHR and 5 mg/kg in dogs) the maximal increases in DA/NE ratio were 14, 11 and 3.2 fold (in SHR) and 95, 151 and 80 fold (in dogs) in the artery, left ventricle and cerebral cortex, respectively. When tested at 30 mg/kg in SHR, SKF 102698 (1) increased the DA/NE ratio by 5.5-fold, 3.5-fold and 2.7-fold, whereas 2a, at the same dose, increased the ratio by 8.3, 7.5 and 1.5 fold in the mesenteric artery, left ventricle and cerebral cortex, respectively. The *R*-enantiomer 2b, at 30 mg/kg in SHR, produced only 2.6, 3.5 and 1.1 fold increases in the DA/NE ratio in the mesenteric artery, left ventricle and cerebral cortex, respectively. These data suggest that 2a produces the expected biochemical effects in both SHR and dogs but is more potent in the latter species. Furthermore, 2a is more potent than its *R*-enantiomer 2b and SKF 102698 (1) in SHR.

[0418] The chronic effects of 2a (14.5 day treatment) on the plasma DA/NE ratio were investigated in normal dogs. Oral administration of 2a (2 mg/kg; b.i.d) produced a significant increase in the DA/NE ratio that attained its peak effect at approximately 6-7 days, then plateaued to a new steady-state between 7-14 days (Fig 12).

[0419] The *in vivo* hemodynamic activity of 2a was further assessed in conscious, restrained SHR, a model having high sympathetic drive to cardiovascular tissues. Oral dosing of 2a resulted in a dose-dependent antihypertensive effect (Fig 13). A maximal decrease in mean blood pressure of 53 ± 4 mmHg (33% reduction relative to vehicle control) was observed at the 10 mg/kg dose. The response was slow in onset, reaching its plateau in 3-4 h. The precise reason for the loss of anti-hypertensive efficacy at the highest dose (30 mg/kg) is unclear at present. Heart rate was not significantly affected except for a slight yet significant decrease at 10 and 30 mg/kg, (9.8 and 10.5 %, respectively). Following this study, the rats were pithed and the effects of 2a on the pressor response to pre-ganglionic nerve stimulation (PNS) of the spinal cord were evaluated 5 h after dosing. The frequency-pressor response curve was shifted significantly ($p < 0.05$) to the right in a dose-dependent manner (maximum shift of ~ 5 fold in the frequency-response curve). The heart rate response to PNS was not significantly affected. These data suggest that 2a inhibits the sympathetic drive to the vasculature and is the probable mechanism for its anti-hypertensive effect in SHR.

[0420] Since the heterocyclic portion of 2a is structurally similar to methimazole, a known potent suppressor of mammalian thyroid function, the effects of 2a on thyroid function were evaluated at doses of 2.0 and 6.2 mg/kg, po, b.i.d in iodine-deficient

Sprague-Dawley rats (n = 9-12) for 10 days. Methimazole (1 mg/kg, po, b.i.d.), used as a positive control, caused a significant reduction in serum levels of T₃ (day 3, 31 %, p < 0.05; days 7 and 9, 42 % and 44 %, p < 0.01) and T₄ (days 3 and 7, 46 % and 58 %, p < 0.01) 4 h post-dose, whereas 2a showed no significant effects throughout the study (days 3, 7 and 9). Both doses of 2a significantly raised the DA/NE ratio in the mesenteric artery (p < 0.01 relative to vehicle controls) but not in the cortex 4 h after the final dose on day 10.

[0421] The findings of this study suggest that 2a (nepicastat) is a potent, selective and orally active inhibitor of DBH. The compound is also devoid of significant behavioral effects in animal models and these findings will be the subject of a future publication. As compound 2a (nepicastat) effectively modulates the sympathetic drive to cardiovascular tissues, it is currently undergoing development for the treatment of CHF.

[0422] Figure 9 shows ^a(a) i: SOCl₂; ii: AlCl₃, CH₂Cl₂, ethylene, -65 °C; (b) (-)-1*R*,2*S*-*N*-methylephedrine, 2-ethylaminopyridine, 1M LAH in Et₂O, <-60 °C for the *R*-enantiomer or (+)-1*S*,2*R*-*N*-methylephedrine, 2-ethylaminopyridine, 1M LAH in Et₂O, <-60 °C for the *S*-enantiomer; (c) MsCl, Et₂O, Et₃N, -15 °C; (d) NaN₃, DMSO, 50 °C; (e) i: H₂, 10% Pd/C, EtOAc, 60 psi; ii: 1M HCl/Et₂O; (f) NaOH, formaldehyde-sodium bisulfite complex, KCN, H₂O, 50-80 °C; (g) i: *n*-butyl formate, 120 °C; ii: *t*-BuOK, ethyl formate, THF, -15 °C; iii: 1M HCl, EtOH, KSCN; (h) i: 1M LAH in THF, 20 °C; ii: HCl/Et₂O, MeOH.

[0423] Figure 10 shows a table describing the interaction of nepicastat at DBH and a range of selected enzymes and receptors.

[0424] Figure 11: (A) - Effects of 2a on tissue DA/NE ratio in spontaneously hypertensive rats. Animals were dosed orally, 12 h apart, and the tissues were harvested 6 h after the third dose. * p < 0.05 vs placebo (vehicle).

[0425] (B) - Effects of 2a on tissue DA/NE ratio in normal beagle dogs. Animals were dosed orally, twice a day, for 4.5 days and the tissues were harvested 6 h after the first dose on day 5. * p < 0.05 vs placebo (empty capsule).

[0426] DA and NE concentrations were assayed by HPLC with electrochemical detection. All data are expressed as mean ± standard error of mean. n = 9 per group.

[0427] Figure 12: Effects of chronic administration of 2a on plasma DA/NE ratio in normal beagle dogs. Animals were dosed orally, b.i.d., for 14.5 d. Blood sampling was done on each day 6 h after the first dose. 2a produced significant (p < 0.05) increases in DA/NE ratio at all time-points compared to the placebo group. DA and NE

concentrations in plasma were assayed by HPLC with electrochemical detection. All data are expressed as mean $\pm$ standard error of mean. $n = 8$ per group.

[0428] Figure 13: Effects of orally administered 2a on mean arterial pressure in conscious, restrained spontaneously hypertensive rats (SHR). SHR were lightly anesthetized with ether and instrumented for measurement of arterial pressure and drug administration. The animals were placed in restrainers and allowed to recover for 30 - 40 minutes. After obtaining baseline measurements, the animals were treated, orally, with either vehicle or the appropriate dose of 2a and hemodynamic parameters were continuously recorded for 4 h. 2a produced significant ($p < 0.05$) lowering of mean arterial pressure at all doses and time points, except at 0.3 mg/kg (180, 210 and 240 min) and 1 mg/kg (30, 210 and 240 min). All data are expressed as mean $\pm$ standard error of mean. $n = 6-8$ per group.

[0429] Melting points were determined on a Uni-Melt Thomas Hoover Capillary Melting Point Apparatus or a Mettler FB 81HT cell with a Mettler FP90 processor and are uncorrected. Mass spectra were obtained with either a Finnigan MAT 8230 (for electron-impact or chemical ionization) or Finnigan MAT TSQ70 (for LSIMS) spectrometer. ^1H NMR spectra were recorded on a Bruker ACF300, AM300, AMX300 or EM390 spectrometer and chemical shifts are given in ppm (δ) from tetramethylsilane as internal standard. IR spectra were recorded on a Nicolet SPC FT-IR spectrometer. UV spectra were recorded on a Varian Cary 3 UV-Visible spectrometer, Leeman Labs Inc. Optical rotations were measured in a Perkin-Elmer Model 141 polarimeter. Chiral HPLC measurements were performed on a Regis Chiral AGP column (4.6 x 100 mm) eluting with 2% acetonitrile-98% 20 mM KH_2PO_4 (pH 4.7) at 1 mL/min at 20 °C.

[0430] 5,7-Difluoro-2-tetralone (3). SOCl_2 (100 mL) was added in one portion to 3,5-difluorophenylacetic acid (100 g, 0.58 mol) and after stirring for 15 h, the volatiles were evaporated under reduced pressure. The resulting oily acid chloride was dissolved in CH_2Cl_2 (200 mL) and added dropwise to a mechanically stirred suspension of AlCl_3 (154 g, 1.16 mol) in CH_2Cl_2 (1.0 L). The stirred suspension was cooled to an internal temperature of -65 °C in a dry ice/acetone bath, and the acid chloride solution was added at such a rate in order to maintain an internal temperature < -60 °C. After the addition was complete, ethylene gas was bubbled through the reaction mixture at a rapid rate for 10 min at -65 °C. The reaction mixture was allowed to warm to 0 °C over 2 h with stirring, and was then cooled to -10 °C and treated with H_2O (500 mL) initially dropwise, followed by rapid addition. The organic layer was separated, washed with brine (100

mL) and dried over MgSO_4 . Evaporation under reduced pressure gave a dark oily residue which was distilled *in vacuo* on a Kugelrohr collecting material boiling between 90-110 °C (1.0 to 0.7 mm Hg). The distillate was redistilled at 100-105 °C (0.3 mm Hg) to give 3 as a white solid, (73.6 g, 0.40 mol; 70%): mp 46 °C; IR (KBr) 1705 cm^{-1} ; ^1H NMR (CDCl_3) δ 2.55 (t, $J=7.5$ Hz, 2H), 3.10 (t, $J=7.5$ Hz, 2H), 3.58 (s, 2H), 6.70 (m, 2H); MS m/z 182 (M^+). Anal. Calcd for $\text{C}_{10}\text{H}_8\text{F}_2\text{O}$: C, 65.93; H, 4.42. Found: C, 65.54; H, 4.42.

[0431] (R)-(+)-2-Hydroxy-5,7-difluoro-1,2,3,4-tetrahydronaphthalene (4a). A solution of (-)-1*R*,2*S*-*N*-methylephedrine (81.3 g, 0.454 mol) in anhydrous Et_2O (1.1 L) was added dropwise (45 min) to 1.0 M lithium aluminum hydride (416 mL, 0.416 mol) in Et_2O at a rate sufficient to maintain a gentle reflux. After the addition was complete, the reaction mixture was heated at reflux for 1 h then allowed to cool to room temperature. A solution of 2-ethylaminopyridine (111 g, 0.98 mole) in anhydrous Et_2O (100 mL) was added (45 min) at such a rate as to maintain a gentle reflux. The reaction mixture was heated at reflux for a further 1 h, during which time a light yellow-green suspension appeared. The mixture was cooled to an internal temperature of -65 °C using a dry ice-acetone bath and a solution of 5,7-difluoro-2-tetralone (23.0 g, 126 mmol) in Et_2O (125 mL) was added dropwise at a rate maintaining the internal temperature below -60 °C. After the addition was complete, the mixture was stirred at -65 °C to -68 °C for 3 h and quenched by the addition of MeOH (100 mL) maintaining the internal temperature below -60 °C. The reaction was stirred for a further 10 min at -65 °C and allowed to warm to approximately -20 °C. A solution of 3N HCl (2 L) was then added at a rate to limit the temperature to <35 °C. After stirring at an increased rate to achieve total dissolution, the layers were separated and the ethereal layer was washed with brine (200 mL) and dried (MgSO_4). The ethereal solution was evaporated under reduced pressure and the residue dissolved in warm Et_2O (20 mL) followed by the addition of hexane (200 mL). The seeded solution was cooled in an ice bath and maintained at 0° C for 1 h whereupon the resulting deposited crystals were collected and dried *in vacuo* to give alcohol 4a (10.9 g, 47 %): mp 85 °C; $[\alpha]_D^{25} +38.1^\circ$ ($c = 1.83$, CHCl_3); 93.4% ee by chiral HPLC: ^1H NMR (CDCl_3) δ 1.70 (br s, 1H), 1.76-1.88 (m, 2H), 1.99-2.06 (m, 2H), 2.63-3.08 (m, 3H), 4.15 (m, 1H), 6.60 (m, 2H). Anal. Calcd for $\text{C}_{10}\text{H}_{10}\text{F}_2\text{O}$: C, 65.21; H, 5.47. Found: C, 65.38; H, 5.42. The spectra for the (*S*)-enantiomer 4b are identical: mp 84-85 °C; $[\alpha]_D^{25} -37.8^\circ$ ($c = 1.24$, CHCl_3); 92.4% ee by chiral HPLC. Anal. Calcd for $\text{C}_{10}\text{H}_{10}\text{F}_2\text{O}$: C, 65.21; H, 5.47. Found: C, 65.47; H, 5.39.

[0432] (R)-(+)-2-Methanesulfonyloxy-5,7-difluoro-1,2,3,4-tetrahydronaphthalene (5a). A solution of *R*-(+)-5,7-difluoro-2-tetralol 4a (59.0 g, 320 mmol) and Et₃N (74.2 mL, 53.9 g, 530 mmol) in anhydrous Et₂O (1.78 L) was cooled (-15 °C) using an ice-MeOH bath and treated under argon with stirring with MsCl (37.2 mL, 55.3 g, 480 mmol) over 5-10 min. After 5 h the reaction was complete (as determined by TLC) and water was added to dissolve the solids. A small amount of EtOAc was added to help complete dissolution of the solids. The organic phase was separated and washed sequentially with 1N HCl, aq. NaHCO₃, brine and dried over MgSO₄. Evaporation of the solvent gave an off-white solid 5a (87.1 g, 332 mmol), used directly in the next step. Trituration of a small sample with *i*-Pr₂O gave an analytical sample: mp 78.8-80.5 °C; $[\alpha]^{25}_{\text{D}} +16.8^{\circ}$ (c = 1.86, CHCl₃); ¹H NMR δ 2.13-2.28 (m, 2H), 2.78-2.96 (m, 2H), 3.07 (s, 3H), 3.09 (dd, *J* = 17.1 Hz, 4.7, 1H), 3.20 (dd, *J* = 17.2, 4.7 Hz, 1H), 5.20 (m, 1H), 6.67 (m, 2H). Anal. Calcd for C₁₁H₁₂F₂O₃S: C, 50.37; H, 4.61. Found: C, 50.41; H, 4.64. The spectra for the (*S*)-enantiomer 5b are identical: mp 79.9-80.9 °C; $[\alpha]^{25}_{\text{D}} -16.6^{\circ}$ (c = 2.23, CHCl₃). Anal. Calcd for C₁₁H₁₂F₂O₃S: C, 50.37; H, 4.61. Found: C, 50.41; H, 4.65.

[0433] (*S*)-(-)-2-Amino-5,7-difluoro-1,2,3,4-tetrahydronaphthalene hydrochloride (8a). Sodium azide (40.0 g, 0.62 mol) was added to DMSO (1 L) with stirring until a clear solution was obtained. The mesylate 5a (138 g, 0.53 mol) was added in one portion and the mixture heated at 50° C for 16 h under a N₂ atmosphere. The reaction mixture was diluted with H₂O (1.8 L) and extracted with pentane (4 x 250 mL) followed by sequentially washing the combined pentane extracts with H₂O (2 x 100 mL), brine (100 mL) and drying over MgSO₄. Evaporation of the solvent under reduced pressure gave a volatile oil which was rapidly chromatographed on silica using pentane as the eluent to give dihydronaphthalene 7 (8.50 g, 51.2 mmol) as a volatile oil. Further elution with pentane/CH₂Cl₂ (9:1) afforded the azide 6a (101 g, 483 mmol) as a colorless oil: IR (CHCl₃) 2103 cm⁻¹; *m/z* 171 (M⁺). The azide 6a was dissolved in EtOAc (1200 mL) and hydrogenated over 10% Pd/C (6 g) in a 2.5 L Parr bottle (60 psi) for 6 h. After each hour, the bottle was evacuated and recharged with hydrogen to remove evolved N₂. The resulting mixture was filtered through Celite, stirred with ethereal HCl (1N, 500 mL), and the fine precipitate filtered off and washed with EtOAc, and then anhydrous ether. (The filtration took about 4 h). The moist solid was transferred to a round-bottom flask, and the remaining solvent removed *in vacuo* to give a white solid 3 (90.4 g, 412 mmol; 77.9%): mp >280 °C; $[\alpha]^{25}_{\text{D}} -60.2^{\circ}$ (c = 2.68, MeOH); ¹H NMR (*d*₆-DMSO) δ 1.79 (m, 1H), 2.33 (m, 1H), 2.63 (m, 1H), 2.83-2.92 (m, 2H), 3.14 (dd, *J* = 16.7, 5.0 Hz, 1H), 3.46

(m, 1H), 6.93 (d, $J = 9.4$ Hz, 1H), 7.00 (dt, $J = 9.4, 2.5$ Hz, 1H). Anal. Calcd for $C_{10}H_{12}ClF_2N$: C, 54.68; H, 5.51; N, 6.37. Found: C, 54.31; H, 5.52; N, 6.44. The spectra for the (*R*)-enantiomer 8b are identical: mp >280 °C; $[\alpha]^{25}_D +58.5^\circ$ ($c = 1.63$, MeOH). Anal. Calcd for $C_{10}H_{12}ClF_2N$: C, 54.68; H, 5.51; N, 6.37. Found: C, 54.64; H, 5.51; N, 6.40.

[0434] (*S*)-(-)-(5,7-Difluoro-1,2,3,4-tetrahydronaphth-2-yl)(cyanomethyl)amine (9a). The amine hydrochloride 8a (50.27 g, 229 mmol) was treated with a solution of NaOH (10.0 g, 250 mmol) in water (150 mL), followed by a few additional pellets of NaOH sufficient to obtain a solution. Further water (300 mL) was added and the mixture placed in a 50 °C bath and treated with formaldehyde sodium bisulfite complex (30.8 g, 230 mmol). After the mixture had been stirred for 30 min, KCN (15.0 g, 230 mmol) was added. The reaction mixture was stirred for a further 1 h at 80 °C, cooled to room temperature, and extracted with EtOAc to give an oil (51.3 g) which solidified. TLC (5% MeOH- CH_2Cl_2) showed ca. 10-15% of starting amine remained. Chromatography on silica gave 9a (39.4 g) and starting free amine (7.12 g), which quickly forms the carbonate in air. Recycling this amine gave an additional 5.35 g of product. Combined yield (44.8 g, 202 mmol; 87.5%): mp 73.1-76.5 °C; $[\alpha]^{25}_D -58.0^\circ$ ($c = 1.63$, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.50 (br s, 1H), 1.70 (m, 1H), 2.05 (m, 1H), 2.55-3.04 (m, 4H), 3.22 (m, 1H), 3.70 (s, 2H), 6.62 (m, 2H); MS m/z 222 (M^+). Anal. Calcd for $C_{12}H_{12}F_2N_2$: C, 64.85; H, 5.44; N, 12.60. Found: C, 65.07; H, 5.47; N, 12.44. The spectra for the (*R*)-enantiomer 9b are identical: mp 64.4-73.6 °C; $[\alpha]^{25}_D +52.3^\circ$ ($c = 2.12$, $CHCl_3$). Anal. Calcd for $C_{12}H_{12}F_2N_2$: C, 64.85; H, 5.44; N, 12.60. Found: C, 65.14; H, 5.54; N, 12.53.

[0435] (*S*)-(-)-1-(5,7-Difluoro-1,2,3,4-tetrahydronaphth-2-yl)-5-cyano-2,3-dihydro-2-thioxo-1*H*-imidazole (10a). The nitrile 9a (44.7 g, 201 mmol) in butyl formate (240 mL) was heated at reflux (120 °C bath) under N_2 for 19 h, and the solvent then removed under reduced pressure. Toluene was added and evaporated to remove last traces of solvent, and the residue was dried under high vacuum to give an oil (53.2 g). The resulting formamide and ethyl formate (48.7 mL, 44.7 g, 604 mmol) in anhydrous THF (935 mL) were cooled in ice/MeOH (-15 °C) and stirred while *t*-BuOK (1M in THF, 302 mL, 302 mmol) was added over 20 min. After the reaction had been stirred for 18 h, the solvent was evaporated, the residue dissolved in 1N HCl (990 mL) and ethanol (497 mL), and treated with KSCN (78.1 g, 804 mmol). The mixture was stirred for 135 min at 85 °C and then placed in an ice bath to give a precipitate. The filtered solid was loaded as a slurry in 10% MeOH/ CH_2Cl_2 on to a silica (1 kg) column packed in hexane. Elution with

10% acetone/CH₂Cl₂ gave 10a (18.05 g, 62.1 mmol; 30.8%): m.p. 240.7-249.2 °C; $[\alpha]_D^{25}$ -69.1° (c = 1.18, DMSO); ¹H NMR (*d*₆-DMSO) δ 2.18 (br m, 1H), 2.47 (m, 1H), 2.75 (m, 1H), 3.03-3.35 (m, 3H), 5.19 (m, 1H), 6.94 (d, *J* = 9.3 Hz, 1H), 7.03 (dt, *J* = 9.3, 2.4 Hz, 1H), 8.29 (s, 1H), 13.3 (br s, 1H); MS *m/z* 291 (M⁺). Anal. Calcd for C₁₄H₁₁F₂N₃S: C, 57.72; H, 3.80; N, 14.42. Found: C, 57.82; H, 3.92; N, 14.37. (Further elution of the column with 1:1 MeOH/CH₂Cl₂ gave the primary amide 11a: mp 261.9-262.7 °C; $[\alpha]_D^{25}$ -90.5° (c = 0.398); IR (KBr) 1593, 1630 cm⁻¹; ¹H NMR (*d*₆-DMSO) δ 2.14 (m, 1H), 2.15-2.28 (m, 1H), 2.74-3.05 (m, 4H), 5.64 (m, 1H), 6.90 (d, *J* = 9.2 Hz, 1H), 7.05 (dt, *J* = 9.5, 2.4 Hz, 1H), 8.73 (s, 1H), 9.70 (br s, 1H), 13.7 (br s, 1H); MS *m/z* 309 (M⁺). Anal. Calcd for C₁₄H₁₃F₂N₃OS•0.25H₂O: C, 53.57; H, 4.33; N, 13.39. Found: C, 53.32; H, 3.96; N, 13.24. The spectra for the (*R*)-enantiomer 10b are identical: mp 243.1-244.7 °C; $[\alpha]_D^{25}$ +74.9° (c = 2.14, DMSO). Anal. Calcd for C₁₄H₁₁F₂N₃S: C, 57.72; H, 3.80; N, 14.42. Found: C, 57.85; H, 3.85; N, 14.45.

[0436] (*S*)-1-(5,7-Difluoro-1,2,3,4-tetrahydronaphth-2-yl)-5-aminomethyl-2,3-dihydro-2-thioxo-1*H*-imidazole. The above nitrile (5.00 g, 17.2 mmol) in THF (75 mL) was stirred under argon in an ice bath until a homogeneous solution was obtained. A solution of LAH in THF (1 M, 34.3 mL, 34.3 mmol) was added dropwise over 10 min, then the solution was stirred for 30 min at 0 °C and allowed to come to room temperature for 1.5 h. The reaction was again cooled to 0 °C and treated with a saturated solution of sodium potassium tartrate until the mixture became freely stirrable. Further tartrate solution (30 mL) was added, followed by 10% MeOH/CH₂Cl₂ (200 mL) and the mixture stirred for 15 min and treated with water (100-150 mL). The organic layer was separated and the aqueous phase extracted with 10% MeOH/CH₂Cl₂ (2 x 125 mL). The combined extracts were washed, dried (MgSO₄), and evaporated. Chromatography of the residue (5.2 g) on silica eluting with 5% MeOH/CH₂Cl₂ gave 2a as the free amine (2.92 g, 9.89 mmol; 58%): mp 170 °C; $[\alpha]_D^{25}$ -11.0° (c = 1.59, DMSO). Anal. Calcd for C₁₄H₁₅F₂N₃S•0.25H₂O: C, 56.07; H, 5.21; N, 14.01. Found: C, 56.11; H, 5.10; N, 14.14.

[0437] (*S*)-1-(5,7-Difluoro-1,2,3,4-tetrahydronaphth-2-yl)-5-aminomethyl-2,3-dihydro-

[0438] 2-thioxo-1*H*-imidazole hydrochloride (2a). The hydrochloride salt was prepared by the addition of ethereal HCl (1M, 20 mL, 20 mmol) to the free amine 2a (3.12 g, 10.6 mmol) which had been dissolved in MeOH (250 mL) by warming. The solvent was partially removed under reduced pressure and displaced by co-evaporation with EtOAc

several times without evaporating to dryness. The resulting precipitate was treated with EtOAc (150 mL) and ether (150 mL), filtered off, washed with ether, and dried under nitrogen and then under high vacuum at 78 °C for 20 h to give the hydrochloride salt (3.87 g): mp 245 °C (dec); $[\alpha]_D^{25} +9.65^\circ$ ($c = 1.70$, DMSO); (93% ee by chiral HPLC); ^1H NMR ($T = 320^\circ\text{K}$, DMSO) δ 2.07 (m, 1H), 2.68-3.08 (m, 4H), 4.09 (m, 3H), 4.77 (m, 1H), 6.84 (m, 2H), 7.05 (s, 1H), 8.57 (br s, 3H), 12.4 (br s, 1H). Anal. Calcd for $\text{C}_{14}\text{H}_{16}\text{ClF}_2\text{N}_3\text{S}\cdot 0.5\text{H}_2\text{O}$: C, 49.33; H, 5.03; N, 12.33. Found: C, 49.44; H, 4.96; N, 12.18. The spectra for the (*R*)-enantiomer 2b are identical; mp 261-263 °C; $[\alpha]_D^{25} -10.8^\circ$ ($c = 1.43$, DMSO), 91.6% ee by chiral HPLC. Anal. Calcd for $\text{C}_{14}\text{H}_{16}\text{ClF}_2\text{N}_3\text{S}\cdot 0.35\text{H}_2\text{O}$: C, 49.73; H, 4.98; N, 12.42. Found: C, 49.80; H, 4.93; N, 12.39.

[0439] *In vitro* assay of DBH activity. DBH activity was assayed by measuring the conversion of tyramine to octopamine. Bovine DBH from adrenal glands was obtained from Sigma Chemical Co (St Louis, MO). Human secretory DBH was purified from the culture medium of the neuroblastoma cell line SK-N-SH. The assay was performed at pH 5.2 and 32 °C in 0.125 M NaOAc, 10 mM fumarate, 0.5 -2 μM CuSO_4 , 0.1 mg/mL catalase, 0.1 mM tyramine and 4 mM ascorbate. In a typical assay, 0.5 - 1 milliunits of enzyme were added to the reaction mixture and then a substrate mixture containing catalase, tyramine and ascorbate was added to initiate the reaction (final volume of 200 μL). Samples were incubated with or without the appropriate concentration of the inhibitor at 37 °C for 30 - 40 min. The reaction was quenched by the stop solution containing 25 mM EDTA and 240 μM 3-hydroxytyramine (internal standard). The samples were analysed for octopamine by reverse phase HPLC using UV detection at 280 nM. The remaining percent activity was calculated based on controls (without inhibitor), corrected using internal standards and fitted to a non-linear 4-parameter concentration-response curve to obtain IC_{50} values.

[0440] *In vitro* assay of selected enzymes and neurotransmitter receptors. The activity of 2a at eleven different enzymes was determined using established assays (PanLabs Inc, Foster City, CA). The affinity of 2a for thirteen selected receptors was determined by radioligand binding assays using standard filtration techniques and membrane preparations. Binding data were analyzed by iterative curve fitting to a four parameter logistic equation. K_i values were calculated using the Cheng-Prusoff equation.

[0441] *In vivo* biochemical studies in spontaneously hypertensive rats (SHR) and normal dogs. Male SHR (15 - 16 week old, Charles River, Wilmington, MA) were used in the

study. On the day of the study, the animals were weighed and randomly assigned to receive either placebo (vehicle) or the appropriate dose of 2a, 2b or 1. Each rat was dosed orally three times, 12 h apart, beginning in the morning. At 6 h after the third dose, the rats were anesthetized with halothane, decapitated, and the tissues (cerebral cortex, mesenteric artery and left ventricle) were rapidly harvested, weighed, placed in iced 0.4 M perchloric acid, frozen in liquid nitrogen and stored at -70°C until analysis. Tissue NE and DA concentrations were assayed by HPLC using electrochemical detection.

[0442] Male beagle dogs (10 - 16 kg, Marshall Farms USA Inc, North Rose, NY) were used in the study. On the day of the study, dogs were randomly assigned to receive either placebo (empty capsule) or the appropriate dose of 2a. Each dog was dosed twice a day for 4.5 days. 6 h after the first dose on day 5, the dogs were euthanized with pentobarbital and the tissues (cerebral cortex, renal artery, left ventricle) harvested, weighed, placed in iced 0.4 M perchloric acid, frozen in liquid nitrogen and stored at -70 °C until analysis. Tissue NE and DA concentrations were assayed by HPLC using electrochemical detection.

[0443] A separate study was conducted in dogs to determine the effects of chronic administration of 2a on the plasma DA/NE ratio. Animals were randomized to receive, orally, either placebo (empty capsule) or 2a (2 mg/kg, b.i.d) for 14.5 days. Daily blood samples were drawn, 6 h after the first dose, for the measurement of plasma concentrations of DA and NE. The samples were collected in tubes containing heparin and glutathione, centrifuged at -4 °C and stored at -70 °C until analysis.

[0444] Hemodynamic study in SHR. Male SHR (15 - 16 week old) were used in the study. The animals were lightly anesthetized with ether and the left femoral artery and vein were catheterized for measurement of blood pressure and drug administration, respectively. The animals were placed in restrainers and allowed to recover for 30 - 40 min. After obtaining baseline measurements, the animals were treated, orally, with either vehicle or the appropriate dose of 2a and hemodynamic parameters were continuously recorded for 4 h. The animals were then anesthetized with pentobarbital, placed on a heating pad (37 °C) and ventilated with a Harvard rodent ventilator. After administration of atropine (1 mg/kg, iv) and tubocurarine (1 mg/kg, iv), the animals were pithed through the orbit of the eye with a stainless steel rod. The pithing rod was stimulated electrically with 1 ms pulses of 80V at different frequencies (0.15, 0.45, 1.5, 5, 15 Hz) to obtain frequency -pressor response curves.

Example 7

[0445] Concentrations of dopamine and norepinephrine were determined in 942 samples of plasma collected from congestive heart failure (CHF) patients. The objectives of the study were:

[0446] 1. to evaluate the effects of various doses of nepicastat on transmyocardial (arterial-coronary sinus) and coronary sinus catecholamine levels after four weeks, and to evaluate the safety and tolerability of nepicastat over 12 weeks.

[0447] 2. to evaluate the effects of nepicastat on changes from baseline in:

[0448] a) Plasma (venous) catecholamine levels after four weeks and 12 weeks

[0449] b) Quality of life (QoL), CHF symptoms, Global Assessments, and NYHA class after four weeks and 12 weeks

[0450] c) Hemodynamic parameters, including cardiac output, systemic vascular resistance, MVO₂, pulmonary artery pressures, and pulmonary artery wedge pressure after four weeks

[0451] d) Hospitalizations and changes in medication dosages for the treatment of CHF over 12 weeks

[0452] e) Blood pressure and heart rate at four and 12 weeks

[0453] f) Six-Minute Walk Test after four weeks and 12 weeks

[0454] g) Left ventricular ejection fraction, left ventricular end systolic, and left ventricular end diastolic volumes at 12 weeks.

[0455] Samples of blood were collected from patients from a peripheral vein, whilst they were supine, at 2 hours post-dose during weeks 4 and 12. Further samples from supine patients were collected on day 0 (i.e. the day prior to the start of dosing) at a time corresponding to 2 hours post-dose. In addition, a group of patients underwent right heart and coronary sinus catheterization during week 4 at 2 hours post-dose and on day 0 (i.e. the day prior to the start of dosing) at a time corresponding to 2 hours post-dose. Triplicate samples of blood were collected from the arterial vein and coronary sinus of these patients.

[0456] Concentrations of the free base of dopamine and norepinephrine were determined by a radioenzymatic method. The method involves the incubation of the plasma samples with catechol-O-methyl transferase and tritiated S-adenosyl methionine. On completion of the incubation, the O-methylated catecholamines are extracted from the plasma by liquid/liquid extraction and then separated by thin layer chromatography. The relevant bands for each catecholamine are marked and then scraped into scintillation vials for

counting. The quantitation limit of the method is 1 pg of dopamine or norepinephrine per mL of plasma. The linear range is 1 to 333000 pg of dopamine or norepinephrine per mL of plasma using aliquots of 0.045 mL to 1 mL. A complete description of the method can be found in the publication "Radioenzymatic Microassay for Simultaneous Estimations of Dopamine, Norepinephrine and Epinephrine in Plasma, Urine and Tissues" by Benedict et al (Clinical Chemistry, Vol. 31, No. 11, 1985, pp. 1861-1864).

[0457] A pooled human plasma sample was used as the Quality Control sample (QC) and was analyzed in singlicate each day during routine use of the method to monitor the performance of the method.

[0458] The analytical results have been unrandomized and are presented in Figures 14-27. Upon completion of the analysis of the samples, the results were reviewed. Figure 28 shows a table that denotes data that should be discounted from further statistical analysis together with the reason for such an action.

Example 8

[0459] Preclinical *in vitro* and *in vivo* pharmacology studies were conducted with nepicastat. The *in vitro* studies assessed the ability of the compound to inhibit DBH activity, and its binding affinity at selected receptors. The *in vivo* studies are subdivided into four categories: 1) biochemical effects (i.e. the ability to decrease tissue norepinephrine levels and increase dopamine levels), 2) effects on thyroid function, 3) cardiovascular effects, and 4) behavioral effects.

[0460] Nepicastat was a potent inhibitor of both bovine and human DBH. The IC₅₀ for nepicastat on human DBH was 9 nM (CL 6960), significantly lower than that for the DBH inhibitor SKF102698 (85 nM). The S enantiomer of RS-nepicastat (denoted as RS-nepicastat-197) was more potent than the R enantiomer (18 nM), denoted as (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride.

[0461] The binding affinity for nepicastat was screened at selected receptors. Nepicastat showed a binding affinity of less than 5.0 for M1, D1 and D2, and 5HT_{1A}, 2A and 2C. The N-acetyl metabolite of nepicastat in rats and monkeys, showed a similar lack of binding affinity for these receptors. Thus nepicastat and its primary metabolite RS-47831-007 were not potent inhibitors for the receptors listed above.

[0462] The aortic contractile response *in vitro* to phenylephrine is impaired in spontaneously hypertensive rats (SHR) relative to normotensive Wistar-Kyoto rats. Daily

treatment with nepicastat (10 mg/kg, p.o.) in SHR for 21 days restored phenylephrine responsiveness to values comparable to the Wistar-Kyoto rats.

[0463] Overall, nepicastat was an effective inhibitor of DBH in rats and dogs. Oral or intravenous administration resulted in a significant ($p<0.05$) decrease in tissue norepinephrine, an increase in dopamine, and an increase in the dopamine/norepinephrine levels in the heart, mesenteric or renal artery, and the cerebral cortex in both species.

[0464] In studies with male spontaneously hypertensive rats (SHR), nepicastat significantly decreased norepinephrine and increased dopamine and the dopamine/norepinephrine ratio in the mesenteric artery from 0.5 to 4 hours following oral or i.v. administration at 6.2 mg/kg. Significant changes in these parameters were also observed in the left ventricle of male Sprague-Dawley rats 6 hours after the second of two i.v. injections (15 mg/kg) given 12 hours apart. The 24 hour time course of tissue catecholamines was studied in male SHR following oral administration of either 10 or 30 mg/kg, respectively. The increase in the dopamine/norepinephrine ratio was significant at 1 hour, and was long lasting (12 hours at 10 mg/kg, mesenteric artery, and 24 hours at 30 mg/kg, left ventricle). Significant changes in mesenteric artery dopamine and norepinephrine levels were observed following 10 days of dosing to male Sprague-Dawley rats at 2.0 and 6.2 mg/kg p.o. b.i.d., with no significant effects observed in the cerebral cortex. SHR dosed at 1 or 10 mg/kg/d p.o. for either 7 or 25 days had significant increases in dopamine and the dopamine/norepinephrine ratio in the mesenteric artery and cerebral cortex. Taken together, nepicastat resulted in a significant decrease in norepinephrine and an elevation in dopamine and the dopamine/norepinephrine ratio in the mesenteric artery in rats with either acute or chronic (up to 25 days) dosing.

[0465] The effects of nepicastat in male SHR and Sprague-Dawley rats were found to be dose responsive when assessed 6 hours following a single oral dose at 0.3, 1, 3, 10, 30, and 100 mg/kg. In SHR there were significant changes in the dopamine/norepinephrine ratio in the mesenteric artery at doses of 0.3 mg/kg, in the left ventricle at 3.0 mg/kg, and in the cerebral cortex at 10 mg/kg. In Sprague-Dawley rats there were significant increases in the dopamine/norepinephrine ratio in the mesenteric artery at 3.0 mg/kg, in the left ventricle at 1.0 mg/kg, and in the cerebral cortex only at 100 mg/kg. In a second dose-response study in SHR, three doses were administered 12 hours apart at either 3.0, 10, 30, or 100 mg/kg, and tissue was harvested six hours after the third dose. nepicastat caused a significant dose dependent decrease in norepinephrine (10 mg/kg) and increase in dopamine (3.0 mg/kg) and the dopamine/norepinephrine ratio (3.0 mg/kg) in the left

ventricle and mesenteric artery. The effects of nepicastat on dopamine and norepinephrine concentrations, and the dopamine/norepinephrine ratio in the cerebral cortex were significant only at 30 and 100 mg/kg. Similar significant dose-response effects in the left ventricle were seen in female Wistar rats dosed with nepicastat for 7 days via the drinking water (0.3, 0.6, and 1.0 mg/ml). In conclusion, nepicastat was less potent in inhibiting DBH in the cerebral cortex of rats (60-100 mg/kg/d) than in the left ventricle and mesenteric artery (1-6 mg/kg/d).

[0466] Nepicastat (the S enantiomer) was significantly more potent than the R enantiomer in the left ventricle and mesenteric artery in SHR after three doses given 12 hours apart (30 mg/kg p.o.). nepicastat was significantly more potent than the DBH inhibitor SKF102698 in decreasing norepinephrine and increasing dopamine and the dopamine/norepinephrine ratio in the left ventricle and mesenteric artery in SHR after a single dose, or three doses at 30 mg/kg. The potency relationships in the left ventricle and mesenteric artery resulting from these *in vivo* studies strongly parallel those obtained from *in vitro* studies using purified DBH (see above). However, nepicastat had significantly less effects than SKF 102698 in decreasing norepinephrine levels and increasing dopamine levels in the cerebral cortex. Norepinephrine has been shown to stimulate the release of renin and increase plasma renin activity. It was therefore of interest to assess whether decreasing norepinephrine levels with nepicastat would result in a decrease in plasma renin activity. However, nepicastat (30 and 100 mg/kg/d p.o. for 5 days) did not alter plasma renin activity in male SHR. Thus, nepicastat, when given at doses that lower tissue norepinephrine levels, does not alter plasma renin activity in SHR.

[0467] Nepicastat caused a significant decrease in norepinephrine levels and an increase in the dopamine/norepinephrine ratio, but did not alter dopamine levels, in the mesenteric artery from male beagle dogs 5 hours after administration of 30 mg/kg intraduodenally. When nepicastat was given to male beagle dogs for 4.5 days (5, 15, and 30 mg/kg b.i.d., or 10, 30, and 60 mg/kg/d) there was a significant decrease in norepinephrine, and an increase in dopamine and the dopamine/norepinephrine ratio in the renal artery, renal cortex, and renal medulla, with a plateau in response beginning at 10 mg/kg/d and extending through 60 mg/kg/d. Similar results were observed in the left ventricle, except that there was no significant increase in dopamine. In the cerebral cortex, norepinephrine significantly decreased at 30 and 60 mg/kg/d, and dopamine and the dopamine/norepinephrine ratio significantly increased at all doses. In conclusion,

nepicastat was a potent, orally active inhibitor of DBH in dogs at doses of at least 10 mg/kg/d.

[0468] Nepicastat has structural similarities to methimazole, a potent inhibitor of thyroid peroxidase *in vivo*. nepicastat doses of 4 or 12.4 mg/kg/d, p.o. had no effect on serum levels of triiodothyramine or thyroxine in male Sprague-Dawley rats fed a low iodine diet and dosed for 10 days, while methimazole (2 mg/kg/d) significantly reduced serum levels of triiodothyramine or thyroxine. Thus, nepicastat, unlike methimazole, did not affect serum levels of triiodothyramine or thyroxine.

[0469] Nepicastat induced a significant antihypertensive effect for up to 4 hours in conscious, restrained SHR (1.0-30 mg/kg, p.o.), and significantly reduced heart rate (10 and 30 mg/kg). The antihypertensive effects of nepicastat in conscious, restrained SHR (10 mg/kg, p.o.) were not attenuated by pretreatment with the dopamine receptor (DA-1) antagonist SCH-23390. nepicastat (10 mg/kg) also reduced blood pressure 4 hours after dosing in conscious, restrained normotensive Wistar-Kyoto rats; however, the decrease in pressure was less (-13 mmHg) than with SHR (-46 mmHg). To summarize together, nepicastat causes a decrease in blood pressure in both SHR and normotensive rats, though the antihypertensive effect is more pronounced in SHR. The antihypertensive effects in SHR do not appear to be mediated via DA-1 receptors.

[0470] Nepicastat also significantly attenuated the hypertensive and tachycardic responses to preganglionic nerve stimulation in pithed SHR 5 hours after dosing (3 mg/kg p.o.). Thus, nepicastat reduces the rise in blood pressure in response to sympathetic nerve stimulation.

[0471] Acute intravenous treatment of anesthetized SHR with nepicastat (3.0 mg/kg, i.v.) decreased mean arterial pressure over a 3 hour period, but did not lower renal blood flow or alter urine production or urinary excretion of sodium or potassium. The calculated renal vascular resistance was decreased following dosing. An attempt was made using the DA-1 antagonist SCH-23390 to assess if the renal vasodilatory effects of nepicastat were mediated by DA-1 receptors. However this compound reduced blood pressure when given alone, thus making the results uninterpretable. Overall, nepicastat did not impair renal function in anesthetized SHR, and did not decrease renal blood flow despite causing a decrease in arterial blood pressure.

[0472] Daily treatment with nepicastat (1 and 10 mg/kg, p.o.) in SHR for 21 days did not alter heart rate, or systolic blood pressure as measured by the tail cuff method. However,

nepicastat(10 mg/kg, p.o.) induced a significant antihypertensive effect when the rats were restrained and their blood pressure measured directly via an arterial cannulae.

[0473] nepicastat significantly lowered blood pressure in SHR instrumented with radio-telemetry blood pressure transducers at doses of 30 and 100 mg/kg/d for 30 days, but produced no significant effects were observed at 3 and 10 mg/kg/d. The effect at 30 and 100 mg/kg/d persisted over a 24-hour period after a single dose, and there was no loss of effect over 30 days. Heart rate was not increased, and motor activity was unaffected. A combination of a dose of the angiotensin converting enzyme inhibitor enalapril (1 mg/kg, p.o.) that failed to lower blood pressure with nepicastat (30 mg/kg) caused a potentiation of the antihypertensive effects of nepicastat over 30 days of dosing, and resulted in a significant reduction in left ventricular mass. A reduction in left ventricular mass did not occur with enalapril alone. Thus, 30 days of treatment of SHR with nepicastat at 30 and 100 mg/kg/d resulted in a decrease in blood pressure and, when combined with enalapril, additional blood pressure decreases along with a reduction in left ventricular mass.

[0474] The blood pressure lowering effect of nepicastatin normotensive Wistar rats instrumented with radio-telemetry blood pressure transducers was less than the effect observed in SHR at doses of 30 and 100 mg/kg/d for 7 days. At 30 mg/kg/d the peak decrease in blood pressure was -10 mmHg, compared to -20 in SHR. At 100 mg/kg/d the peak decrease in blood pressure was -17 mmHg, compared to -42 in SHR. Thus, nepicastat had a greater blood pressure lowering effect in SHR than in normotensive rats.

[0475] Studies in normal anesthetized dogs showed no cardiovascular effects of nepicastat following acute intravenous dosing (1-10 mg/kg i.v.) with no changes in arterial blood pressure, left ventricular pressures (including peak dp/dt), heart rate, cardiac output or renal blood flow for up to five hours after dosing. A similar lack of effect was observed in chronically instrumented, conscious dogs studied for 12 hours after a single dose (3-30 mg/kg i.v.).

[0476] Nepicastat(30 mg/kg intraduodenally) did not significantly inhibit either the decrease in renal blood flow in response to direct renal nerve stimulation, or the increase in arterial blood pressure in response to carotid artery occlusion up to 5 hours after dosing in anesthetized male beagle dogs. However, nepicastat caused a significant decrease in norepinephrine levels and an increase in the dopamine/norepinephrine ratio, but not dopamine levels, in the mesenteric artery 5 hours after dosing. Thus, although tissue norepinephrine levels were significantly reduced, there was no significant inhibition of sympathetically-evoked functional responses.

[0477] When nepicastat was given to male beagle dogs for 4.5 days at 10 mg/kg/d there was no statistically significant decrease in the degree of blood pressure and heart rate increases in response to carotid artery occlusion in anesthetized animals. nepicastat treatment significantly reduced the increase in heart rate in response to an i.v. tyramine challenge, but produced only slight and non-significant inhibition of blood pressure increases. Thus, chronic dosing with nepicastat at a dose that has been shown to result in a maximal decrease in tissue norepinephrine levels, does not have a major inhibitory effect on sympathetically-evoked functional responses.

[0478] Nepicastat caused no significant effects on gross motor behavior in mice following acute dosing at 1.0-30 mg/kg, p.o., and it did not effect locomotor activity in mice (10-100 mg/kg i.p.). Acute administration to rats did not effect locomotor activity or acoustic startle reactivity (3-100 mg/kg i.p.).

[0479] No behavioral effects were observed in rats following 10 days of dosing at 10, 30, and 100 mg/kg/d, p.o. (AT 6867). Rectal temperature was also unaffected. Motor activity and auditory startle reflex were significantly reduced by treatment with the DBH inhibitor SKF102698 (100 mg/kg/d, p.o.), and by the centrally acting α -adrenergic agonist clonidine (20 mg/kg, b.i.d., p.o.). Motor activity was also unaffected over 30 days of dosing in SHR (3-100 mg/kg/d, p.o.) (AT 6829). Thus, nepicastat did not cause detectable changes in central nervous system mediated behavioral effects in rats.

[0480] Nepicastat is a potent competitive inhibitor of human DBH *in vitro*, and in rats and dogs *in vivo*. In rats, oral treatment with nepicastat resulted in significant evidence for DBH inhibition in the heart and mesenteric artery at a dose 6 mg/kg/d. In contrast to another DBH inhibitor, SKF 102698, nepicastat showed some selectivity to the left ventricle and mesenteric artery relative to the cerebral cortex. No behavioral effects were observed with nepicastat in rats. In dogs, a plateau effect for DBH inhibition occurred at 10 mg/kg/d in the heart, renal artery and kidney; the minimal dose for significant effects has not been identified. nepicastat significantly reduced the hypertensive response to sympathetic nerve stimulation in rats (3 mg/kg p.o.), and it significantly lowered blood pressure throughout the day when dosed once daily (30 mg/kg/d p.o.) for 30 days in SHR. In conclusion, nepicastat is a potent DBH inhibitor that modulates the action of the sympathetic nervous system.

Example 9

[0481] The studies described here were designed to evaluate the pharmacokinetics of higher oral doses of nepicastat, to compare the pharmacokinetics in male and female rats,

and to determine penetration of nepicastat into the CNS by quantitating levels of nepicastat in brain.

[0482] Male rats (Crl: CD BR Vaf+) weighing 180 - 220 g were fasted overnight before dosing and until 4 hr after dosing. Doses were formulated in water containing 2% 1-hydroxypropyl methylcellulose (50 centipoises viscosity), 1% benzyl alcohol, and 0.6% Tween 80 (all obtained from Sigma Chemical Company). Concentration of drug in the dose solutions was 5, 15, and 50 mg/ml for the 10, 30, and 100 mg/kg doses, respectively, and was verified by liquid chromatography (LC). The 5 mg/ml dose was a clear solution and the higher concentrations were a translucent suspension. Dose volumes were 2.0 ml/kg. At various times after dosing, samples of blood were obtained by cardiac puncture with heparinized syringes, and plasma was prepared by centrifugation. Brains of rats were surgically excised, and all samples were frozen at -20°C until analysis.

[0483] Aliquots of plasma (0.05 or 0.5 ml) were mixed with internal standard (50 µl of methanol containing 5 µg/ml a monofluoro analog of nepicastat, and 5 mg/ml dithiothreitol). Samples were mixed with 200 mM sodium phosphate buffer, pH 7.0, (0.5 ml) and extracted with 3 ml of ethyl acetate/hexane (1/1, v/v). The organic phase containing analytes was back extracted with 250 µl of 250 mM acetic acid and 100 µl aliquots of the aqueous phase were assayed by LC. The LC system used a Keystone Hypersil BDS 15 cm C₈ column at ambient temperature. Mobile phase A was 12.5 mM potassium phosphate, pH 3.0, with 5 mM dodecanesulfonic acid and mobile phase B was acetonitrile. Solvent composition was 40% B and was pumped at a flow rate of 1 ml/min. Detection was by UV absorption at 261 nm. Concentrations of analytes were determined from a standard curve generated from the analysis of plasma from untreated rats fortified with known concentrations of analyte. Plasma concentration data are expressed as µg (free base) per ml.

[0484] Brains were rinsed briefly with saline, blotted on a paper towel, then weighed (1.5 - 2.0 g). Internal standard was added (50 µl of methanol containing 20 µg/ml a monofluoro analog of nepicastat), and brains were homogenized in 5 ml of 200 mM sodium phosphate, pH 7.0, containing 0.5 mg/ml dithiothreitol. Aliquots of homogenate (2 ml) were extracted with 10 ml of ethyl acetate / hexane (1/1, v/v). The organic phase was gently back extracted with 150 µl of 250 mM acetic acid.

[0485] Following addition of 100 µl of methanol to the aqueous phase (to disperse any emulsion), 100 µl aliquots were assayed by LC as described for plasma. Level in brain are expressed as µg (free base) per g of brain tissue.

[0486] Pharmacokinetic parameters were calculated from mean plasma concentrations. Plasma half-life ($T_{1/2}$) was calculated as $0.693/\beta$, where β is the elimination rate constant determined by linear regression of the log plasma concentration vs. time data within the terminal linear portion of the data. Areas under the plasma concentration vs. time curve (AUC) from zero to the time of the last quantifiable plasma concentrations were calculated by the trapezoidal rule. AUC from zero to infinity (AUC_{total}) was calculated as:

[0487] $AUC_{total} = AUC(0-C_{last}) + C_{last}/\beta$ where C_{last} is the last quantifiable plasma concentration.

[0488] Figure 29 shows pharmacokinetic parameters of nepicastat in rat plasma and brain.

[0489] Concentrations of nepicastat in plasma of male rats given 10, 30, or 100 mg/kg single oral doses are shown in Figures 30-32 and plotted in Figure 33. Concentrations of nepicastat in plasma increased with increasing dose, and the relationship between AUC_{total} and dose was linear (Figure 34). The elimination half-life appeared to increase slightly at higher doses (1.70, 2.09, and 3.88 hr following the 10, 30, and 100 mg/kg oral doses to male rats, respectively). Following a 30 mg/kg oral dose of nepicastat to female rats, the plasma AUC_{total} of nepicastat was 77% higher in female rats than in male rats given an equivalent dose of nepicastat (Figure 33 and 35). Levels of nepicastat in brain (expressed as $\mu\text{g/g}$) were initially lower than those in plasma (expressed as $\mu\text{g/ml}$). From 2 hr following dosing onward, however, concentrations of nepicastat in brain exceeded those in plasma (Figure 36-37).

[0490] Plasma levels of nepicastat in male rats increased linearly with increasing doses between 10 and 100 mg/kg, based on values of AUC_{total} .

[0491] Plasma levels of nepicastat were higher in female rats than in male rats following a 30 mg/kg oral dose.

[0492] Following administration of a 10 mg/kg oral dose of nepicastat to male rats, levels of nepicastat in brain were initially lower than those in plasma, but from 2 hr onward, levels of nepicastat in brain were greater than in plasma.

Example 10

[0493] The purpose of this study was to determine the 24 hours time course of the effects of nepicastat (10 mg/kg) on dopamine and norepinephrine levels in the mesenteric artery following a single oral dose in spontaneously hypertensive rats. Catecholamine levels

were measured at 1, 2, 4, 6, 8, 12, 16, and 24 hours after a single oral administration of either nepicastat (10 mg/kg) or vehicle (dH₂O; 10 ml/kg).

[0494] Sixteen-17 week old, male spontaneous hypertensive rats (SHR) rats weighing 300-400 grams were allowed food and water ad libitum. Animals were weighed and randomly assigned, the afternoon before the study, to one of the following treatment groups (n=9 per group): a single oral administration of nepicastat at 10 mg/kg or a single oral administration of vehicle (10 ml/kg) to be sacrificed at 1, 2, 4, 6, 8, 12, 16, or 24 hours.

nepicastat was synthesized as the hydrochloride salt by the Institute of Organic Chemistry, Syntex Discovery Research and obtained from Syntex Central Compound Inventory. nepicastat was dissolved in vehicle (dH₂O) to yield an oral dose that could be administered in repeated volumes of 10 ml/kg. All doses of nepicastat were administered as free base equivalents and prepared the morning of administration. Animals were dosed every minute the morning of sacrifice. At 1, 2, 4, 6, 8, 12, 16 and 24 hours following administration, 9 treated animals and 9 vehicle animals were anesthetized with halothane, decapitated, and the left ventricle and mesenteric artery were rapidly harvested and weighed. The mesenteric artery was put in 0.5 ml of 0.4M perchloric acid in a centrifuge tube and the left ventricle put into an empty cryotube. Both tissues were immediately frozen in liquid nitrogen and stored at -70° C. Mesenteric artery catecholamine levels were determined using HPLC with electrochemical detection. At the time of decapitation, plasma samples were taken by draining blood from the carcass into a tube containing heparin, and centrifuging at 4°C.

[0495] Each treatment group was compared to vehicle at each time point. A two way analysis of variance (ANOVA) with effects TRT, HARVEST and their interaction was performed. A one way ANOVA with factor TRT was performed for each harvest time. Pairwise analyses between treated and vehicle animals, at each time point, were carried out using Fisher's LSD strategy to control the experiment-wise error rate. Norepinephrine values were significantly ($p<0.05$) lower than vehicle only at the 4 hr time point. Levels were marginally ($0.05<p<0.1$) lower at the 6 hour time point (Figure 38). Dopamine levels were significantly ($p<0.05$) higher than those of vehicle at the 2 and 6 hr harvest times (Figure 39). The dopamine/norepinephrine ratio was significantly ($p<0.05$) greater than those of vehicle treated animals at the 1, 2, 4, 6 and 12 hour time points (Figure 40).

[0496] In general, nepicastat had few statistically significant effects on mesenteric artery norepinephrine or dopamine levels following a single oral administration at 10 mg/kg in

spontaneously hypertensive rats at 1, 2, 4, 6, 8, 12, 16 or 24 hours following dosing. However, a consistent increase in the dopamine/norepinephrine ratios were observed across most of the first 12 hours of treatment. At the 16 and 24 harvest time no changes in any of the three parameters were observed.

Example 11

[0497] The purpose of this study was to determine the effects of intravenous administration of nepicastat (hereafter referred to as nepicastat) on the levels of dopamine and norepinephrine in the left ventricle in Sprague-Dawley rats. Animals received two intravenous (iv) administrations, 12 hours apart, of either vehicle (75% propylene glycol + 25% DMSO; 1.0 ml/kg) or 15 mg/kg of nepicastat. Tissue norepinephrine and dopamine levels were measured six hours after the last compound administration.

[0498] Sixteen to 17 week old male Sprague-Dawley rats, weighing 300-400 grams, were allowed food and water ad libitum. Animals were weighed and randomly assigned, the afternoon before the study, to one of the following treatment groups (n=10 per group): vehicle (1.0 ml/kg) or nepicastat at 15 mg/kg. Nepicastat was synthesized by the Institute of Organic Chemistry, Syntex Discovery Research and obtained from Syntex Central Compound Inventory. nepicastat was dissolved in the appropriate amount of vehicle (75% propylene glycol + 25% DMSO) to obtain a dosing volume of 1.0 ml/kg. nepicastat was administered as the free base equivalent and prepared the afternoon prior to the first administration.

[0499] Each rat was dosed iv in the tail vein the afternoon before harvest. The dosing was repeated 12 hours later the following morning. Six hours after the final administration rats were anesthetized with halothane, decapitated, and the left ventricle was rapidly harvested and weighed. The ventricle was placed in 1.0 ml iced 0.4 M perchloric acid. Tissues were immediately frozen in liquid nitrogen and stored at -70°C. Tissue dopamine and norepinephrine concentrations were assayed by high performance liquid chromatography using electrochemical detection.

[0500] A one-way analysis of variance (ANOVA) with a main effect for treatment was performed for norepinephrine. A Kruskal-Wallis was performed for dopamine and their ratio primarily due to heterogeneous variances among treatment groups. Subsequent pairwise comparisons between nepicastat treated rats and vehicle were performed using Fisher's LSD test. A Bonferroni adjustment was performed on all p-values to ensure an overall experiment-wise type 1 error rate of 5%.

[0501] Nepicastat administered at 15 mg/kg significantly ($p < 0.01$) decreased norepinephrine levels by 51% (figure 41), and significantly ($p < 0.01$) increased dopamine levels by 472% (figure 42), and significantly ($p < 0.01$) increased the dopamine/norepinephrine ratio by 1117% (figure 43), compared to vehicle treated animals.

[0502] In conclusion, intravenous administration of nepicastat resulted in significant inhibition of DBH in the left ventricle of Sprague-Dawley rats.

Example 12

[0503] This study assessed the effectiveness of nepicastat in altering the levels of dopamine and norepinephrine in the cortex, left ventricle, and mesenteric artery of male spontaneously hypertensive rats (SHR). Animals were given three doses, 12 hours apart at 3, 10, 30 or 100 mg/kg p.o..

[0504] This study also compared the efficacy of the S enantiomer (nepicastat) with the R enantiomer ((R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride) following three doses (30 mg/kg).

[0505] This study also compared the effects of nepicastat with SKF102698, a DBH inhibitor previously shown to be orally active in rats.

[0506] Compounds were prepared and administered as the free base equivalent. Nepicastat, (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride and SKF 102698 were obtained from Syntex Central Compound Inventory. Nepicastat was dissolved in the appropriate amount of vehicle (dH₂O for nepicastat and PEG 400:dH₂O, 50:50 vol:vol for SKF102698. Doses of 3, 10, 30, and 100 mg/kg of nepicastat, and 30 mg/kg SKF 102698 were prepared in 10.0 ml/kg dosing volumes.

[0507] Fifteen to sixteen week old male spontaneously hypertensive rats (SHR) (Charles River Labs) were allowed food and water ad libitum. Animals were weighed and randomly assigned to one of the following treatment groups: 1) distilled water vehicle (dH₂O), or nepicastat at 3, 10, 30, and 100 mg/kg, 2) (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride at 30 mg/kg in distilled water, or 3) PEG 400:dH₂O vehicle or RS-2643 1-000 at 30 mg/kg. Each rat was dosed orally (p.o., using a gavage needle) three times 12 hours apart, beginning in the morning. At six hours after the third dose rats were anesthetized with halothane, decapitated, and the cortex, mesenteric artery, and left ventricle were rapidly harvested, weighed, placed in iced 0.4 M perchloric acid, frozen in liquid nitrogen, and

stored at - 70°C. Tissue dopamine and norepinephrine concentrations were assayed by high performance liquid chromatography and electrochemical detection.

[0508] Four series of statistical analyses were performed. The first series compared the rats treated with various doses of nepicastat, and (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride at 30 mg/kg to the vehicle control animals. A nonparametric one-way analysis of variance (ANOVA) with factor Dose and blocking factor Day was performed for each tissue and strain separately. Overall results are reported. Pairwise analysis between treated and controls at each dose were carried out using Dunnett's test to control the experiment-wise error rate. The second statistical test compared SKF 102698 to the PEG-dH₂O vehicle treated group using a nonparametric t-test. The third statistical test compared (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride to NEPICASTAT at doses of 30 mg/kg using a nonparametric t-test. A fourth statistical analysis compared RS25560-197 to SKF 102698 at doses of 30mg/kg. Since two different vehicles were used, a linear contrast was developed which calculates the difference of differences as follows:

[0509] $\text{Change} = (30\text{mg/kg} - \text{Vehicle})_{\text{NEPICASTAT}} - (30\text{mg/kg} - \text{Vehicle})_{\text{SKF102698}}$

[0510] This new variable was tested for equality to zero by the SAS procedure General Linear Models.

[0511] All data in the figures is presented $\pm$ the standard deviation.

[0512] The dopamine concentration in the cerebral cortex was significantly ($p < 0.05$) greater (Figure 44), the norepinephrine concentration was significantly ($p < 0.05$) lower (Figure 45), and the dopamine/norepinephrine ratios significantly ($p < 0.05$) greater (Figure 46) than vehicle at doses of 30 and 100 mg/kg of nepicastat.

[0513] Dopamine concentration in the left ventricle was significantly ($p < 0.05$) greater than vehicle at doses of 3, 10, 30 and 100 mg/kg (Figure 47). Norepinephrine concentration was significantly ($p < 0.05$) lower than vehicle at doses of 10, 30 and 100 mg/kg (Figure 48). The dopamine/norepinephrine ratio in the left ventricle was significantly ($p < 0.05$) greater than vehicle at doses of 3, 10, 30, and 100 mg/kg (Figure 49) of nepicastat.

[0514] Dopamine concentration in the mesenteric artery of SHR was significantly ($p < 0.05$) greater than vehicle at doses of 3, 10, 30 and 100 mg/kg (Figure 50). Norepinephrine concentration was not significantly less ($p > 0.05$) than vehicle at 10, 30,

and 100 mg/kg (Figure 51). The dopamine/norepinephrine ratios in the mesenteric artery were significantly ($p<0.05$) greater than vehicle at all doses (Figure 52) of nepicastat.

[0515] In the cerebral cortex, relative to treatment with vehicle, (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride resulted in significant increase in both dopamine (Figure 53) and norepinephrine (Figure 54) ($p<0.01$), and had no effect on the dopamine/norepinephrine ratio (Figure 55). Norepinephrine levels were significantly lower with nepicastat compared to (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride ($p<0.01$) (Figure 54).

[0516] In the left ventricle, relative to treatment with vehicle, (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride resulted in a significant increase in dopamine (Figure 56) and the dopamine/norepinephrine ratio (Figure 58) ($p<0.01$), but did not significantly lower norepinephrine levels (Figure 57). NEPICASTAT was significantly more effective ($p<0.01$) than (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride at lowering norepinephrine levels (Figure 57), and increasing dopamine and the dopamine/norepinephrine ratio (Figures 56 and 58).

[0517] In the mesenteric artery, relative to treatment with vehicle, (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride resulted in a significant increase in dopamine (Figure 59) and the dopamine/norepinephrine ratio (Figure 61) ($p<0.01$), but did not significantly lower norepinephrine levels (Figure 60). nepicastat was significantly more effective ($p<0.01$) than (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride at lowering norepinephrine levels (Figure 60), and increasing dopamine and the dopamine/norepinephrine ratio (Figures 59 and 61).

[0518] Comparing nepicastat with SKF102698 at 30 mg/kg) in the cerebral cortex, dopamine concentration in the cortex was significantly greater ($p<0.01$) than vehicle for SKF 102698 at a dose of 30mg/kg (Figure 53). The increase above vehicle was greater for SKF102698 than for nepicastat ($p<0.01$). Norepinephrine concentration was significantly lower than vehicle for SKF 102698, and the decrease was greater for SKF 102698 than for nepicastat ($p<0.01$) (Figure 44). The dopamine/norepinephrine ratios in the cortex were significantly ($p<0.01$) greater than vehicle for SKF 102698 (Figure 55), and the increase above vehicle was greater for SKF 102698 than for nepicastat ($p<0.01$).

[0519] The dopamine concentration in the left ventricle was significantly greater ($p < 0.01$) than vehicle for SKF102698 (Figure 56), and the increase above vehicle was greater for nepicastat than for SKF 102698 ($p < 0.01$). Norepinephrine concentration was not different from vehicle with SKF 102698 treatment, however treatment with nepicastat significantly lowered norepinephrine relative to vehicle more than SKF 102698 ($p < 0.01$) (Figure 57). The dopamine/norepinephrine ratios in the left ventricle were significantly ($p < 0.05$) greater than vehicle for SKF102698 (Figure 58), and the increase above vehicle was greater for nepicastat than for SKF 102698 ($p < 0.05$).

[0520] The dopamine concentration in the mesenteric artery was significantly greater than vehicle for SKF102698 (Figure 59), and the increase above vehicle was greater for NEPICASTAT than for SKF102698. Norepinephrine concentration was significantly lower than vehicle with SKF 102698 treatment, and treatment with nepicastat significantly lowered norepinephrine relative to vehicle more than SKF102698 (Figure 60). The dopamine/norepinephrine ratios in the left ventricle were significantly greater than vehicle than for SKF 102698 (Figure 61), and the increase above vehicle was greater for nepicastat than for SKF102698.

[0521] In conclusion, the data show that nepicastat is a potent inhibitor of DBH in vivo in the mesenteric artery, left ventricle, and cerebral cortex of SHR six hours after the third of three oral doses administered 12 hours apart. The S enantiomer, nepicastat was more potent than the R enantiomer ((R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride) in all three tissues at 30 mg/kg. Furthermore, nepicastat was more effective than SKF 102698 in the mesenteric artery and left ventricle, but less effective in the cerebral cortex, following three doses at 30mg/kg administered over 24 hours.

Example 13

[0522] Nepicastat was prepared and administered as the free base equivalent. Nepicastat and methimazole were dissolved in vehicle (66.7% propylene glycol:33.3% dH₂O) to yield dosing solutions of appropriate concentrations so that all doses could be administered in a 1.0 ml/kg volume.

[0523] Male Sprague-Dawley rats, weighing 180-200 grams, were fed an iodine deficient diet (Purina, 5891C, Lot 1478, 0.066 ± 0.042 mg iodine/kg sample) ad libitum 14 days prior to treatment. Animals were weighed and randomly assigned to one of the following treatment groups (n=12 per group): Nepicastat at 2.0 mg/kg, Nepicastat at 6.2 mg/kg, R, Methimazole at 1 mg/kg, or vehicle at 1 ml/kg. Each group of rats was dosed orally in

the evening and the following morning, approximately 12 hours apart, for 10 consecutive days.

[0524] At four hours after the second dose, on day 10, rats were anesthetized with halothane, decapitated, and the cortex, striatum, and mesenteric artery were harvested and weighed. Tissue samples were not harvested from the methimazole groups as they only served as positive controls for determination of thyroid function. The mesenteric artery, cortex, and striatum were immediately placed in 0.4M iced perchloric acid and analyzed for norepinephrine and dopamine levels the same day using HPLC.

[0525] Orbital blood samples were taken at day -3, 0, 3, 7, and 9 (day 0 was the first day of dosing). Serum samples were analyzed for T₃ and T₄ levels using a radioimmunoassay.

[0526] To statistically evaluate changes in T₃ and T₄ levels, a change from baseline was calculated from the day -3 time point. A non-parametric two-way within subject analysis of variance (ANOVA) was conducted. Also a one-way ANOVA was performed to detect if a significant difference from control occurred. Pairwise analyses between controls and each treatment group were carried out using Fisher's LSD strategy to control the experiment-wise error rate. For statistical analysis of catecholamine levels, a one-way ANOVA with factor DOSE was performed. Pairwise analyses between treated and controls at each dose were carried out using Fisher's LSD strategy to control the experiment-wise error rate.

[0527] Norepinephrine levels in the nepicastat treated animals were not significantly ($p>0.05$) different in the cortex compared to vehicle control at doses of 2.0 and 6.2 mg/kg. Norepinephrine levels in the mesenteric artery were significantly ($p<0.05$) lower at the 2.0 and 6.2 mg/kg dose groups, and norepinephrine levels in the striatum were marginally ($p<0.10$) lower in both the 2.0 and 6.2 mg/kg dose groups, compared to vehicle control (Figure 62).

[0528] Dopamine levels in all three tissues were not significantly ($p>0.05$) different from vehicle control at either the 2.0 or 6.2 mg/kg dose group of nepicastat (Figure 62).

[0529] The dopamine/norepinephrine ratio of the cortex and striatum at 2.0 and 6.2 mg/kg RS-25560-197 were not significantly ($p>0.05$) different from vehicle control, while the ratio of the mesenteric artery at both 2.0 and 6.2 mg/kg nepicastat were significantly ($p<0.05$) higher than vehicle control (Figure 62).

[0530] Neither 2.0 or 6.2 mg/kg nepicastat affected thyroid function by altering free T₃ or total T₄ levels in the rat serum. A dose of 1.0 mg/kg of Methimazole, the positive control,

significantly ($p < 0.05$) lowered T_3 levels on all treatment days (Figure 63) and T_4 levels at day 3 and 7 (Figure 64), compared to vehicle control. T_4 levels of the methimazole treated animals were only marginally ($p < 0.10$) lower on day nine.

[0531] nepicastat (2.0 or 6.2 mg/kg) did not cause any significant ($p > 0.05$) changes in dopamine or the norepinephrine levels, or dopamine/norepinephrine ratio when compared to vehicle. In the striatum, a marginally significant ($p < 0.10$) decrease in norepinephrine level was observed in the 6.2 mg/kg dose group, but no other significant changes were observed. In the mesenteric artery, both 2.0 and 6.2 mg/kg of nepicastat produced significantly ($p < 0.05$) lower norepinephrine levels and significantly ($p < 0.05$) higher dopamine/norepinephrine ratios, compared to vehicle, with no significant changes observed in dopamine levels. Thus nepicastat appears to be an effective inhibitor of dopamine β -hydroxylase in vivo, with greater effect in the mesenteric artery than the cerebral cortex or striatum following 10 days of dosing in Sprague-Dawley rats.

Example 14

[0532] This study was performed to determine the dopamine and norepinephrine concentrations in kidney medulla and kidney cortex from dogs dosed with nepicastat. Adult male beagle dogs were randomly assigned to four groups of 8 dogs per group and dosed by oral administration with nepicastat. Nepicastat was delivered in doses of 5, 15 and 30 mg/kg placed in single capsules. Vehicle was an empty capsule. Each dog received 2 doses daily, morning and afternoon (8-10 hours apart) for four days. On the fifth day, each dog received a single dose in the morning and the dogs were euthanized six hours after the last dose. Samples of kidney medulla and kidney cortex were rapidly harvested, weighed, placed in cold 0.4 M perchloric acid, frozen in liquid nitrogen and stored at -70°C .

[0533] To quantitate concentrations of norepinephrine (NE) and dopamine (D), each tissue was homogenized by brief sonication in 0.4 M perchloric acid. After sonication, the homogenates were centrifuged at 13,000 rpm in a microfuge for 30 minutes at 4°C . An aliquot of each supernatant was removed and spiked with 3,4-dihydroxybenzylamine (DHBA) as internal standard. The extract from each sample was subjected to HPLC separation using electrochemical detection. The method has a quantitation limit of 2.0 ng/mL and a linear range of 2.0 ng/mL to 400 ng/mL for each analyte.

[0534] The analytical results are presented in Figure 65 and 66. Each analyte determination was normalized to the weight of the tissue sample and expressed as μg of analyte per gram of tissue. The table contains concentrations of dopamine,

norepinephrine and the ratio of dopamine concentration to norepinephrine concentration (D/NE) obtained for each dog. In addition, the calculated means and standard deviations for each analyte and D/NE ratio are provided for each treatment group.

Example 15

[0535] Male Beagle dogs (Marshall farms, North Rose, NY) weighing between 9-16 kg were used in the study. The animals were allowed water ad libitum and given food once daily at ~ 10.00 AM. Animals were randomly assigned to one of the following treatment groups (n = 8/group): placebo (empty capsule), or nepicastat at 2 mg/kg b.i.d (4 mg/kg/day). Each animal received 2 doses daily, morning and afternoon (8-10 hours apart). Daily blood samples (10 ml) were drawn 6 h after the AM dose for measurement of plasma levels of nepicastat and catecholamines. The blood was collected in tubes containing heparin and glutathione and centrifuged at - 4°C within 1h of collection. The plasma was separated and divided into two samples, one for the measurement of plasma catecholamines and the other for analysis of nepicastat .

[0536] Tissue samples were also taken from the dogs at the end of the study in case it was deemed necessary to analyse tissue catecholamines at a later point. On day 15, 6 hours after the AM dose, a final blood sample (10 ml) was taken. Dogs were anesthetized with sodium pentobarbital (40 mg/kg, iv), placed on a necropsy table and euthanized with a second injection of pentobarbital (80 mg/kg,iv). A rapid bilateral transthoracotomy and abdominal incision was performed. Biopsies were taken from the renal artery and left ventricle. The skull was opened to expose the frontal lobe of the cerebral cortex and a biopsy was taken. Tissue samples were weighed, placed on iced 0.4 M perchloric acid, frozen in liquid nitrogen and stored at - 70°C until analyzed.

[0537] Plasma NE, DA and EPI were analysed by HPLC using electrochemical detection. Plasma concentration of nepicastat was determined by HPLC using electrochemical detection.

[0538] The Box-Cox transformations indicated that the logarithm was an appropriate variance stabilizing transformation; hence all analyses were performed on the log-values. The BQL (below quantitation limit) in the DA concentration of dog 1 at day 10 was set to 0; ln (0) was set to missing. The analysis was performed using a mixed model (using PROC MIXED) with the day and treatment categorical variables being fixed and the dog within treatment being a random factor. For the fixed effects, the interaction between the day and the treatment was included, since the difference between the drug and placebo groups varies from day to day. Contrasts were calculated using the CONTRAST

statement, which correctly takes into account the error terms for each particular contrast. In particular, the contrasts comparing the treatment group to the drug group uses the dog mean square for its error term, while the comparisons used to establish steady state are all within dog comparisons, and require the error mean square.

[0539] The time period of steady state was calculated using the Helmert transformation (cf. SAS PROC GLM manual). These transformations compare each treatment mean with the average of the treatment means of the time points following. The steady state period is defined to start at the first time point following the maximum time at which the Helmert contrast is statistically significant. However, since this method can fail to detect a smoothly changing process, as appears might be the case here, the slope of the analyte concentration during the steady state period also was calculated. The slope during the steady state period was calculated for each dog individually, yielding one slope per animal. Univariate statistics on the slopes were then calculated, with Normal theory confidence intervals built on the mean slope, and the hypothesis of slope equalling zero was tested, and its Normal theory p-value was calculated. This slope analysis was used as the basis for determining whether the steady state period was a period of changing concentration.

[0540] When compared to the placebo group, nepicastat (2 mg/kg, b i d) produced significant decreases in plasma NE (2.1 fold) and EPI (1.91 fold) and significant increases in plasma DA (7.5 fold) and DA/NE ratio (13.6 fold) (see Figure 67-71). The peak decreases in plasma NE and EPI were observed at day 6 and day 8, respectively, whereas the peak increases in plasma DA and DA/NE ratio were observed at day 7 and day 6, respectively. The effects on plasma NE, DA and EPI attained steady-state at approximately 4, 8 and 6 days post-dose, respectively. The changes in plasma DA and DA/NE ratio were significantly different from placebo on all days post-dose. The changes in plasma NE were significantly different from placebo on days 4-9 and days 11-13 post dose. The changes in plasma EPI were significantly different from placebo on days 7-9 and day 12 post-dose.

[0541] Administration of nepicastat (2 mg/kg, bid) produced significant plasma levels of the drug on all days (Figure 72). The peak levels were observed at 2 days post-dose. No significant levels of the N-acetyl metabolite of nepicastat were detected on any of the days.

[0542] Chronic (14.5 days) administration of nepicastat (2 mg/kg, bid, po) produced significant decreases in plasma NE and EPI and significant increases in plasma DA and

DA/NE ratio. These changes reflect inhibition of the sympatho-adrenal system via inhibition of the enzyme dopamine- β -hydroxylase.

Example 16

[0543] Nepicastat was weighed and put into capsules (size 13 - Torpac; East Hanover, NJ) to yield doses of 5, 15, and 30 mg/kg per capsule (given b.i.d. to yield doses of 10, 30 and 60 mg/kg/day). The initial dog weight was used to determine the dose for each animal. Dogs receiving 0 mg/kg/day received empty capsules (placebo). All doses of nepicastat were administered as free base equivalents.

[0544] Thirty-two male beagle dogs, weighing 10-12 kg, were randomly assigned to one of the following 4 treatment groups (n=8 per group): nepicastat at 0 mg/kg/day (placebo), 10 mg/kg/day (5 mg/kg b.i.d.), 30 mg/kg (15 mg/kg b.i.d.), or 60 mg/kg/day (30 mg/kg b.i.d.). Dog numbers 1-16 were assigned as dose group A and dog numbers 17-32 as dose group B. The terminal surgery for tissue harvest was performed over 2 days with 16 animals studied per day. Two or 3 days before the first compound administration each dog was weighed and skin regions overlying both cephalic, saphenous and jugular veins were shaved. Dosing consisted of oral administration of one capsule with the second given 8-10 hr later. Dogs were dosed as scheduled on days 1-3. On day 4, prior to the AM dose, 3 ml of blood were obtained from a jugular vein for determination of baseline plasma compound levels. The dog was then administered the AM dose, and at 1, 2, 4 and 8 hr following the dose additional 3 ml blood samples were collected for determination of plasma compound levels. Blood samples were put into tubes containing heparin, centrifuged at 4°C and stored at -20°C until analysis. The PM dose was then administered as scheduled.

The AM dose was administered as scheduled on the days of surgery. Approximately 6 hr after the AM dose, a final 3 ml blood sample was taken from the jugular vein for determination of plasma compound levels. The dog was then anesthetized with pentobarbital Na (~40 mg/kg), given iv in a cephalic or saphenous vein, and delivered to the necropsy room where an additional dose of pentobarbital Na was given (~80 mg/kg, iv). The left ventricle, renal artery, kidney, renal medulla, renal cortex and cerebral cortex were then rapidly harvested, weighed, put into 2 ml iced 0.4M perchloric acid, frozen in liquid nitrogen and stored at -70°C until analysis for catecholamines by HPLC using electrochemical detection. All tissue samples were divided into 2 portions, the second of which were immediately frozen in liquid nitrogen and stored at -70°C for determination of tissue compound levels. A third transmural sample taken from the left

ventricle was immediately frozen in liquid nitrogen and stored at -70°C for use in receptor binding studies.

[0545] Ventricles were homogenized in 50 mM Tris-HCl, 5 mM Na₂EDTA buffer (pH 7.4 at 4°C) using a Polytron P-10 tissue disrupter (setting 10, 2 x 15 second bursts). Homogenates were centrifuged at 500 x g for 10 minutes and the supernatants stored on ice. The pellets were washed by resuspension and centrifugation at 500 x g and the supernatants combined. The combined supernatants were centrifuged at 48,000 x g for 20 minutes. The pellets were washed by resuspension and centrifugation once in homogenizing buffer and twice in 50 mM Tris-HCl, 0.5 mM EDTA buffer (pH 7.4 at 4°C). Membranes were stored at -70°C until required. Saturation experiments were conducted using [³H] CGP-12177 in buffer containing 50 mM Tris-HCl, 0.5 mM EDTA (pH 7.4 at 32°C). Non-specific binding was defined by 10 uM isoproterenol. Total bound, non-specific bound and total count tubes were set up for eight concentrations of [³H] CGP-12177 ranging from 0.016 nM to 2 nM. Samples were incubated at 32°C for 60 minutes. Samples were filtered over 0.1% PEI pre-treated GF/B glass fiber filtermats using a Brandel cell harvester. Samples were washed with room temperature water three times for 3 seconds. Aquasol scintillation fluid was added to each vial and radioactivity determined by liquid scintillation counting. Saturation binding isotherms were analyzed after first converting total ligand concentrations to free ligand concentrations (total - bound = free). Individual saturation isotherms were completed for each tissue. Membranes were assayed for protein using the Bio-Rad protein binding method and using gamma globulin as the standard. Receptor densities were expressed, per mg protein, as mean for each treatment group. Tissue catecholamine levels were analyzed by comparing nepicastat-treated groups with the placebo (control) treated groups. A nonparametric one-way analysis-of-variance (ANOVA) with factor DOSE was performed for each tissue and each catecholamine measure separately. Pairwise analyses between treated and controls at each dose were carried out using Dunnett's test to control the experiment-wise error rate. Student-Neuman-Kuels and Fisher's LSD tests were performed as validation. Analysis of tissue and plasma compound levels were performed in 2 ways. First, individual t-tests were run to compare each dose level to a factored level of its partner dose for each parameter. For example, three times the level of compound present at 10 mg/kg in a particular tissue or plasma should be comparable to the compound level observed in the 30 mg/kg group. Additionally, a linear orthogonal contrast was calculated for all three doses within the

context of a one-way ANOVA. A paired t-test was used to determine any differences in binding between the vehicle treated group and the 10 mg/kg/day nepicastat group.

[0546] In the renal artery, nepicastat administered at doses of 10, 30 and 60 mg/kg/day significantly ($p<0.01$) decreased norepinephrine levels by 86%, 81% and 85%, respectively (Figure 73). Dopamine levels were significantly ($p<0.01$) increased at doses of 10, 30 and 60 mg/kg/day by 180%, 273% and 268%, respectively (Figure 74). Doses of 10, 30 and 60 mg/kg/day nepicastat significantly ($p<0.01$) increased the dopamine/norepinephrine ratio by 1711%, 1767% and 1944%, respectively, compared to placebo (figure 75).

Following administration of 10 and 60 mg/kg/day nepicastat, dopamine levels were significantly ($p<0.01$) increased 632% and 411%, respectively in the cerebral cortex (figure 76). The dopamine/norepinephrine ratio was significantly ($p<0.01$) increased 531% after 10 mg/kg/day nepicastat and 612% following administration of 60 mg/kg/day nepicastat (figure 78). Norepinephrine levels were not significantly ($p>0.01$) affected at these 2 doses (figure 77). At 30 mg/kg/day, norepinephrine was significantly ($p<0.01$) reduced by 63% and the ratio significantly ($p<0.01$) elevated by 86%, while dopamine levels marginally ($0.05<p<0.10$) increased 174%, compared to placebo (Figures 76-78). Following administration of 10, 30 and 60 mg/kg/day nepicastat, norepinephrine levels were significantly ($p<0.01$) decreased by 85%, 58% and 79%, respectively in the left ventricle (Figure 80). The dopamine/norepinephrine ratio significantly ($p<0.01$) increased 852%, 279% and 607%, respectively, compared to placebo animals (figure 81). No significant changes were observed in dopamine levels at doses of 10, 30, and 60 mg/kg/day nepicastat (Figure 79).

[0547] In the renal cortex, compared to placebo, norepinephrine levels were significantly decreased ($p<0.01$) by 86%, 66% and 85%, respectively, following doses of 10, 30 and 60 mg/kg/day nepicastat (figure 83). Dopamine levels were significantly ($p<0.01$) increased 156%, 502% and 208%, respectively, at these doses (figure 82). The dopamine/norepinephrine ratio significantly ($p<0.01$) increased by 1653%, 1440% and 1693%, respectively, at doses of 10, 30, and 60 mg/kg/day (Figure 84). In the renal medulla, the dopamine/norepinephrine ratios were significantly ($p<0.01$) increased by 555%, 636% and 677%, respectively, at doses of 10, 30 and 60 mg/kg/day nepicastat, compared to placebo (Figure 87). Dopamine levels were significantly ($p<0.01$) increased 522% at 30 mg/kg/day and marginally ($0.05<p<0.10$) increased by 150% and 156%, respectively, at 10 and 60 mg/kg/day (Figure 85). Norepinephrine

levels were significantly ($p < 0.01$) decreased 72% following administration of 10 mg/kg/day nepicastat, compared to placebo, and marginally ($0.05 < p < 0.10$) decreased by 69% following 60 mg/kg/day (Figure 86).

[0548] Statistical analysis indicated that the concentration of nepicastat in plasma obtained on Day 4 and tissue and plasma obtained on Day 5 was dose-proportional between each dose level and factored levels of its partner dose. Therefore, dose points were determined to be linear, with the following exceptions (a significant result would suggest the data are not linear):

[0549] Kidney medulla: $3 \times 10 < 30$ ($p < 0.05$)

Kidney medulla: $6 \times 10 < 60$ ($p = 0.077$)

Plasma (day 4): $2 \times 30 > 60$ ($p = 0.076$)

[0550] On Day 5, levels of nepicastat in all tissues examined were higher than those in plasma (Figures 88-90).

[0551] The results demonstrated no difference between left ventricular samples from the 10 mg/kg/day nepicastat treated group and vehicle treated group (Figure 91).

Example 17

[0552] Nepicastat was evaluated for its activity at a range of enzymes including tyrosine hydroxylase, NO synthase, phosphodiesterase III, phospholipase A₂, neutral endopeptidase, Ca²⁺/calmodulin protein kinase II, acetyl CoA synthetase, acyl CoA-cholesterol acyl transferase, HMG-CoA reductase, protein kinase (non-selective) and cyclooxygenase-I. As shown in Figure 92, nepicastat had an IC₅₀ of $> 10 \mu\text{M}$ at all the 12 enzymes studied, therefore is a highly selective (> 1000 -fold) inhibitor of dopamine- β -hydroxylase.

Example 18

[0553] Bovine DBH from adrenal glands was obtained from Sigma Chemicals (St. Louis, MO). Human secretory DBH was purified from the culture medium of the neuroblastoma cell line SK-N-SH and was used to obtain the inhibition data. A lentil lectin-sepharose column containing 25 ml gel was prepared and equilibrated with 50 mM KH₂PO₄, pH 6.5, 0.5 M NaCl. The column was eluted with 35 ml of 10% methyl α , D-mannopyranoside in 50 mM KH₂PO₄, pH 6.5, 0.5 M NaCl at 0.5 ml/min. Fraction containing most enzymatic activities were pooled and concentrated with an Amicon stirred cell using a YM30 membrane. Methyl α , D-mannopyranoside was removed by buffer exchange with in 50 mM KH₂PO₄, pH 6.5, 0.1 M NaCl. The concentrated enzyme solution was aliquoted and stored at -25°C .

[0554] An HPLC assay was used to measure DBH activity using tyramine and ascorbate as substrates. The method is based on the separation and quantitation of tyramine and octopamine by reverse phase HPLC chromatography (Feilchenfeld, N.B., Richter, H. & Waddell, W.H. (1982). *Anal. Biochem.* A time-resolved assay of dopamine β -hydroxylase activity utilizing high-pressure liquid chromatography. 122: 124-128.). The assay was performed at pH 5.2 and 37°C in 0.125 M NaAc, 10 mM fumarate, 0.5~2.0 μ M CuSO₄, 0.1 mg/ml catalase (6,500 u, Boeringer Mannheim, Indianapolis, IN), 0.1 mM tyramine, and 4 mM ascorbate. In a typical assay, 0.5-1.0 milli-units of enzyme were added to the reaction mixture and then a substrate mixture containing catalase, tyramine and ascorbate was added to initiate the reaction (final volume 200 μ l). Samples were incubated at 37°C for 30~40 minutes. The reactions were quenched by the stop solution containing 25 mM EDTA and 240 μ M 3-hydroxytyramine (internal standard). The samples (150 μ l) were loaded to a Gilson autosampler and analyzed by HPLC using UV detection at 280 nm. PC-1000 software (Thermo Separations products, Fremont, CA) was used for integration and data analysis. The HPLC run was carried out at the flow rate of 1 ml/min using a LiChroCART 125-4 RP-18 column and isocratic elution with 10 mM acidic acid, 10 mM 1-heptanesulfonic acid, 12 mM tetrabutylammonium phosphate, and 10% methanol. The remaining percent activity was calculated based on the control without inhibitor, corrected using internal standards and fitted to a nonlinear 4 parameter dose response curve to obtain the IC₅₀ values.

[0555] Purification of [¹⁴C]-Tyramine. [¹⁴C]Tyramine hydrochloride was purified by a C18 light load column (two columns combined into one) that was washed with 2 ml of MeOH, 2 ml of 50 mM KH₂PO₄, pH 2.3, 30% acetonitrile, and then 4 ml of 50 mM KH₂PO₄, pH 2.3. A vacuum manifold (Speed Mate 30, from Applied Separations) was used to wash and elute the column by vacuum. After loading of [¹⁴C]tyramine, the column was washed with 6 ml of 50 mM KH₂PO₄, pH 2.3 and eluted with 2 ml of 50 mM KH₂PO₄ containing 30% acetonitrile. The eluate was lyophilized to remove acetonitrile, resuspended in H₂O, and stored at -20°C.

[0556] Enzyme Assay by Radioactive Method. Enzymatic activity was assayed using [¹⁴C]tyramine as substrate and a C18 column to separate the product. The assay was performed in 200 ml volume containing 100 mM NaAc, pH 5.2, 10 mM fumaric acid, 0.5 μ M CuSO₄, 4 mM ascorbic acid, 0.1 mg/ml catalase and various concentrations of tyramine. The total counts of each reaction was ~150,000 cpm. Bovine DBH (0.18 ng for each reaction) was mixed with tyramine and inhibitor in the reaction buffer at 37°C.

The reaction was initiated by the addition of ascorbate/catalase mixture and was incubated at 37°C for 30 minutes. The reaction was stopped by the addition of 100 ml of 25 mM EDTA, 50 mM KH₂PO₄, pH 2.3. Entire mixture was loaded to a C18 light load column (two combined into one) that was pre-washed with MeOH and equilibrated with 50 mM KH₂PO₄, pH 2.3. Elution into scintillation vials was carried out with 1 ml of KH₂PO₄, pH 2.3 buffer twice, followed by 2 ml of the same buffer. ReadySafe scintillation fluid (16 ml) was added to the scintillation vials and the samples were counted for ¹⁴C radioactivity.

[0557] Nepicastat concentrations of 0, 1, 2, 4, 8 nM were used to study inhibition kinetics at the following tyramine concentrations: 0.5, 1, 2, 3, 4 mM. The ¹⁴C counts were identical in each reaction which was carried out as described above. A blank control without the enzyme was used to obtain the background. The data were corrected for background, converted to activity in nmol/min, and plotted (1/V vs 1/S). Km' was calculated from the slopes and Y intercepts and linear regression was used to obtain Ki value.

[0558] The IC₅₀ values for SKF102698, nepicastat and (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride against human and bovine DBH were obtained using the HPLC assay at the substrate concentrations of 0.1 mM tyramine, 4 mM ascorbate at pH 5.2 and 37°C. All three compounds caused a dose-dependent inhibition of DBH activity on both bovine and human enzyme (Figures 93 and 94).

[0559] The IC₅₀ values for nepicastat, (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride and SKF102698 are given in Figure 95. The S enantiomer (nepicastat) was more potent than the R enantiomer ((R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride by 3-fold against bovine DBH and 2-fold against the human enzyme. nepicastat was more potent than SKF 102698 by 8-fold against bovine enzyme, and 9-fold against human DBH.

[0560] Figure 96 shows the Lineweaver-Burk plot of the inhibition data against bovine DBH (upper panel) and the plot of apparent Km versus inhibitor concentration (lower panel). A Km of 0.6 mM was determined from the plot. nepicastat (1-8 nM) caused a major shift in Km, as would be predicted for a competitive inhibitor. The inhibition of bovine DBH by nepicastat appears to be competitive with tyramine. A Ki of 4.7 ± 0.4 nM was calculated by linear regression.

[0561] Nepicastat was a potent inhibitor of both human and bovine DBH. It was 8-9-fold more potent than SKF102698. nepicastat (the S enantiomer) is 2-3 fold more potent than (R)-5-Aminomethyl-1-(5,7-difluoro-1,2,3,4-tetrahydronaphth-2-yl)-2,3-dihydro-2-thioxo-1H-imidazole hydrochloride (the R enantiomer). The inhibition of bovine DBH by nepicastat appeared to be competitive with tyramine, with a K_i of 4.7 ± 0.4 nM.

Example 19

[0562] The affinity of nepicastat was determined in the bindings assays outlined in Figure 97. Standard radioligand filtration binding methods were used.

[0563] Competition binding data were analyzed by iterative curve fitting to a four parameter logistic equation. Hill coefficients and IC_{50} were obtained directly. pK_i (-log of K_i) of competing ligands were calculated from IC_{50} values using the Cheng-Prusoff equation.

[0564] Nepicastat had moderate affinity for α_1 receptors (pK_i of 6.9 - 6.7). The affinity at all other receptors examined was relatively low ($pK_i < 6.2$) (Figure 98).

Example 20

[0565] Vehicle and nepicastat monohydrate powder were obtained from the Center for Pharmaceutical Development, Syntex Preclinical Research and Development. At the time of dosing, a 60-mg/ml Nepicastat formulation was prepared by mixing vehicle with Nepicastat powder, followed by shaking. The 6- and 20-mg/ml Nepicastat formulations were prepared by diluting the 60-mg/ml formulation with vehicle. The reconstituted Nepicastat formulations retained potency for the duration of use. The aqueous vehicle and nepicastat formulations contained hydroxypropylmethylcellulose, benzyl alcohol, and polysorbate 80.

[0566] Dose selection was based on an acute toxicity study in which mice were administered single oral doses of 250, 1000, or 2500 mg/kg of Nepicastat. Clinical signs of toxicity and death occurred at 1000 and 2500 mg/kg.

[0567] A single oral dose of vehicle or Nepicastat formulation was administered by gavage to each mouse using a rodent intubator. The oral route was selected because it is a proposed clinical route of administration. Dose volumes were calculated on the basis of individual body weights recorded before dosing (body weight data are not tabulated in this report). Food and water were withheld from the mice 2.5 to 3.5 hours before dosing, instead of 1.5 hours as specified in the protocol. This deviation did not affect the integrity of the study.

[0568] Clinical observations were recorded before dosing. Beginning 60 minutes after dosing, mice in each treatment group were evaluated in groups of up to 3 over an interval of approximately 10 minutes each for clinical observations and protocol-specified behavioral tests.¹ One mouse in the 30-mg/kg group and 1 mouse in the 100-mg/kg group died after dosing and they were removed from the study. Surviving mice were euthanatized and removed from the study at the end of the observation/testing period.

[0569] Mice in groups of 6 males each were administered single oral doses of 0 (vehicle), 30, 100, or 300 mg/kg of nepicastat by gavage. Clinical observations and behavioral tests were initiated 60 minutes after administration of the test formulation. At the end of the observation period, all surviving mice were euthanatized and removed from the study.

[0570] Lower body temperatures were present in the 30-, 100-, and 300-mg/kg groups compared with the vehicle-control group. No treatment-related clinical or gross behavior changes were present. Rectal body temperature data are summarized in Figure 99; observation and behavioral test data are summarized in Figure 100. No treatment-related clinical or gross behavioral changes were present. (See Figures 101-103) Abnormal social grouping (listed as other reaction) occurred among mice in the 100-mg/kg group, but not the 300-mg/kg group; this finding was considered incidental. Clinical/behavioral changes in 1 mouse in the 100-mg/kg group included inactivity, abnormal gait and posture, decreased induced activity, abnormal passivity, and soft/continuous vocalization; these changes were not attributed to Nepicastat. One mouse each in the 30- and 100-mg/kg group died after dosing; the deaths were considered incidental and the mice were removed from the study.

Example 21

[0571] The purpose of this study is to determine if the DBHIs SKF-102698 and nepicastat produce changes in locomotor activity or acoustic startle reactivity. Changes in these behaviors may therefore reflect activity of these compounds in the central nervous system.

[0572] Adult male Sprague Dawley rats (250-350 g on study day) were obtained from Charles Rivers Labs. Rats were housed under a normal light/dark cycle with lights on between 0900 Hrs. and 2100 Hrs.. Animals were housed in pairs in standard metal wire cages, and food and water were allowed ad libitum.

[0573] The locomotor activity boxes consisted of a Plexiglas[®] box measuring 18" x 18" by 12" high. Surrounding the Plexiglas[®] boxes were Omnitech Digiscan Monitors (model # RXXCM 16) which consisted of a one inch band of photobeams and

photosensors numbering 32 per box. The number of photobeam breaks were analyzed by an Omnitech Digiscan Analyzer (model # DCM-8). The animals were tested in an enclosed room with a white noise generator running to mask extraneous noise.

[0574] Acoustic startle reactivity tests were conducted in eight SR-Lab (San Diego Instruments, San Diego, CA) automated test stations. The rats were placed individually in a Plexiglas[®] cylinder (10 cm diameter) which is housed in a ventilated sound-attenuating enclosure. Acoustic noise bursts (a broad band noise with a rise time and fall time of 1 msec) was presented via a speaker mounted 30 cm above the animal. A piezoelectric accelerometer transforms the subject's movement into an arbitrary voltage on a scale of 0 to 4095.

[0575] Prior to drug administration, each of seventy-two rats was placed in the startle apparatus, and after a 5 minute adaption period they were presented with an acoustic noise burst every 20 seconds for 15 minutes (45 startles total). The average startle was calculated for each rat by taking the mean of startle number 11 through 45 (the first ten startles will be disregarded). Sixty-four of these rats were then placed in one of eight treatment groups such that each group had similar mean startle values. The eight treatment groups were as follows: SKF-102698 (100 mg/kg) and its vehicle (50% water/50% polyethylene glycol), clonidine (40 µg/kg), nepicastat (3, 10, 30 and 100 mg/kg), and their vehicle, dH₂O. Previous work has shown that this matching procedure to be the most appropriate for startle since there is significant variability in startle response between rats, but a high degree of consistency within rats from one day to the next.

[0576] Each day after this testing procedure, eight rats (one rat from each of the eight treatment groups) was injected with their assigned drug treatment and immediately placed individually in a motor activity box. The rats motor activity was monitored for four hours. Next, the rats were placed in a transfer cage for fifteen minutes. At the beginning of this fifteen minutes the rat that has been assigned the clonidine treatment will receive another injection of 40 µg/kg. Next, the rats were placed in the startle apparatus, and after a five minute acclimation period they were presented with a 90 dB noise burst every minute for four hours.

[0577] To evaluate motor activity, horizontal activity (number of photobeams broken), number of movements, and rest time were measured. Each parameter was analyzed separately. At each time interval (or called sample), a two-way analysis of variance (ANOVA) was performed using the ranked data (nonparametric technique) to test for the

treatment effect blocked by day. Pairwise comparisons for 4 RS-treated groups to the vehicle control were also performed using Dunnett's t-test.

[0578] To evaluate startle reactivity, for the 200 milliseconds immediately succeeding each startle the average force exerted by each startled rat over the entire 200 milliseconds, and the maximum force, were measured. The mean maximum and average voltages (MAXMEAN and AVGMEAN) were computed for each treatment (TREAT) at each trial (TRIALN), and then these values were plotted against trial number for each treatment. The plots are attached to the report. Trials 1-60 were set to time=1, trials 61-120 to time=2, trials 121-180 to time=3 and trials 181-240 to time=4. The mean maximum and average startle responses was computed within each time and for each treatment. The means were then used in the statistical analysis. The startle responses were analyzed using analysis of covariance. Treatment comparisons within time were of interest to the investigators, but not time effects within treatments. Therefore, the startle responses were analyzed by time. The model included terms for the day the rat was tested (date), baseline startle response, and treatment. Date was a blocking factor and baseline startle response was a covariate. There were three separate models for each of the objectives stated above. The varying doses of nepicastat were compared to vehicle using Dunnett's procedure in order to control for multiple comparisons.

[0579] The results of analyses for the 3 parameters (horizontal activity, no. of movements and rest time) are presented in Figures 104-115. The plots of each parameter versus hours by treatment group are displayed in Figures 116-118.

[0580] When the four nepicastat -treated groups were compared to the vehicle-treated controls, there were no overall no pairwise significant differences at any time examined in any of the 3 parameters.

[0581] When compared to the vehicle-treated controls, the clonidine-treated group had significantly more horizontal activities at 2 and 2.5 hours, significantly more movements at 2 hours, and significantly less rest time at 2 hours (all $p < 0.05$, see Figures 104-115 respectively). Note that the clonidine-treated group had significantly more rest time than the vehicle-treated controls at 1 hour ($p < 0.05$).

[0582] When compared to the vehicle-treated controls, the SKF-102698-treated group had significantly less horizontal activities and significantly less movements at 2.5 hours (both $p < 0.05$, see Figures 104-115. Note that the SKF-102698-treated group had significantly more movements than the vehicle-treated controls at 1.5 and 4 hours (both p

< 0.05). No significant differences between SKF-102698 and vehicle were detected at any time examined in the rest time (see Figures 104-115)

[0583] In general, the horizontal activity and number of movements decreased for the first 2 hours and stayed low for the last 2 hours. Similarly, the rest time increased for the first 2 hours and remained elevated for the last 2 hours.

[0584] nepicastat had no significant effects on the locomotor activity in rats. Animals treated with 3, 10, 30 or 100 mg/kg of nepicastat were not significantly different from the vehicle-treated controls at any time examined in the horizontal activity, no. of movements or rest time.

[0585] Animals treated with the α_2 -adrenoceptor agonist clonidine had significantly more rest time than the vehicle-treated controls at 1 hour. However at 2 hours, animals treated with clonidine had significantly more horizontal activities and movements, and significantly less rest time, as compared to the vehicle-treated controls.

[0586] Animals treated with SKF-102698 had significantly less horizontal activities and movements than the vehicle-treated controls at 2.5 hours. The rats treated with SKF-102698 had significantly more movements at 1.5 and 4 hours, as compared to the vehicle-treated controls. No significant differences between SKF-102698 and controls were detected at any time examined in the rest time.

[0587] In startle response, the overall treatment effects for nepicastat and vehicle were not significant ($p > 0.05$) at any time for either response. The overall treatment effect for average startle response at time 2 was marginally significant ($p = 0.0703$), and Dunnett's test revealed that nepicastat 30 mg/kg had a significantly higher average startle response than the vehicle group ($p < 0.05$). Baseline average startle response was statistically significant at times 3 and 4 for both responses ($p \leq 0.05$), and marginally significant at times 1 and 2 for maximum startle response, and at time 2 for average startle response ($p \leq 0.10$).

[0588] Figures 123-124 show the mean maximum and average startle responses versus time for each of these five treatment groups.

[0589] SKF-102698 (100 mg/kg) was not statistically significantly different from vehicle at any time for either startle response measurement.

[0590] Figures 125 and 126 show the time course for mean maximum and average startle responses for SKF-102698 and vehicle.

[0591] Clonidine had statistically significantly lower maximum and average startle responses than vehicle at time 1 ($p < 0.01$) and at time 2 for average startle only ($p =$

0.0352). The maximum startle response at time 2 and the average startle response at time 3 for the clonidine group were marginally significantly lower than the water group.

[0592] Figures 127-128 show the time course for mean maximum and average startle responses for clonidine and water.

[0593] nepicastat administered at 3, 10, 30, or 100 mg/kg does not appear to effect the maximum or average startle response in rats at any time when compared to vehicle. SKF-102698 behaved similarly to vehicle (PEG) for both startle responses at all times. Clonidine successfully lowered both maximum and average startle response during earlier times, and behaved similarly to vehicle during later times.

[0594] Figures 119-122 show summary statistics and significance assessments for maximum startle response.

Example 22

[0595] The effects of chronic dosing of nepicastat were examined. Between three and thirteen days prior to the first dosing day the rats were placed inside the startle apparatus and after a five minute acclimation period they were presented with a 118dB noise burst on average once a minute (a variable inter-trial interval ranging between 30 and 90 seconds will be used) for 20 minutes. The startle responses were measured and a mean for the last twenty startle response was calculated for each rat. The rats were randomly placed in one of the eight treatment groups (nepicastat, 5, 15 or 50 mg/kg, bid; SKF-102698, 50 mg/kg, bid; clonidine, 20 ug/kg, bid; d-amphetamine, 2 mg/kg, bid; dH₂O or cyclodextrin (SKF-102698's vehicle). Rats were dosed by oral gavage with a 10 ml/kg dosing volume. The rats were dosed in the morning and in the evening every day for ten day. The time in between morning and evening dosing will be between 6 and 10 hours. Previous work has shown that this matching procedure to be the most appropriate for acoustic startle reactivity since there is significant variability in startle response between rats, but a high degree of consistency within rats from one day to the next.

[0596] Since it was impossible to test all 96 rats (8 treatment groups, n=12) on the same day, the dosing schedule was staggered such that only 8 rats were run every day. These 12 groups of eight rats each consisted of one rat from each of the eight treatment groups so that the treatment groups were balanced across days. Furthermore, all treatment groups were balanced across the eight motor activity chambers, however, treatment groups could not be balanced across the startle chambers.

[0597] The following behavioral tests were administered during and after chronic dosing; body core temperature, motor activity, acoustic startle reactivity, and pre-pulse inhibition of acoustic startle.

[0598] The animals were tested in an enclosed room with a white noise generator running. Motor activity tests were conducted immediately after the body core temperature reading taken on dosing day ten (about 3 hours and 35 minutes after the morning daily dose of nepicastat, and SKF-102698, and 20 minutes prior to the daily administration of clonidine and d-amphetamine on dosing day ten). Motor activity tests were run for one hour. A diagnostic program was run on each of the motor activity chambers prior to each test session to assure that the photo beams and light sensors were operating properly. Motor activity has been shown to be sensitive to changes in central dopamine levels (Dietze and Kuschinsky, 1994) which makes this behavioral test a potential sensitive assay to the effects of DBHI in-vivo. D-amphetamine was used as the positive control for this assay.

[0599] Rat body core temperatures were obtained by inserting the rectal probe 2 cm into the colon of each rat. Each rat's body core temperature was measured three times and the average of the three reading was calculated. Body core temperature readings were obtained immediately prior to the ten day chronic dosing schedule (to obtain a baseline), and three and half hour after the morning daily dose of nepicastat, and SKF-102698, and 15 minutes prior to the daily administration of clonidine and d-amphetamine, on dosing days one, five and ten. Body core temperature has been shown to be sensitive to both dopamine and norepinephrine levels , which makes this behavioral test a potential sensitive assay to the effects of DBHI in-vivo. Both clonidine (an α_2 agonist), and d-amphetamine (a dopamine releaser) were used as the positive controls for this assay.

[0600] Acoustic startle reactivity (a series of muscle contractions elicited by an intense burst of noise with a rapid onset), and pre-pulse inhibition (sensorimotor gating measured by analyzing any decrease in startle reactivity which occurs when a startling stimulus is immediately preceded by a non startling stimulus) were both measured in eight SR-Lab (San Diego Instruments, San Diego, CA) test stations. The rats were placed individually in a Plexiglas cylinder (10 cm diameter) which was housed in a ventilated sound-attenuating enclosure. Acoustic noise bursts (a broad band noise with a rise time and fall time of 1 msec) were presented via a speaker mounted 30 cm above the animal. Also, these speakers produced a 68 dB level of background noise throughout all test sessions. A piezoelectric accelerometer attached below the plexiglas cylinder transduced the

subject's movement into a voltage which was then rectified and digitized (on a scale from 0 to 4095) by a PC computer equipped with SR-Lab software and interface assembly. A decibel meter was used to calibrate the speakers in each of the eight test station to $\pm 1\%$ of the mean. Additionally, a SR-Lab calibrating instrument was used to calibrate each of the eight startle detection apparatuses to $\pm 2\%$ of the mean. Startle reactivity and pre-pulse inhibition tests were run concurrently immediately alter the motor activity test (about 4 hours and 40 minutes after the morning daily injection of nepicastat, and SKF-102698, and 10 minutes after a supplemental administration of clonidine and d-amphetamine on dosing day ten). The startle reactivity and pre-pulse inhibition tests consisted of placing each rat individually into a SR-Lab test station and after a five minute acclimation period the rats were presented with one of three different types of noise bursts (and startle reaction measured) on average once a minute (a variable inter-trial interval ranging between 30 and 90 seconds was used) for an hour (60 total noise bursts and startle reactions). The three different types of noise bursts consisted of a loud noise burst (118 dB), and a relatively quite noise burst (77 dB), the quite burst preceding the loud noise bursts by 100 msec (pre-pulse inhibition trial). These trials were presented in pseudo-random order. Pre-pulse inhibition has been shown to be sensitive to changes in mesolimbic dopamine levels. Furthermore, acoustic startle reactivity has also been shown to be sensitive to changes in dopamine and norepinephrine levels which makes these behavioral test a potential sensitive assay to the effects of DBHI in vivo. Clonidine and d-amphetamine served as the positive control for the acoustic startle reactivity and pre-pulse inhibition of acoustic startle tests.

[0601] The schedule of daily behavioral tests was as follows. At $t=0$, DBHI is injected. At 3.5 hours, the core body temperature is measured. At 3 hr. 35 minutes, motor activity is assessed. At 4 hr. 40 minutes, startle reactivity and pre-pulse inhibition are assessed.

[0602] Three temperature readings were taken from each subject per time of testing. The average of these three readings was then calculated.

[0603] Each rats spontaneous locomotion was obtained by calculating the total number of photobeams that the subject broke during the testing session.

[0604] The subject's reaction was measured during each trial for the 40 msec window after the stimulus was presented. Each startle reaction was calculated by taking the average of 40 readings (one per millisecond) starting immediately after each noise burst. Acoustic startle reactivity was calculated by determining the mean response for each subjects startle elicited by the 118 dB acoustic burst. Pre-pulse inhibition values were

calculated by subtracting the mean startle response elicited by the 77 dB pulse - 118 dB pulse paired trial (pre-pulse inhibition trial described above) from the 118 dB alone trial and then dividing this value by the 118 db alone trial for each rat, i.e. $([118 \text{ dB trial value} - \text{pre-pulse inhibition trial value}] \div 118 \text{ db trial value})$.

[0605] An overall one-way ANOVA with a main effect for treatment was performed at each time on the change from baseline for each animal. Subsequent t-tests were performed for each comparison of interest.

[0606] Spontaneous motor activity was measured for each animal every 15 min for 1 hour. Each time block (every 15 min) was analyzed separately. Kruskal-Wallis test (nonparametric technique) was performed to test for the difference between treatment groups. If the overall significant difference is not detected, Bonferroni's adjustment for multiple comparisons is then made.

[0607] The mean average voltage (AVGMEAN) and mean percent prepulse inhibition (RATIO) were computed for each treatment (TREAT) and trial type (TRIALT) at each trial (TRIALN). Pre-pulse inhibition values were calculated by subtracting the mean startle response elicited by the 77 dB pulse - 118 dB pulse paired trial (pre-pulse inhibition trial described above) from the 118 dB alone trial and then dividing this value by the 118 db alone trial for each rat, i.e. $([118 \text{ dB trial value} - \text{pre-pulse inhibition trial value}] \div 118 \text{ db trial value})$.

[0608] These values were plotted against trial number for each treatment and trial type, and these plots are attached to the report. Note that the y-axis on the plots varies. The trials 1-15 correspond to time 1, 16-30 time 2, 31-45 time 3, and 46-60 time 4. Plots displaying the mean percent prepulse inhibition and the mean average startle of animals over TIME for each treatment are attached also.

[0609] The average startle response and the percent prepulse inhibition were analyzed using Analysis of Variance. The model included terms for treatment, animals nested within treatment, time and treatment by time interaction. Treatment effects were tested using the error term for animals nested within treatment. Overall treatment effects and treatment effects by time were studied. The method of Fisher's Least Significant Differences was used to adjust for multiple comparisons. If the overall treatment or treatment by time effects were not significant ($p\text{-value} > 0.05$) then a Bonferroni adjustment was made. If the overall treatment effects were nonsignificant, then the adjustment was applied to the specific pairwise comparisons. Further, if the specific pairwise treatment effect was not significant ($p\text{-value} > 0.05$), then the adjustment was

also applied to the treatment effects within time. If both the overall treatment and treatment by time effects were not significant ($p\text{-value} > 0.05$) then a Bonferroni adjustment was made for the individual comparisons within time and averaging over time.

[0610] The change from pre-dose in body weights was calculated for each animal for the analysis. A repeated measures two-way ANOVA was used to test for the overall effects of treatment, time and treatment by time interaction. One-way ANOVAS were then performed to test the treatment effect at each day.

[0611] Figure 129-130 show pre-treatment acoustic startle reactivity and starting date for each rat.

[0612] Figure 131 shows that other than the positive controls (d-amphetamine and clonidine) significantly increasing body core temperature on day one of the chronic dosing, no other compound had any significant effect on body core temperature at any time. Figures 132-133 contain the mean body core temperature at each time for each treatment, the mean change in core body temperature from baseline, and significance results.

[0613] As Figure 134 shows, the d-amphetamine group had significantly higher locomotor activity than the vehicle control at all times examined. The clonidine group, however, was not significantly different from the vehicle controls at any time examined. The SKF 102698 50 mg/kg b.i.d. group had significantly lower locomotor activity than its vehicle control at the first 45 minutes (i.e. samples 1 - 3), but not significant after 45 minutes (see Figures 135-136).

[0614] Figure 137 also shows that there was no overall significant treatment effect for nepicastat at any time examined. Pairwise comparisons revealed that none of the nepicastat-treated groups were significantly different from the vehicle controls at any time examined. Also, there was no significant difference between the two vehicle controls ((dH₂O and SKF's vehicle) at any time examined (see Figure 135 and Figure 136).

[0615] As Figure 138 shows, none of the treatment groups produced any significant change in pre-pulse inhibition. The overall time effect was statistically significant for the SKF 102698 group and the cyclodextrin group ($p = 0.0001$). The treatment by time interaction was statistically significant for cyclodextrin versus dH₂O ($p = 0.0283$), but no others. Treatment effects were not significant for any comparisons of interest. However,

the SKF group had marginally higher percent prepulse inhibition compared to the cyclodextrin group ($p = 0.0782$) (see Figure 139).

[0616] During times 1 and 2, the clonidine group had just significantly higher percent prepulse inhibition than the vehicle control and were not significantly different from vehicle during times 3 and 4 (see Figure 140-141). Neither d-amphetamine nor SKF 102698 was significantly different from their own vehicle at any time. None of the nepicastat dose groups were significantly different from dH₂O at any time.

[0617] As Figure 142 shows, only the SKF 102698 treatment group produced a significant change in acoustic startle reactivity. The overall time effect was statistically significant for all comparisons of interest (all $p = 0.0001$). The treatment by time interaction was statistically significant for the comparisons of amphetamine versus dH₂O, clonidine versus dH₂O and cyclodextrin versus dH₂O (all $p < 0.05$), but no others. Treatment effects were significant for SKF 102698 50 mg/kg b.i.d. versus cyclodextrin ($p = 0.0007$) and for nepicastat 50 mg/kg b.i.d. versus SKF 102698 50 mg/kg b.i.d. ($p = 0.0047$), but no others (see Figure 143). The SKF 102698 50 mg/kg b.i.d. group had significantly lower startle response compared to cyclodextrin, and also had significantly lower startle response as compared to the nepicastat 50 mg/kg b.i.d. group.

[0618] The SKF 102698 (50 mg/kg b.i.d.) group had significantly lower startle response than the cyclodextrin group at all times (see Table 144-145). During times 1 and 3, the nepicastat (50 mg/kg b.i.d.) group had significantly higher startle response than the SKF 102698 (50 mg/kg b.i.d.) group. No other significant differences were detected.

[0619] There was no overall or pairwise significant differences in body weight between groups at the pre-dose baseline.

[0620] As shown in Figures 146-147, the d-amphetamine group had a significantly smaller change in body weight from pre-dose than the vehicle controls ($p < 0.01$). When analyzed within each day, the vehicle controls had a significantly greater increase from pre-dose in body weight than the amphetamine group at treatment days 4-10. The clonidine group, however, was not significantly different from the vehicle controls at any time examined. As illustrated in Figure 146, the SKF 102698 (50 mg/kg b.i.d.) group showed a significantly smaller increase ($p < 0.01$) in body weight from pre-dose baseline than its vehicle control (SKF-vehicle). When analyzed within each day, the SKF-vehicle controls had a significantly greater increase from pre-dose in body weight than the SKF 102698 group at treatment days 2-10, except days 3 and 6. Importantly, there was no

difference in changes in body weight between the SKF-vehicle and the vehicle control groups on any day.

[0621] As shown in Figure 147, there was no overall significant treatment effect for any dose of nepicastat at any time examined. Pairwise comparisons revealed that none of the nepicastat-treated groups were significantly different from the vehicle controls at any time examined. Interestingly, there was a significant ($p < 0.05$) overall difference between the SKF 102698 (50 mg/kg b.i.d.) group and the nepicastat (50 mg/kg b.i.d.) group with respect to changes in body weight. When analyzed within each day, the SKF 102698 (50 mg/kg b.i.d.) group had significantly lower body weights than the nepicastat (50 mg/kg b.i.d.) group at days 7-9.

Example 23

[0622] 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was purchased from RBI, Inc, (Natick, MA). For administration, MPTP was suspended in water at a concentration of 2 mg/ml (free base) and was injected subcutaneously in a volume (ml) equal to the weight (kg) of each animal. For example, a 950 gram animal received an injection of 0.95 ml of MPTP at 2 mg/ml resulting in 2.0 mg/kg final per injection.

[0623] Twenty-seven (27) mature squirrel monkeys (*Saimiri sciureus*), sixteen females and eleven males were used for this study. The monkeys were maintained on a 13h/11h light-dark cycle, with food and water available *ad libitum*. All procedures used in this study followed NIH guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC). Animals were individually housed and allowed a minimum of one month to acclimate to the colony prior to commencing behavioral studies.

[0624] A total of six squirrel monkeys, three non-lesioned and three lesioned (received 2 mg/kg MPTP 3 months prior), were used for these studies. To determine the optimal route of administration of drug nepicastat, three different approaches were examined including (i) insertion into treats, (ii) oral syringe, and (ii) oral gavage. (i) Insertion of RS solution (5 mg/ml) into marshmallows was tested in 3 non-lesioned monkeys and proved to be a poor route of drug administration due to failure of animals to ingest treats probably due to adverse taste. (ii) Oral syringe injection of drug (0.5, 2, and 5 mg/kg) into the mouth of three non-lesioned and three lesioned monkeys was also not an acceptable route since animals tended to spit out the solution at the highest drug concentration. (iii) Oral gavage administration was carried out in 3 MPTP-lesioned monkeys at the highest dose (5 mg/kg) and was well accepted.

[0625] These results (oral syringe and oral gavage delivery) are summarized in Figure 148 and Figure 149.

[0626] A total of six squirrel monkeys, three non-lesioned and three lesioned (received 2 mg/kg MPTP 3 months prior), were used for these studies. Animals received drug at a concentration of 0.5, 2.0, or 5.0 mg/kg twice daily, (10 am and 2 pm), for 5 days with a two-day washout between the different dose levels. Drug was administered via oral syringe at the 0.5, 2.0 and 5.0 mg/kg doses and as oral gavage at the 5.0 mg/kg dose. Drug was well tolerated at the two lower doses. One non-lesioned monkey receiving 5.0 mg/kg had light beige colored loose stools on the final two days of administration that resolved upon one day withdrawal of drug. These results are summarized in Figures 148 and 149.

[0627] A total of 24 squirrel monkeys (*Saimiri sciureus*), fourteen females and ten males were used in this study. The twenty-four animals were randomly assigned to one of four treatment groups, with 6 animals per group. The groups consisted of the following (1) group A: placebo (2) group B: 1 mg/kg/day; (3) group C: 4 mg/kg/day; and (4) group D: 10 mg/kg/day. In Group B, one animal died acutely following MPTP-lesioning, and was not replaced.

[0628] Prior to lesioning, animals were subjected to quantitative assessment of spontaneous motor activity using an infrared activity monitor (IRAM) cage. All recording sessions were 60 minutes in length and were carried out for 10 sessions over a period of 2 weeks. The behavior of animals was also assessed by 1 to 3 clinical raters using a parkinsonian clinical rating scale (CRS) once per day (12 noon to 1 pm) for 3 to 5 consecutive days. Normal animals did not typically score greater than 3 on the CRS (see Figure 150 for the clinical rating scale used in these studies). Both the activity monitoring (IRAM) and clinical rating assessments established the mean base-line activity of each animal.

[0629] Animals were lesioned by the administration of MPTP at a concentration of 2.0 mg/kg (free-base) via subcutaneous injection to achieve a parkinsonian state (see Figures 151-156). A post-MPTP lesioning behavioral assessment was carried out 2 to 4 weeks after the last MPTP-lesioning. Locomotor activity was monitored by IRAM in 60-minute sessions for 3 to 5 days. Clinical behavior (CAS) was assessed by one to three individuals rating over a period of 3 to 5 days.

[0630] In some cases, animals required additional doses of MPTP (2 mg/kg) to obtain a sufficient degree of lesioning to display parkinsonian symptoms, defined as an average total clinical rating score greater than 3.

[0631] All animals received a final post-MPTP behavioral assessment (IRAM and CRS), within three weeks of starting the efficacy study. This final post-MPTP evaluation was used to establish a baseline clinical parkinsonian state and used as a pretreatment value for statistical analysis.

[0632] Animals were tested for response to L-Dopa and the efficacy of drug nepicastat. Testing was carried out 4 to 12 weeks after the last MPTP dose. Twenty-four squirrel monkeys were randomly assigned to 4 groups; Group A (6 animals) received placebo (water) treatment; Group B (5 animals) received drug nepicastat at 1 mg/kg/day (0.5 mg/kg twice daily); Group C (6 animals) received 4 mg/kg/day (2 mg/kg twice daily); and Group D (6 animals) received 10 mg/kg/day (5 mg/kg twice daily).

[0633] L-Dopa was administered at a concentration of either 2.5, 5, or 7.5 mg/kg by oral gavage twice daily (at 10 am and 2 pm) for 7 consecutive days. Behavior was determined by IRAM and CRS. Clinical rating was carried out 60 to 90 minutes following the 10 am morning dose on the last 4 days of treatment. Raters (one to three individuals) were blinded to the different treatment groups. IRAM assessment were preformed for 90 minutes immediately following drug administration at 2 pm on the last 2 to 5 days of drug treatment. There was a minimum 2 day washout period between each treatment dose.

[0634] Drug nepicastat or water (as placebo) was administered for 12 days following a minimum 2 day washout after L-Dopa dosing. Drug was administered twice daily at 10 am and 2 pm by oral gavage. Behavior was rated by IRAM and CRS. The CRS was conducted in the morning, 60 to 90 minutes after the 10 am dose of nepicastat on the last 5 days of drug treatment. Raters (one to three individuals) were blinded to the different treatment groups. IRAM assessments were preformed for 90 minutes immediately following drug administration at 2 pm on the last 5 days of drug treatment.

[0635] A pharmacokinetic study was carried out to determine the plasma concentration of nepicastat in the squirrel monkey. This study was carried out concurrently with the safety and tolerability study. Three MPTP-lesioned squirrel monkeys (#353, 358 and 374) were used. One milliliter of blood (drawn from the femoral vein of each animal) was collected for analysis. Nepicastat was administered at concentrations of 1, 4, and 10 mg/kg for 5 days with a 2-day washout between each drug concentration. Blood was collected for

analysis 1 hour prior to the first dose to establish baseline and at 6 hours after this first drug dose of each of the different drug levels.

[0636] A second pharmacokinetic study was carried out to determine the steady-state plasma level of nepicastat. This study was carried out concomitantly with the efficacy study where animals were tested on each of three different drug concentrations for 12 days. One milliliter of blood was drawn from the femoral vein 6 hours after the first dose on day 1, then 6 hours after the first dose on day 7, and finally 6 hours after the first dose on day 12. Baseline plasma levels were determined on samples collected the week prior to drug dosing.

[0637] The average locomotor activity was calculated pre- and post-MPTP-lesioning for each animal. The pre-MPTP-lesioning baseline was determined by averaging ten 1-hour monitoring sessions. The post-MPTP (pre-treatment) behavioral assessment was obtained within three weeks of commencing the efficacy study. The post-MPTP-lesioning locomotor activity was determined by averaging three to five 1-hour monitoring sessions (IRAMS). Activity monitoring was reported as "movements/10 minutes". A higher score was considered a faster animal. The Wilcoxon sign rank test was used to compare pre- and post-MPTP-lesioning activity for each group of animals (groups A through D). (b) Clinical Rating Score: No pre-MPTP-lesioned animal scored greater than three on the CRS. A post-MPTP clinical rating score was determined within three weeks of commencing the efficacy study by averaging the total CRS of 1 to 3 individual raters from data over 3 to 5 consecutive days.

[0638] Eight parkinsonian features were rated in each animal and the total score was derived from the sum of these eight features. For each animal, a single clinical rating score was obtained for each drug dose by averaging the clinical rating scores of all raters (one to three) conducted over the consecutive multiple dosing (with the same dose) days. This average CRS was used for statistical analysis. A lower score was considered a less parkinsonian behavioral state.

[0639] Statistical analysis consisted of:

[0640] (1) comparisons between the average CRS of placebo to nepicastat at 1, 4, and 10 mg/kg/day using the Kruskal-Wallis (non-parametric analysis of variance). This comparison was repeated using the average CRS for each experimental drug concentration corrected by the final post-MPTP ratings for each animal. The corrected clinical scores are clinical scores of experimental drug at each concentration as a ratio of post-MPTP clinical scores.

[0641] (2) Pairwise comparisons between the average CRS post-MPTP lesioning (pre-treatment) to 2.5, 5.0, and 7.5 mg/kg L-Dopa and placebo treatment using Friedman's analysis (non-parametric analysis of variance, repeated measures). The same analysis was performed for nepicastat at concentrations of 1, 4, and 10 mg/kg. Dunnett's post hoc analysis for non-parametric data was performed when needed.

[0642] IRAM Locomotor activity was monitored every 10 minutes for a minimum of 90 minutes following each drug level. A higher rating is considered a faster (less parkinsonian) animal.

[0643] Statistical analysis consisted of descriptive statistics and graphing the mean of each 10 minutes data blocks of placebo and experimental drug at 1, 4, and 10 mg/kg. The graph was then examined to detect any trends. Further statistical analysis was not performed since no difference was determined from graphical analysis.

[0644] Statistical analysis comparing post-MPTP lesioning (pre-treatment) to 2.5, 5.0, and 7.5 mg/kg L-Dopa and nepicastat (1,4,10mg/kg/day or placebo) was not performed due to insufficient IRAM data collection. Only 60 minutes sessions were collected at Post-MPTP, versus 90 minutes for 1nepicastat.

[0645] Both IRAM (activity monitoring) and CRS (clinical rating scale) were used to assess the degree of MPTP-lesioning in each squirrel monkey. The following section tabulates these results for Groups A through D showing IRAM and CRS. Overall there was no significant difference in the locomotor activity as measured by IRAM between base-line (pre-MPTP-lesioning) and post-MPTP-lesioning within groups due to the high degree of variability of the RAM results for each animal. The CBS results showed a difference between pre-MPTP and post-MPTP-lesioning states. Pre-MPTP-lesioned animals scored no greater than 3 in the CRS. Post-MPTP-lesioned animals all scored greater than 3. All groups (A through D) had an average CRS ranging from 8 to 10 out of a total possible CRS score of 24.

[0646] Figure 153A shows results for Group A: Placebo Treatment. There was no significant difference between pre-lesioned and post-lesioned IRAM groups due to the high degree of variability of movements per 10 minutes per animal. Wilcoxon signed rank test: $W = 19$, $N = 6$, $P < 0.06$ Accept Null Hypothesis.

[0647] Figure 153B shows Clinical Rating Score (GRS). The average GRS for group A was 8.9, range 4.8 to 15.4. All animals showed substantial increase in the clinical rating scores after MPTP-lesioning. Normal animals (non-lesioned) typically have scores less than 3.

[0648] Figure 154 A and B show results for Group B: 1 mg/kg/day Treatment. There was no significant difference between pre-lesioned and post-lesioned IRAM groups due to the high degree of variability of movements per 10 minutes per animal. Wilcoxon signed rank test: $W = 9$, $N = 5$, $p < 0.06$ Accept Null Hypothesis Clinical Rating Score (CRS). The average CRS for group B was 10.32, range 4.3 to 16.1. All animals showed substantial increase in the clinical rating scores after MPTP-lesioning. Normal animals (non-lesioned) typically have scores less than 3.

[0649] Figure 155 shows results for Group C: 4 mg/kg/day Treatment. There was no significant difference between pre-lesioned and post-lesioned IRAM groups due to the high degree of variability of movements per 10 minutes per animal. Wilcoxon signed rank test: $W = 17$, $N = 6$, $P > 0.06$ Accept Null Hypothesis The average CRS for group C was 8.97, range 6.5 to 17.3. All animals showed substantial increase in the clinical rating scores after MPTP-lesioning. Normal animals (non-lesioned) typically have scores less than 3.

[0650] Figure 156 shows results for Group D: 10 mg/kg/day treatment. There was no significant difference between pre-lesioned and post-lesioned IRAM groups due to the high degree of variability of movements per 10 minutes per animal. Wilcoxon signed rank test: $W = 21$, $N = 6$, $P > 0.06$ Accept Null Hypothesis. All animals showed substantial increase in the clinical rating scores after MPTP-lesioning. The average CRS for group C was 8.02, range 4.0 to 15.6. Normal animals (non-lesioned) typically have scores less than 3.

[0651] There were no detectable differences between placebo treatment and three different concentrations of nepicastat (1, 4, 10 mg/kg/day) in the MPTP-lesioned squirrel monkey. Both 4 and 10 mg/kg/day of nepicastat and placebo showed a significant improvement over the post-MPTP (pre-treatment) state. All groups of animals showed significant improvement with both 5 mg/kg and 7.6 mg/kg L-Dopa when compared to post-MPTP (pre-treatment), with the exception of Group C for the 7.5 mg/kg dose and Group B for the 5 mg/kg/dose. No groups of animals demonstrated significant improvement at 2.5 mg/kg L-Dopa when compared to post-MPTP.

[0652] Figures 157-170 show comparisons of treatment groups and L-DOPA, Friedman test results, descriptive statistics, and Dunnett's test post hoc analysis.

[0653] Figures 171-172 show the comparison between the activity monitoring of placebo treatment to all other concentrations of nepicastat at time points 10 to 90 minutes post-dosing. Ten-minute intervals were plotted for each drug dose level. There was no

difference of drug (nepicastat) treatment at the 4 and 10 mg/kg/day dose level when compared to placebo. At 1 mg/kg/day animals were slower than placebo treatment. Based on a non-pairwise comparative analysis of 4 different treatment groups (1,4, and 10mg/kg of nepicastat and placebo), nepicastat produced no significant effect in parkinsonian symptoms compared to placebo (water treatment) in the MPTP-lesioned non-human primate model of PD. Based on a pairwise comparative analysis of animals, (animals of the same group examined pre and post treatment), nepicastat at 4 and 10 mg/kg/day concentrations showed a significant effect in parkinsonian symptoms compared to post-MPTP lesioning, (pre-treatment evaluation). Placebo had a borderline significant effect. Using the same pairwise comparison, 5 and 7.5 mg/kg of L-Dopa demonstrated a significant effect when compared to the post-MPTP lesioned state in all groups with the exception of Group B (no effect at 5 mg/kg L-Dopa) and Group C (no effect at 7.5 mg/kg L-Dopa) animals. However, 2.5 mg/kg of L-Dopa demonstrated no significant effect.

[0654] This study also demonstrated that, a pairwise analysis, which reduces animal to animal variability by comparing the same animal pre- and post- treatment, is better suited for detecting a significant drug effect than a non-pairwise study design when a small number of animals is used.

Example 24

[0655] Male, spontaneous hypertensive rats (280-345g; Charles River Labs, Kingston, NY) were fasted overnight then anesthetized with ether. A femoral artery and femoral vein were cannulated with PE50 tubing for recording of blood pressure and administration of compounds, respectively. Animals were then placed in MAYO restrainers and their feet loosely taped to the restrainer. Heparinized saline (50 units sodium heparin/ml) was used to maintain patency of each cannula throughout the experiment. The following parameters were continuously recorded using Modular Instruments MI² BioReportTM software installed on an IBM personal computer: mean arterial pressure (MAP), heart rate (HR), and the change from baseline for each parameter at specified time points in the experiment.

[0656] All compounds were dissolved on the day of use. nepicastat was dissolved in deionized water (vehicle) to a free base concentration of 1 mg/ml. Oral dosing volume for nepicastat or vehicle was 10 ml/kg. SCH-23390 was dissolved in saline (vehicle) to a free base concentration of 0.2 mg/ml. Nepicastat or saline were administered

intravenously as a bolus in a volume of 1.0 ml/kg followed by 0.2 ml flush of isotonic saline.

[0657] Following surgical preparation, each animal was allowed a minimum one hour recovery period. Animals were randomly assigned to four treatment groups: vehicle (iv)/vehicle (po); vehicle (iv)/nepicastat (po); SCH-23390 (iv)/vehicle (po); or SCH-23390 (iv)/nepicastat (po). Once animals were stabilized (minimum one hour), baseline blood pressure and heart rate was determined by taking an average of each parameter over a 15 min period of time. Once baseline blood pressure and heart rate were established, animals were dosed intravenously with either SCH-23390 (200 µg/kg) or vehicle (saline, 1 ml/kg). Fifteen minutes later, animals were orally dosed with either nepicastat (10 mg/kg) or vehicle (deionized water, 10 ml/kg).

[0658] Recorded parameters were measured 15 min prior to intravenous dosing to establish baseline blood pressure and heart rate. Recorded parameters were then measured at 5, 10, and 15 min following intravenous administration of SCH-23390 or vehicle. Following oral administration of nepicastat or vehicle, recorded parameters were measured at 15, 30, 60, 90, 120, 150, 180, 210, and 240 min.

[0659] At the end of the experiment, each animal was anesthetized with halothane and euthanized via decapitation. The cortex, left ventricle (apex), and mesenteric artery were dissected out, weighed, and fixed in 0.4 M perchloric acid. Tissues were then frozen in liquid nitrogen and stored at -70°C. Biochemical analysis will be performed on these tissues at a later date to determine catecholamine levels (specifically, dopamine and norepinephrine). Assay results will be reported at a later date. Blood pressure and heart rate were analyzed separately. The change from baseline for blood pressure and heart rate were analyzed by an analysis of variance (ANOVA) with effects for treatment, time, and their interaction. This analysis was performed both for the post-iv time period and for the post-oral time period. Further analyses were performed at each time by an ANOVA with a main effect for time. Pairwise comparisons were performed following each ANOVA by Fisher's LSD strategy with a Bonferroni correction when the overall treatment effect was not significant.

[0660] An additional analysis was performed to compare the baseline means of each treatment group by an ANOVA with a main effect for treatment and subsequent pairwise comparisons. Comparisons of SCH-23390 (iv)/Vehicle (po) vs. Vehicle (iv)/Vehicle (po), Vehicle (iv)/nepicastat (po) vs. Vehicle (iv)/Vehicle (po), and SCH-23390 (iv)/nepicastat(po) vs. Vehicle (iv)/nepicastat (po) were made.

[0661] There were no significant differences in baseline heart rate or mean arterial pressure between treatment groups (Figure 173).

[0662] Intravenous treatment with SCH-23390 resulted in a significant decrease ($p<0.05$) in heart rate during the post-oral period at 120 min and 240 min compared to vehicle control (Fig. 174). Nepicastat did not decrease the heart rate as much as observed in vehicle treated animals. This was statistically significant ($p<0.05$) at 150 and 180 min post dose (Fig. 174). The large variability in heart rate observed over the course of this experiment should be noted.

[0663] Intravenous administration of SCH-23390 produced a small (5 ± 1 mmHg) yet significant decrease ($p<0.05$) in mean arterial pressure compared to animals that received vehicle during the 15 min post-iv period (Fig. 175). Oral treatment with nepicastat caused a significant decrease ($p<0.05$) in mean arterial pressure by 30 min post dose which continued for the duration of the experiment (Fig. 175). Pretreatment with SCH-23390 did not significantly attenuate the antihypertensive effects observed with nepicastat administration alone (Fig. 175).

Example 25

[0664] Male Crl:COBS(WI)BR rats of 15 weeks old were used. Twenty-four rats were chronically implanted with telemetry implants (TA11PA-C40, Data Sciences, Inc., St. Paul, MN) for measurement of arterial blood pressure, heart rate and motor activity. The rat was anesthetized with pentobarbital sodium (60 mg/kg, ip) and its abdomen shaved. Under aseptic conditions, an incision was made on midline. The abdominal aorta was exposed, and cannulated with the catheter of a telemetry transmitter unit. After the transmitter was sutured to the abdominal musculature, the skin was closed. Each rat was allowed to recover for at least 2 weeks before being subjected to drug administration. Three days prior to the start of the experiment, the rats were randomly divided into 4 treatment groups: Vehicle (p.o.), Hydralazine (10 mg/kg, p.o.), nepicastat (30 mg/kg, p.o.), nepicastat (100 mg/kg, p.o.).

[0665] Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), heart rate (HR), and motor activity (MA) were monitored. After the pre-dose values for these parameters were established, respective groups of rats received a 7 day daily treatment of vehicle, nepicastat or hydralazine. Typical pre-dose data on MBP, HR and MA were presented on Figures 176-178.

[0666] Both nepicastat and hydralazine were prepared in water with traces of Tween 80. All doses were given orally to the rat in 10 ml/kg and were expressed as free base equivalents.

[0667] A computerized data collection system was used to continuously collect data on SBP, DBP, MBP, HR, and MA. Data on each rat were collected every 5 min. for 10 sec. These were then averaged hourly and standard errors of the mean (SE) calculated. In this report, only data on MBP, HR and MA were presented. For clarity, the SE bars and indications of significance levels were omitted from the figures (see Figures 186 for the significance levels for each time point on MBP for rats treated with rufinamide and Figure 187 for rats treated with hydralazine). Body weights were recorded daily.

[0668] All values were expressed as means $\pm$ SEM. Statistical significance was defined as a p level of less than 0.05.

[0669] Data on MBP, HR and MA were analyzed separately. Each analysis was done on 26 time points measured each day. A two-way ANOVA with main effects for treatment and time and their interaction was used. If an overall treatment effect or a significant interaction was detected, a series of one-way ANOVA at each time point would be performed. The pairwise comparisons at each time point were performed using Dunn's procedure. If no overall treatment effect was detected, then the pairwise difference from control would be performed by adjusting the critical value using a Bonferroni adjustment.

[0670] For body weight, a two-way ANOVA with respect to the changes from pre-dose was used to analyze overall effects for treatment, day, and treatment by day interaction. Then a one-way ANOVA was performed for each day, and pairwise comparisons for the drug-treated groups to the vehicle controls were made using Dunn's procedure and Fisher's LSD strategy to adjust for multiple comparisons.

[0671] Oral administration of nepicastat at 30 mg/kg (all doses expressed hereafter are po) tended to slowly lower blood pressure but did not induce a consistent hypotensive effect on day 1 (Figure 179). As the effect progressed, a peak hypotensive effect of -10 mmHg was observed on day 2 at hour 13 (Figure 180). Similar degrees of antihypertensive effects were induced throughout the study. At 100 mg/kg, the compound induced a peak antihypertensive response of -11 mmHg 22 hr after dosing on day 1 ($p < 0.01$; Figure 179). MBP continued to decrease and reached its nadir of approximately -17 mmHg on day 3 ($p < 0.01$; Figure 181). The MBP remained low throughout the study (see Figure 182, day 7).

[0672] Hydralazine at 10 mg/kg caused an immediate hypotensive effect which subsided in 10 hr (Figure 179). A maximal decrease of -24 mmHg ($p < 0.01$) in MBP was observed within 1 hr after dosing on day 1 (Figure 179). Similar transient hypotensive effects were observed throughout the study (see Figures 179-182).

[0673] Nepicastat at 30 and 100 mg/kg, did not consistently affect HR on day 1. On day 2, however, Nepicastat at 100 mg/kg caused a bradycardic response of -100 b/mm 3 hours after dosing (Figure 183). Significant but less pronounced bradycardic responses were observed on days 3-7.

[0674] In comparison, hydralazine at 10 mg/kg induced varying degrees of tachycardia throughout the study (see Figure 183).

[0675] Throughout the study, none of the drug treatments showed a consistent effect on MA (for example, see Figure 184, day 3).

[0676] Compared to that treated with vehicle, none of the drug treatments had any effect on body weights ($p < 0.05$; Figure 185). Although treatment with nepicastat at 100 mg/kg tended to decrease body weight on day 3, it was not statistically significant.

[0677] It will be readily apparent to one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein are suitable and may be made without departing from the scope of the invention or any embodiment thereof. While the invention has been described in connection with certain embodiments, it is not intended to limit the invention to the particular forms set forth, but on the contrary, it is intended to cover such alternatives, modifications and equivalents as may be included within the spirit and scope of the invention as defined by the following claims.

CLAIMS

1. A method of treating a patient diagnosed with post-traumatic stress disorder, comprising administering to the patient a therapeutically effective amount of Compound A.
2. The method of claim 1, wherein the method further comprises coadministering a therapeutically effective amount of at least one other agent, selected from benzodiazepine, a selective serotonin reuptake inhibitor (SSRI), a serotonin-norepinephrine reuptake inhibitor (SNRI), a norepinephrine reuptake inhibitor (NRI), a serotonin 5-hydroxytryptamine_{1A} (5HT_{1A}) antagonist, a dopamine β -hydroxylase inhibitor, an adenosine A_{2A} receptor antagonist, a monoamine oxidase inhibitor (MAOI), a sodium (Na) channel blocker, a calcium channel blocker, a central and peripheral alpha adrenergic receptor antagonist, a central alpha adrenergic agonist, a central or peripheral beta adrenergic receptor antagonist, a NK-1 receptor antagonist, a corticotropin releasing factor (CRF) antagonist, an atypical antidepressant/antipsychotic, a tricyclic, an anticonvulsant, a glutamate antagonist, a gamma-aminobutyric acid (GABA) agonist, and a partial D₂ agonist.
3. The method of claim 2, wherein the at least one other agent is a SSRI selected from paroxetine, sertraline, citalopram, escitalopram, and fluoxetine.
4. The method of claim 2, wherein the at least one other agent is a SNRI selected from duloxetine, mirtazapine, and venlafaxine.
5. The method of claim 2, wherein the at least one other agent is a NRI selected from bupropion and atomoxetine.
6. The method of claim 2, wherein the at least one other agent is the dopamine β -hydroxylase inhibitor disulfiram.
7. The method of claim 1, wherein the patient has abnormal brain levels of at least one catecholamine.
8. The method of claim 1, wherein the Compound A reduces dopamine β hydroxylase activity in the brain of the patient.
9. The method of claim 1, wherein the Compound A modulates brain levels of at least one catecholamine in the patient.

10. The method of claim 1, wherein the Compound A reduces stress associated with memory recall in the patient.
11. The method of claim 1, wherein the Compound A reduces at least one of the frequency and intensity of at least one sign of the post-traumatic stress disorder in the patient.
12. The method of claim 1, wherein the patient is a child or an adolescent.
13. The method of claim 12, wherein the Compound A reduces at least one of the frequency and intensity of at least one sign or symptom of the post-traumatic stress disorder in the patient, wherein the sign or symptom is selected from disorganized or agitated behavior, repetitive play that expresses aspects of the trauma, frightening dreams which lack recognizable content, and trauma-specific reenactment.
14. The method of claim 1, wherein the Compound A reduces the incidence of at least one disorder comorbid with post-traumatic stress disorder selected from drug abuse, alcohol abuse, and depression in the patient.
15. A method of treating post-traumatic stress disorder in a patient comprising:
 - diagnosing the patient with post-traumatic stress disorder;
 - administering to the patient a therapeutically effective amount of Compound A;
 - assessing at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder; and
 - determining that the post-traumatic stress syndrome is improved if the Compound A reduces at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder.

16. The method of claim 15, wherein the method further comprises coadministering a therapeutically effective amount of at least one other agent, selected from benzodiazepine, a selective serotonin reuptake inhibitor (SSRI), a serotonin-norepinephrine reuptake inhibitor (SNRI), a norepinephrine reuptake inhibitor (NRI), a serotonin 5-hydroxytryptamine_{1A} (5HT_{1A}) antagonist, a dopamine β -hydroxylase inhibitor, an adenosine A_{2A} receptor antagonist, a monoamine oxidase inhibitor (MAOI), a sodium (Na) channel blocker, a calcium channel blocker, a central and peripheral alpha adrenergic receptor antagonist, a central alpha adrenergic agonist, a central or peripheral beta adrenergic receptor antagonist, a NK-1 receptor antagonist, a corticotropin releasing factor (CRF) antagonist, an atypical antidepressant/antipsychotic, a tricyclic, an anticonvulsant, a glutamate antagonist, a gamma-aminobutyric acid (GABA) agonist, and a partial D₂ agonist.

17. The method of claim 15, wherein the Compound A reduces at least one of the frequency and intensity of at least one sign of the post-traumatic stress disorder in the patient.

18. The method of claim 15, wherein the Compound A reduces at least one of the frequency and intensity of at least one symptom of the post-traumatic stress disorder in the patient.

19. The method of claim 15, wherein the Compound A reduces at least one of the frequency and intensity of at least one symptom cluster of the post-traumatic stress disorder in the patient, wherein the symptom cluster is selected from re-experiencing/intrusion, avoidance/numbing, and hyperarousal.

20. A method of improving resilience in a patient comprising administering a therapeutically effective amount of Compound A.

21. The method of claim 20, wherein the method further comprises coadministering a therapeutically effective amount of at least one other agent, selected from benzodiazepine, a selective serotonin reuptake inhibitor (SSRI), a serotonin-norepinephrine reuptake inhibitor (SNRI), a norepinephrine reuptake inhibitor (NRI), a serotonin 5-hydroxytryptamine_{1A} (5HT_{1A}) antagonist, a dopamine β -hydroxylase inhibitor, an adenosine A_{2A} receptor antagonist, a monoamine oxidase inhibitor (MAOI), a sodium (Na) channel blocker, a calcium channel blocker, a central and peripheral alpha adrenergic receptor antagonist, a central alpha adrenergic agonist, a central or peripheral beta adrenergic receptor antagonist, a NK-1 receptor antagonist, a corticotropin releasing factor (CRF) antagonist, an atypical antidepressant/antipsychotic, a tricyclic, an anticonvulsant, a glutamate antagonist, a gamma-aminobutyric acid (GABA) agonist, and a partial D₂ agonist.

22. The method of claim 20, wherein the Compound A reduces at least one of the frequency and intensity of at least one sign of the post-traumatic stress disorder in the patient.

23. A method of diagnosing post-traumatic stress disorder in a patient comprising:
administering to the patient a therapeutically effective amount of Compound A and
assessing at least one of sign, symptom, or symptom cluster of post-traumatic stress disorder; and

diagnosing post-traumatic stress disorder in the patient if the Compound A reduces at least one of sign, symptom, and symptom cluster of post-traumatic stress disorder.

24. The method of claim 23, wherein the patient is a child, adolescent, or adult.

Figure 1

ENZYME	ASSAY
Tyrosine hydroxylase	Release of [$^3\text{H}_2\text{O}$] associated with conversion of L-[3,5- ^3H]-tyrosine to DOPA
Acetyl CoA synthetase	Utilization of [^3H]-acetic sodium acetate
Acyl-CoA, Cholesterol acyltransferase	Formation of cholesteryl ester from [1- ^{14}C]-palmitoyl-CoA and endogenous cholesterol
Ca^{2+} /Calmodulin-protein kinase II	Phosphorylation [^{32}P] of BB40 (a synthetic peptide substrate)
Cyclooxygenase-I	Oxidation of arachidonic acid followed by spectrophotometric quantitation of malondialdehyde
HMG-CoA reductase	Formation of [^{14}C]-mevalonic acid from [^{14}C]-HMG-CoA
Neutral endopeptidase (human)	Formation of 4-methoxy-2-naphthylamine from glutaryl-ala-ala-phe-4-methoxy-2-naphthylamide
Nitric oxide synthase (constitutive)	Conversion of [^3H]-arginine to [^3H]-citrulline
Nitric oxide synthase (inducible)	Measurement of iNOS reaction products (NO_2 and NO_3) in cytosol preparation from mouse macrophages induced by interferon- γ and lipopolysaccharide
Phosphodiesterase III (human)	Conversion of [^3H]-cAMP to [^3H]-AMP which is subsequently converted to [^3H]-adenosine
Phospholipase A_2	Formation of [^{14}C]-palmitate from [^{14}C]-3-phosphatidylcholine
Protein kinase C (non-selective)	Phosphorylation [^{32}P] of Histone H1

Figure 2

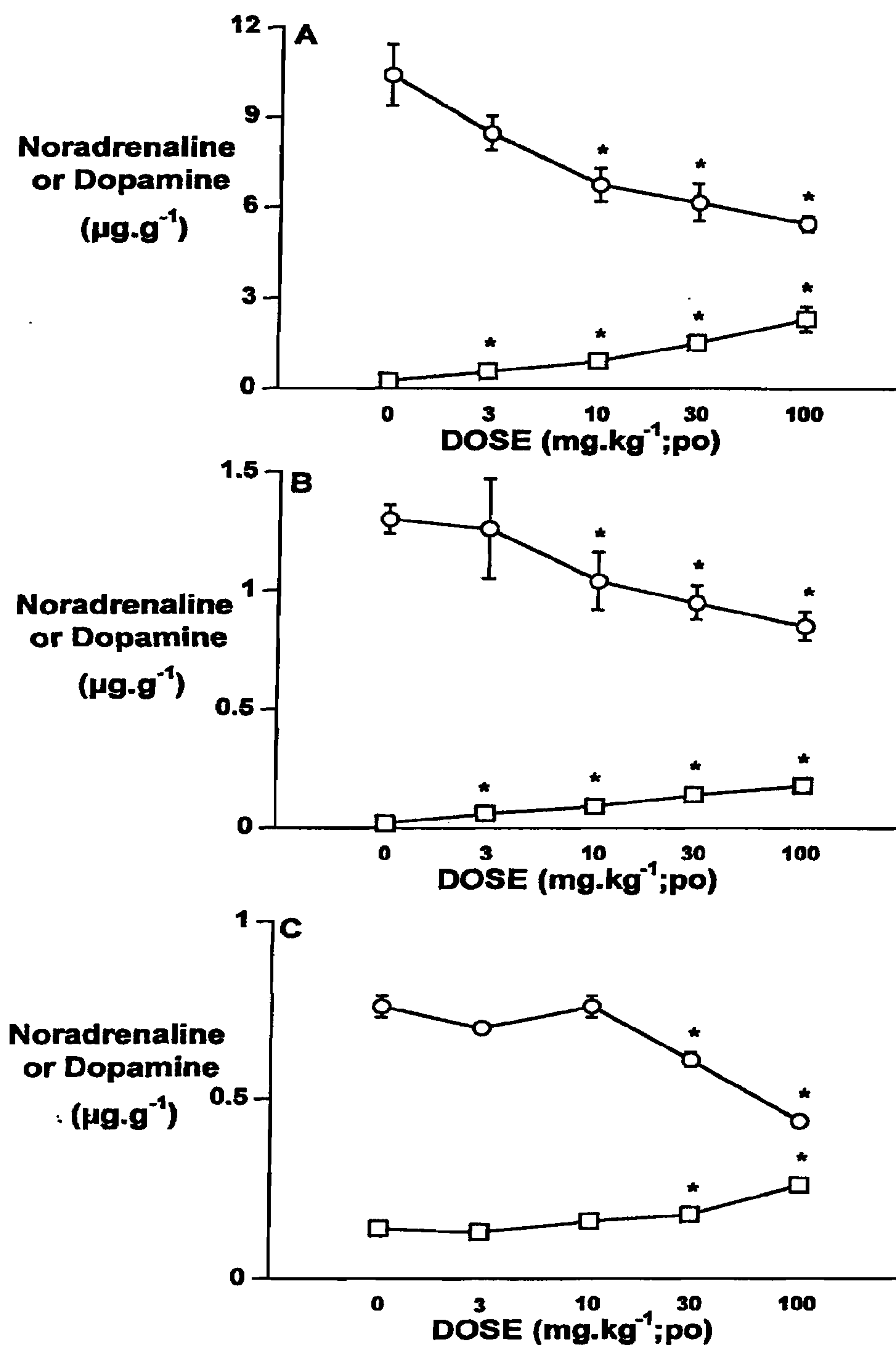


Figure 3

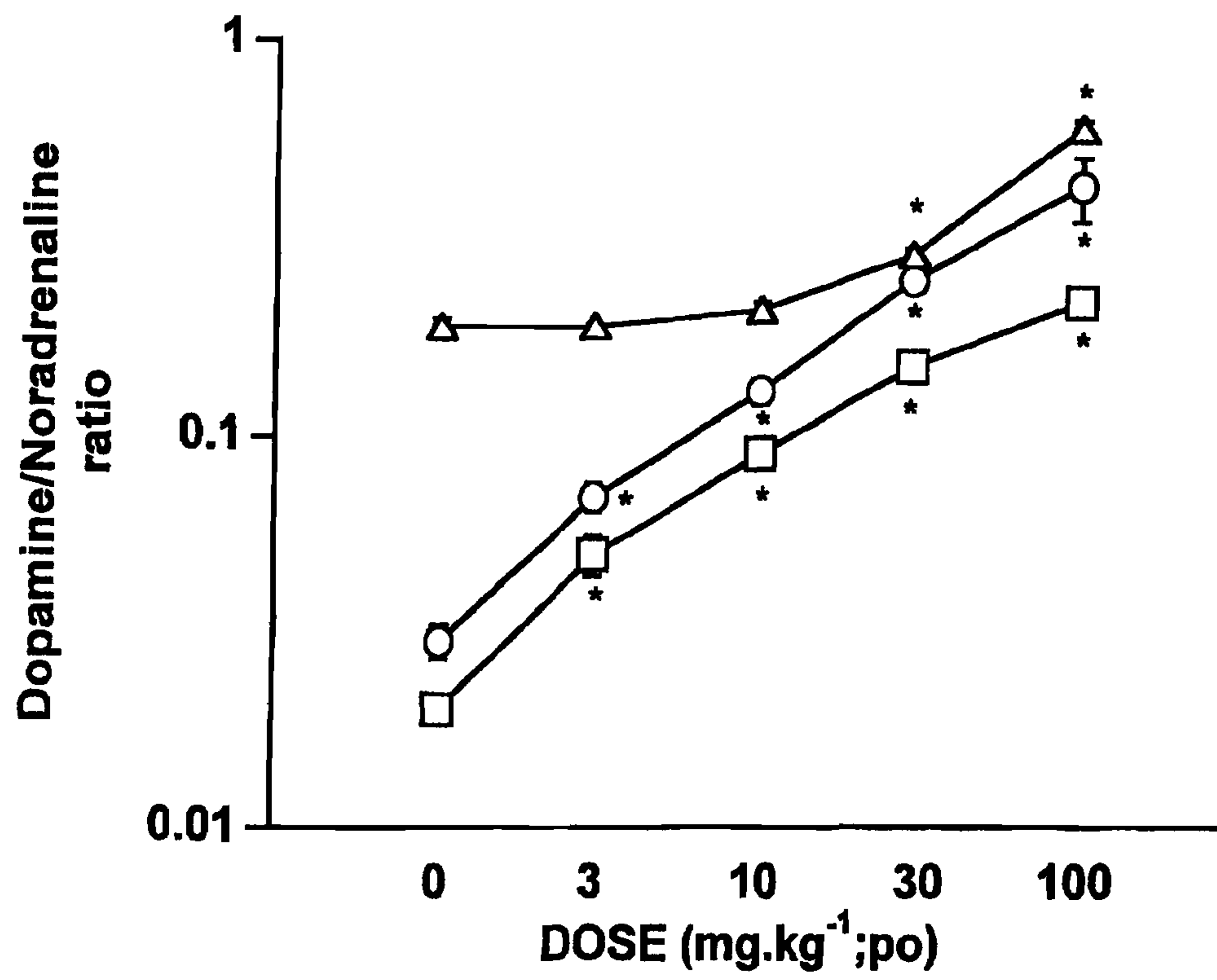


Figure 4

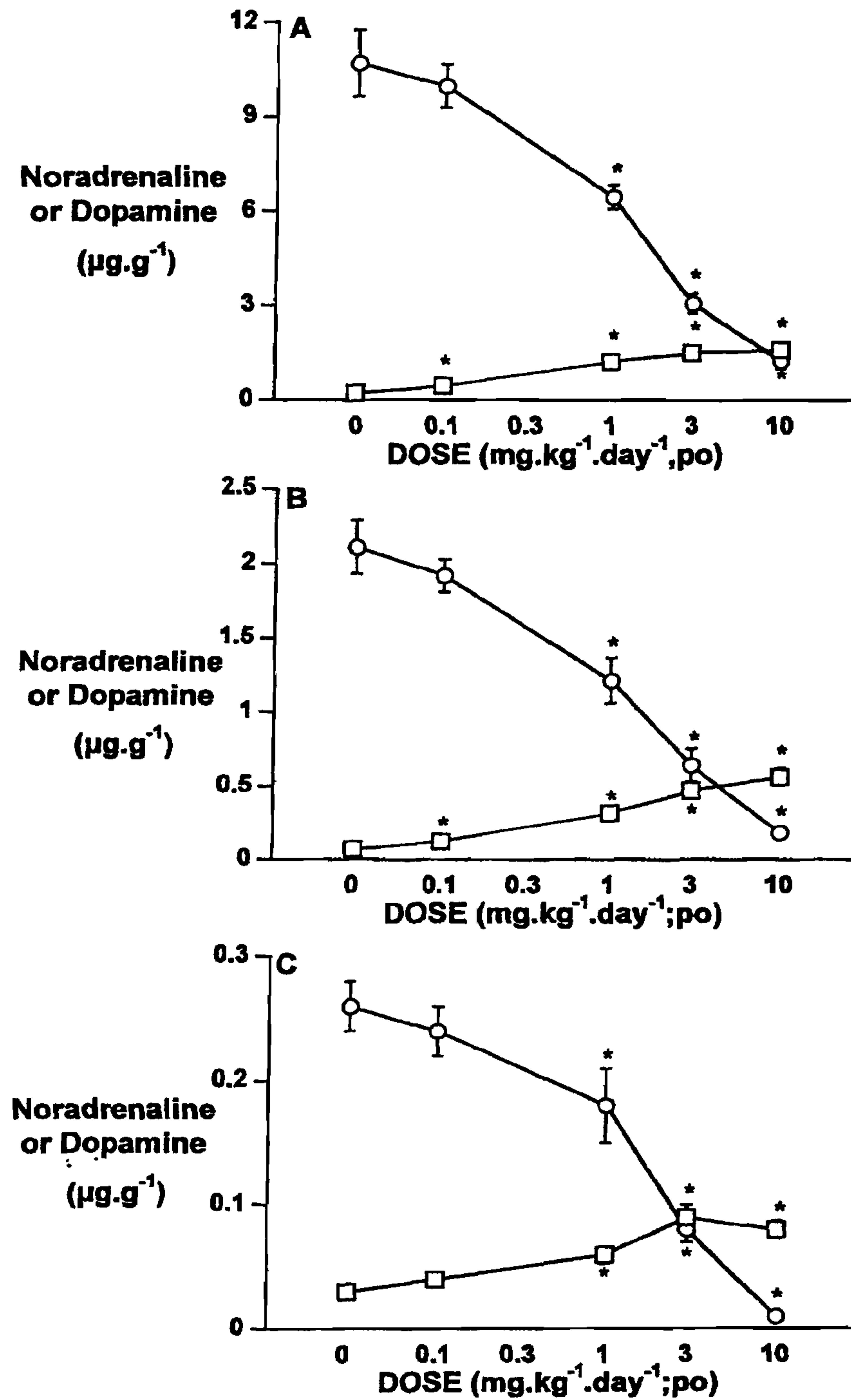


Figure 5

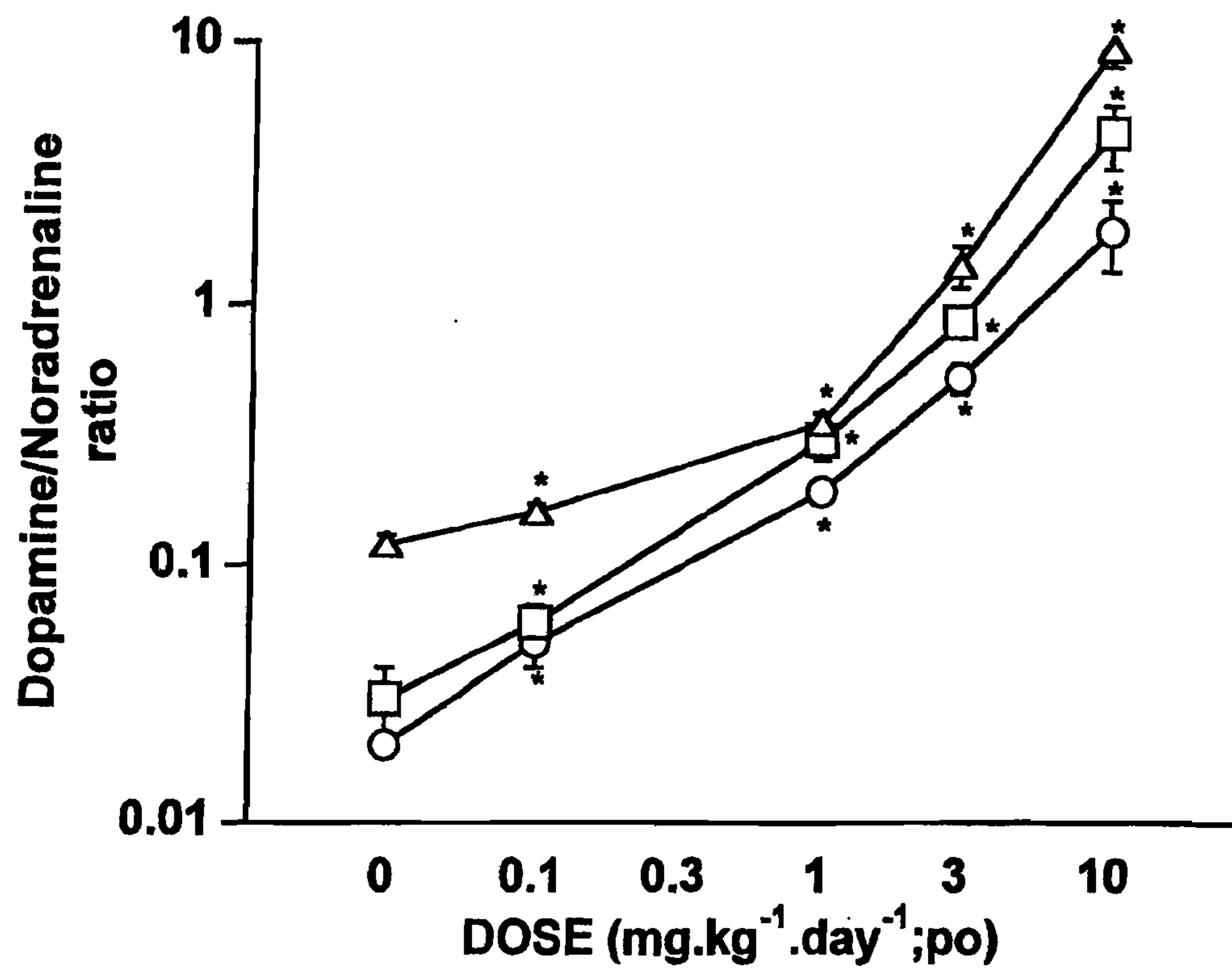


Figure 6

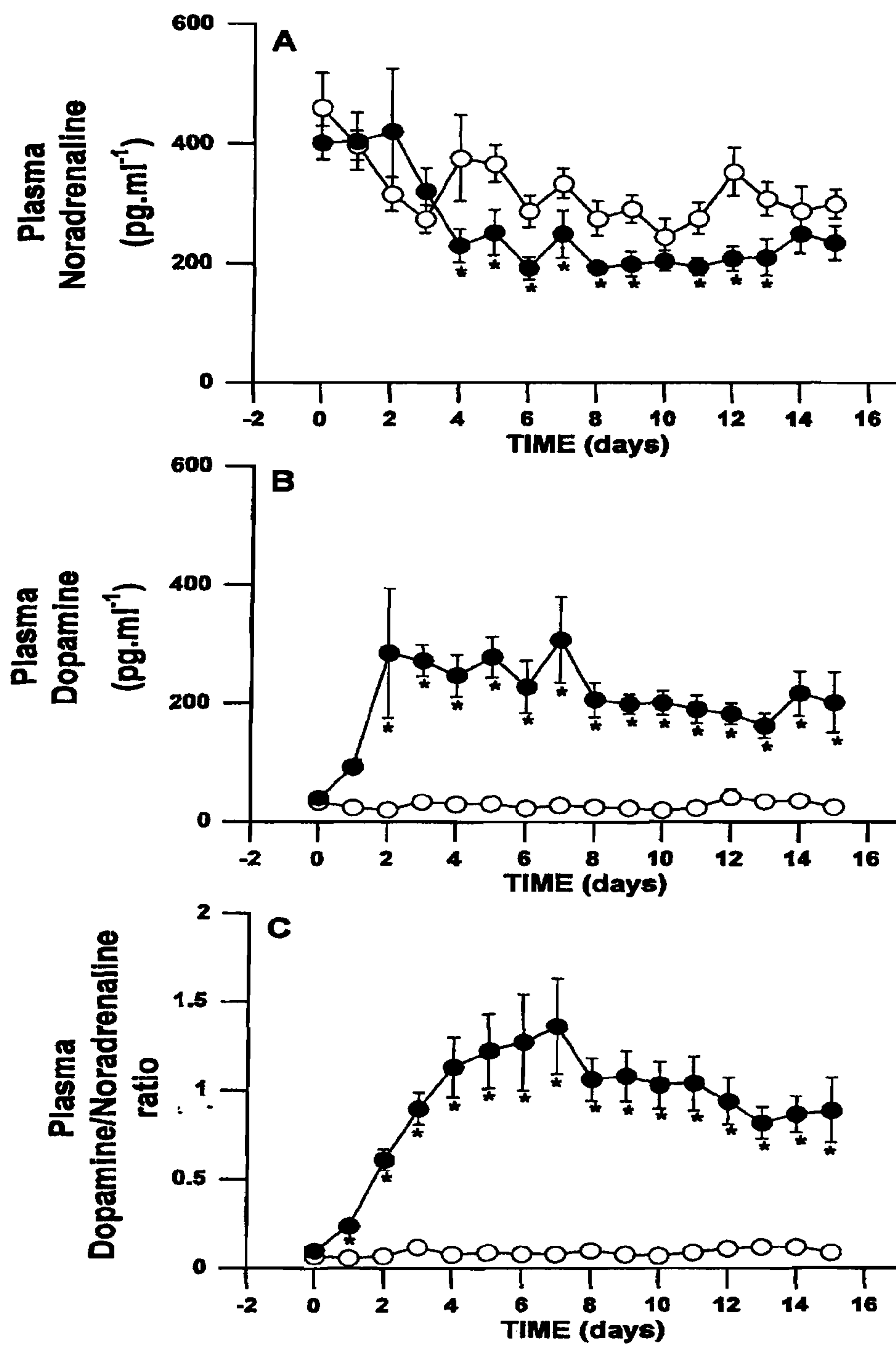


Figure 7

TREATMENT	TISSUE	Noradrenaline ($\mu\text{g.g}^{-1}$)	Dopamine ($\mu\text{g.g}^{-1}$)	Dopamine/ noradrenaline ratio
Control (vehicle) Nepicastat R-5-aminomethyl-1- Nepicastat	Mesenteric artery	10.4 A $\forall$ 1.03 6.15 $\forall$ A 0.62* 8.91 A $\forall$ 0.9#	0.25 A $\forall$ 0.02 1.51 A 0.16* 0.67 $\forall$ A 0.09**	0.03 A $\forall$ 0.00 0.25 $\forall$ A 0.01* 0.08 $\forall$ A 0.01**
Control (vehicle) Nepicastat R-5-aminomethyl-1- Nepicastat	Left ventricle	1.30 $\forall$ A 0.06 0.95 $\forall$ A 0.07* 1.16 $\forall$ A 0.08#	0.02 A $\forall$ 0.00 0.14 A $\forall$ 0.01* 0.07 A $\forall$ 0.00**	0.02 A $\forall$ 0.00 0.15 $\forall$ A 0.01* 0.06 $\forall$ A 0.01**
Control (vehicle) Nepicastat R-5-aminomethyl-1- Nepicastat	Cerebral cortex	1.76 $\forall$ A 0.03 0.61 $\forall$ A 0.02* 0.88 $\forall$ A 0.02**	0.14 $\forall$ A 0.01 0.18 A $\forall$ 0.00* 0.18 A $\forall$ 0.00*	0.19 A $\forall$ 0.00 0.29 $\forall$ A 0.01* 0.20 $\forall$ A 0.01*

All data are expressed as mean A s.e.mean, n = 9 per group.

* p < 0.05 vs control, # p < 0.05 vs Nepicastat

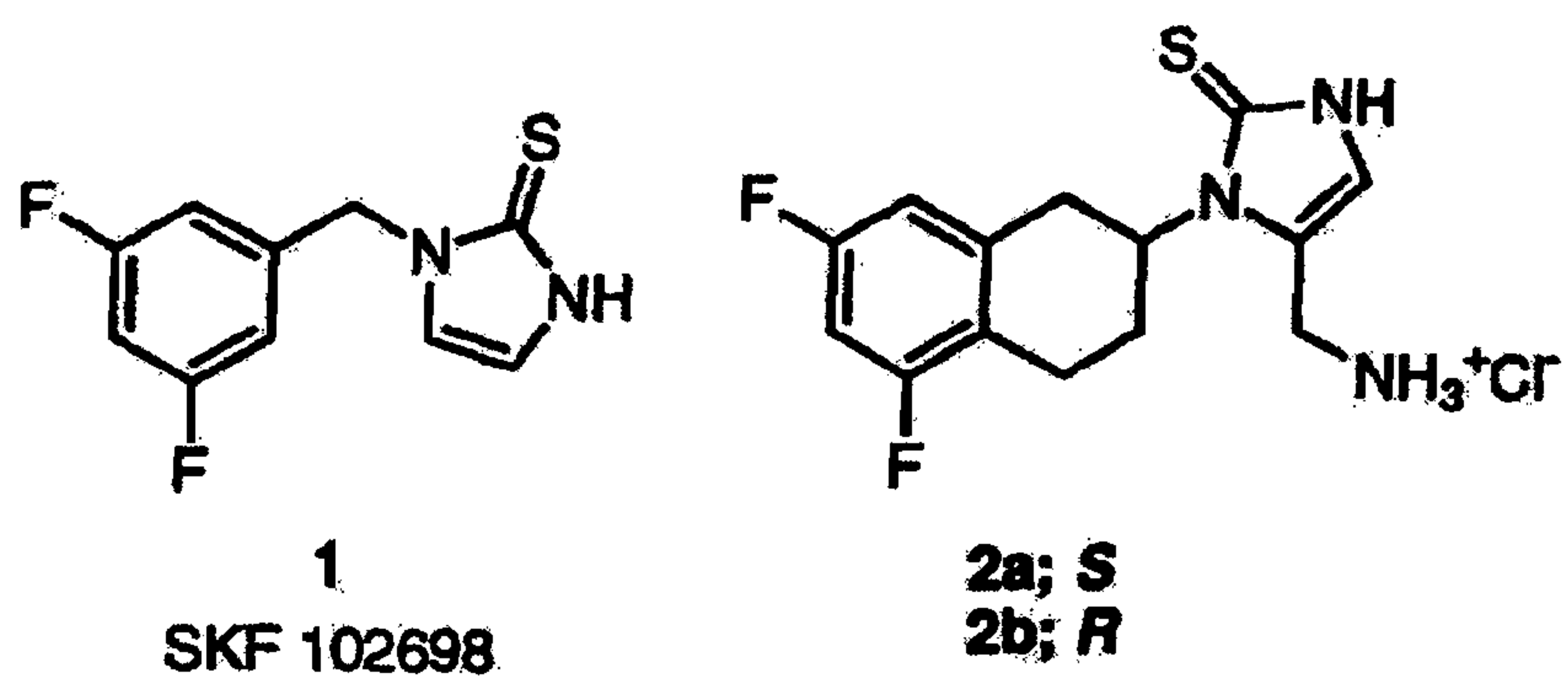
Figure 8

Figure 9

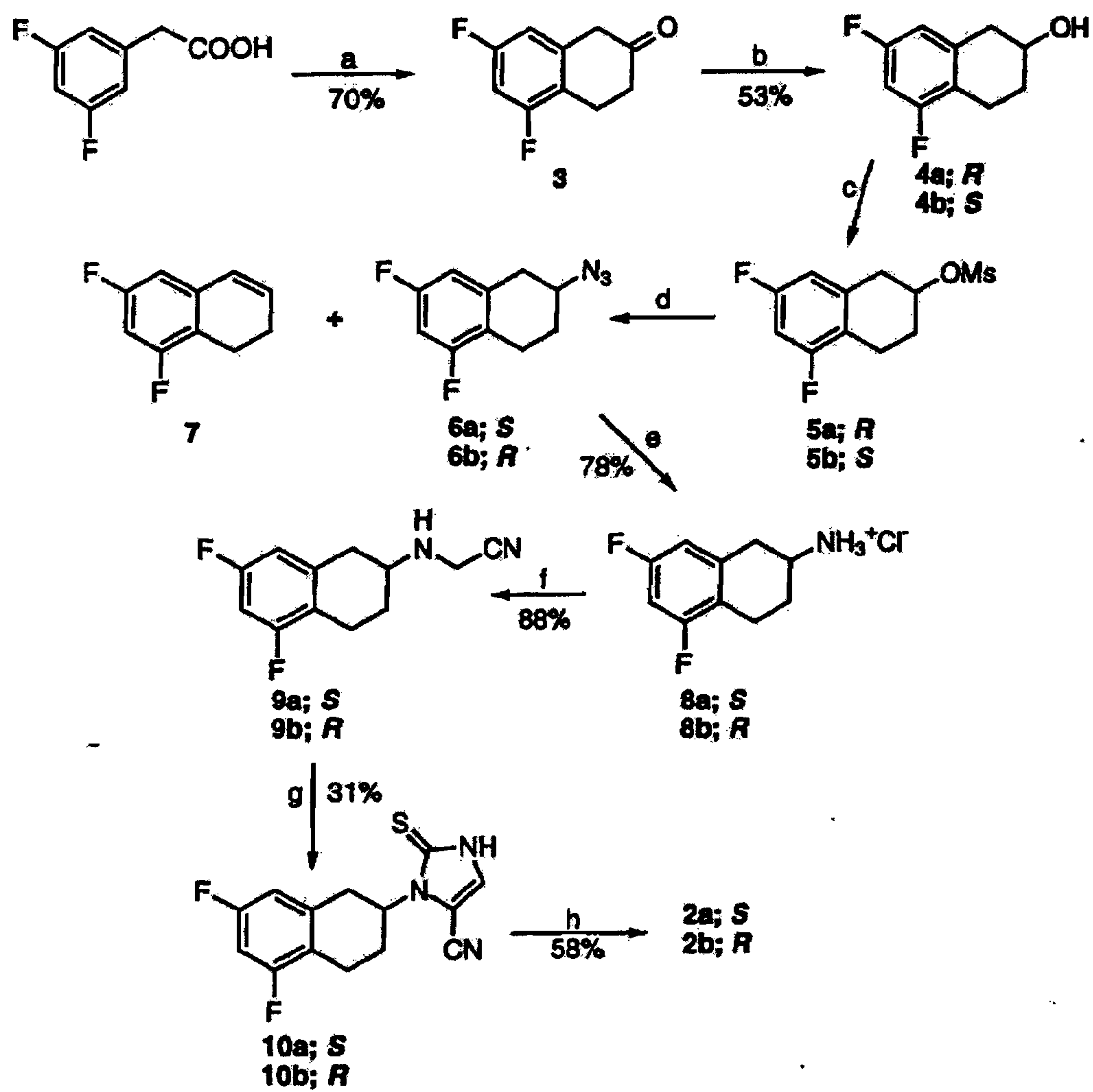
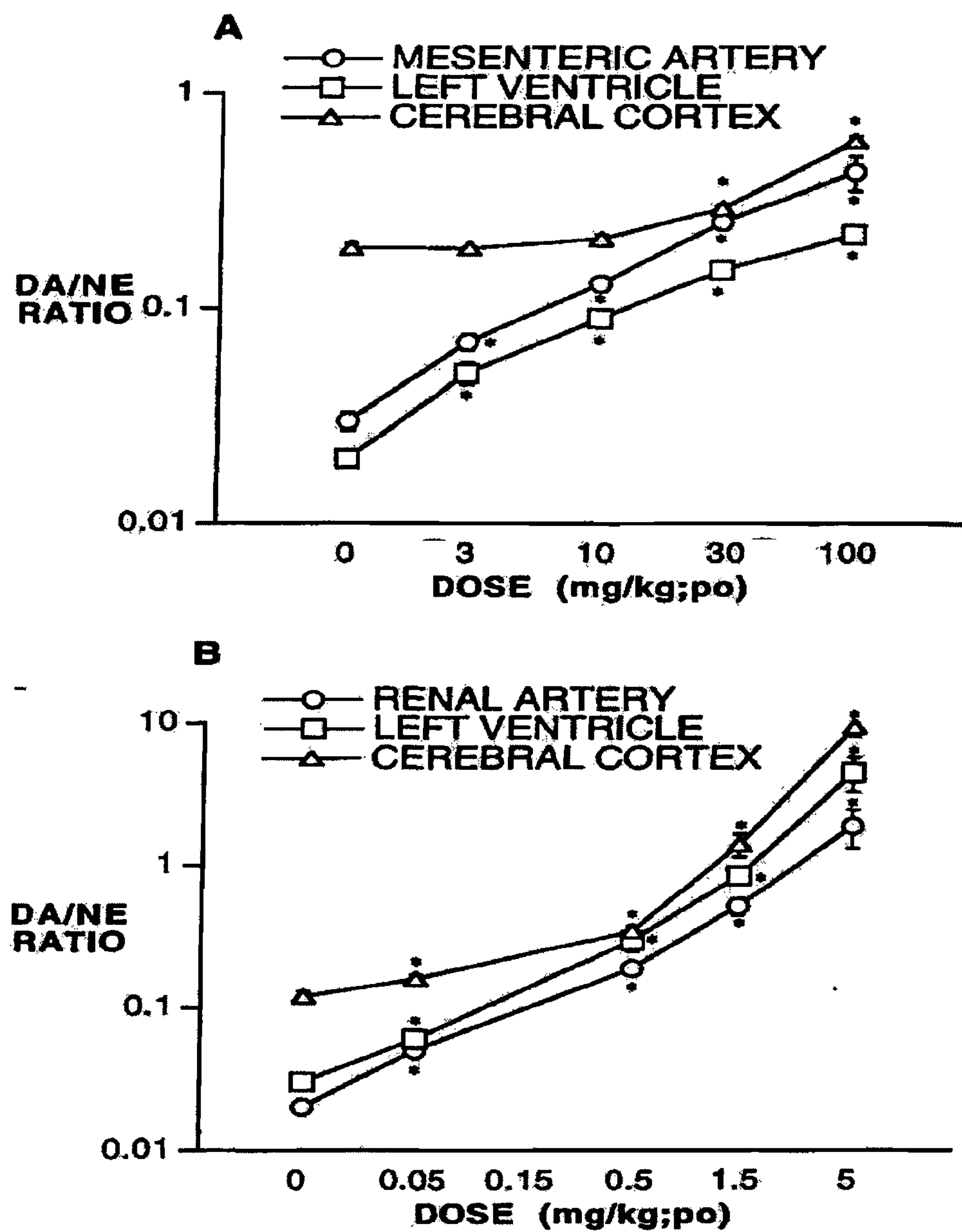
Scheme 1^a

Figure 10

ENZYME OR RECEPTOR	IC ₅₀ (enzyme) or pKi (receptor)
<u>Enzymes</u>	
Dopamine-β-hydroxylase (bovine)	8.5 nM
Dopamine-β-hydroxylase (human)	9 nM
Acetyl CoA Synthetase	< 10 μM
Acyl-CoA: Cholesterol Acyltransferase	< 10 μM
Ca ²⁺ /Calmodulin Protein Kinase II	< 10 μM
Cyclooxygenase-I	< 10 μM
HMG-CoA Reductase	< 10 μM
Neutral Endopeptidase (human)	< 10 μM
Nitric oxide synthase (constitutive)	< 10 μM
Nitric oxide synthase (inducible)	< 10 μM
Phosphodiesterase III (human)	< 10 μM
Phospholipase A ₂	< 10 μM
Protein Kinase (non-selective)	< 10 μM
<u>Receptors</u>	
α _{1A} , α _{1B} adrenoceptors	< 5
α _{2A} , α _{2B} adrenoceptors	< 5
β ₁ , β ₂ adrenoceptors	< 5
M ₁ muscarinic receptors	< 5
D ₁ , D ₂ dopamine receptors	< 5
μ-opioid receptors	< 5
5-HT _{1A} , 5-HT _{2A} , 5-HT _{2C} serotonin receptors	< 5

Figure 11



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Figure 12

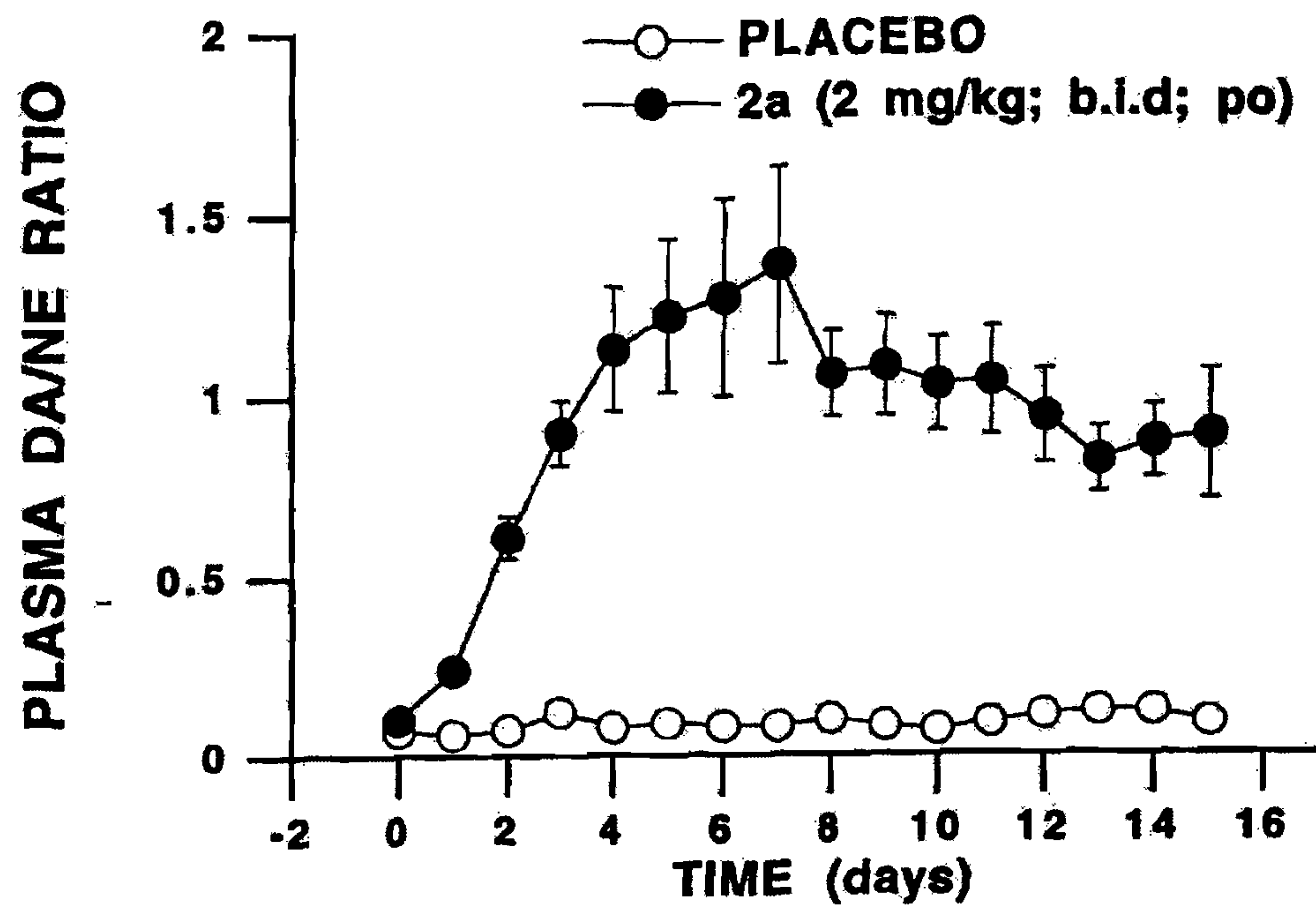


Figure 13

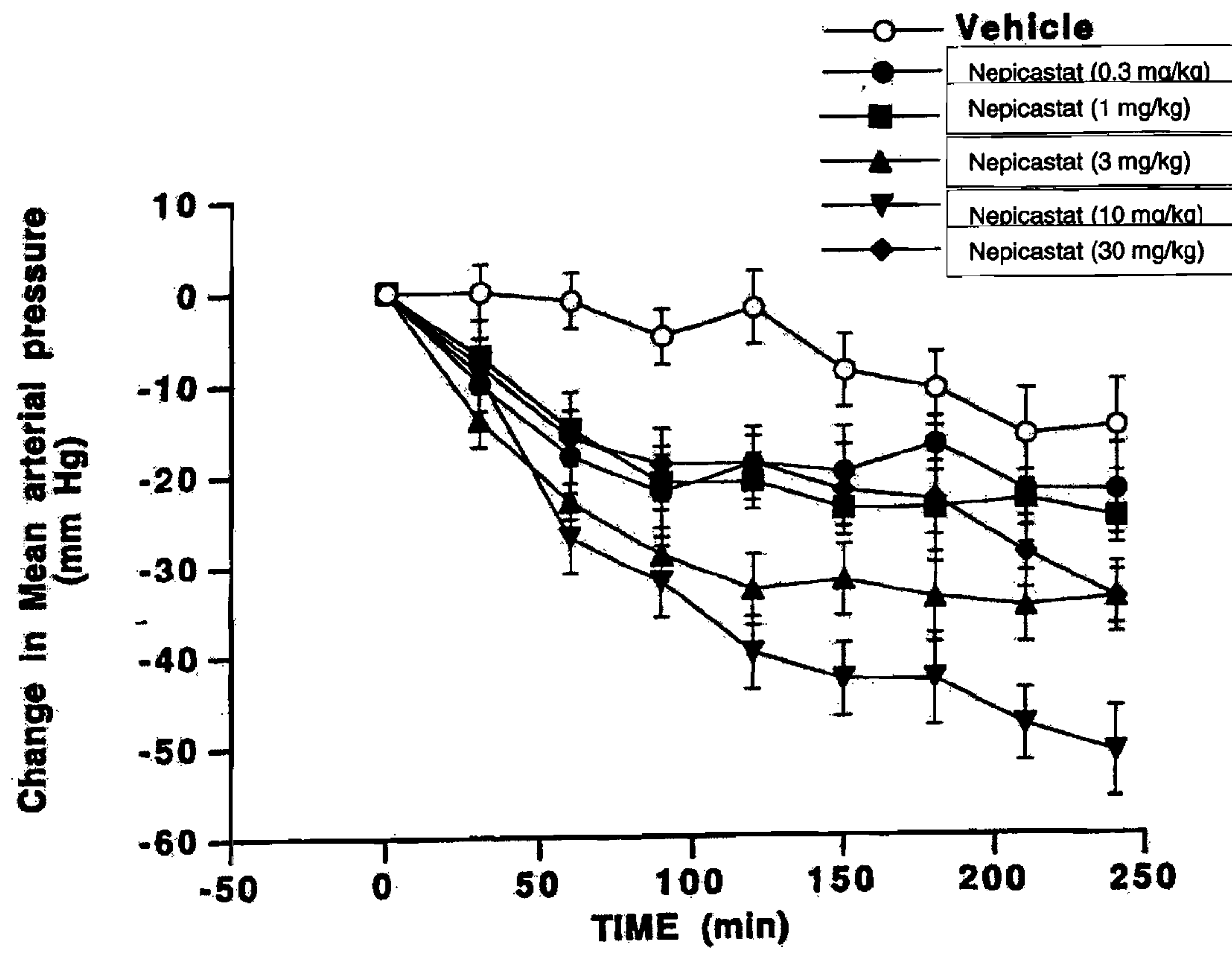


Figure 14

Study Nepicastat

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma			Concentrations (pg/mL) of Dopamine in Plasma		
	Study Day ¹			Study Day		
	Day 0	Week 4	Week 12	Day 0	Week 4	Week 12
101	393	308	422	101	41	84
105	232	188	226	48	43	43
111	119	132	640	54	64	83
1115	262	258	362	54	65	40
117	520	NS ²	NS	82	NS	NS
121	415	404	385	438	91	129
126	249	289	431	71	81	60
130	309	NS	NS	58	NS	NS
1130	225	174	338	41	38	45
135	385	296	414	113	72	373
140	220	280	522	54	62	65
141	557	331	666	76	99	87
145	2083	2295	NS	147	155	NS
152	994	678	946	60	13	42
903	456	273	277	62	50	68
906	454	253	281	68	53	54
910	640	767	653	59	65	59
913	199	220	199	87	91	74
918	236	311	209	363	115	75
924	271	335	251	99	152	679
925	858	756	1666	104	111	115
931 ³	NS	419	590	NS	75	80
935	341	451	580	44	58	66
939	331	251	396	46	39	45
941	436	520	496	79	95	83
946	320	358	854	49	38	60
950	530	198	281	33	29	44
956	217	191	191	22	14	15
958	485	377	283	128	68	43
964	458	441	251	30	25	22

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² NS denotes No Sample

³ Patient 931 was originally Patient 115. Patient was reassigned, as catheterization procedure was unsuccessful

Figure 15**Study Nepicastat**

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma			Concentrations (pg/mL) of Dopamine in Plasma		
	Study Day ¹			Study Day		
	Day 0	Week 4	Week 12	Day 0	Week 4	Week 12
102	174	202	393	51	109	102
108	NS ²	482	53	NS	90	68
110	293	649	624	58	86	62
116	618	673	353	71	102	64
119	65	612	138	45	1574	73
122	190	379	121	58	71	71
128 ³	495	NS	NS	103	NS	NS
1128	149	132	344	64	61	54
131	160	NS	NS	44	NS	NS
1131	293	603	794	26	42	40
136	280	280	NS	66	70	NS
138	329	306	921	70	73	136
143	228	231	467	67	79	76
147	419	316	255	79	79	66

- ¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose
- ² NS denotes No Sample
- ³ Patient 128 was reassigned as Patient 937 after the baseline sample was drawn as catheterization procedure was unsuccessful. The values in this table are also included in Table 3 for Patient 937 Day 0

Figure 16

Study Nepicastat

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma			Concentrations (pg/mL) of Dopamine in Plasma		
	Study Day ¹			Study Day		
	Day 0	Week 4	Week 12	Day 0	Week 4	Week 12
104	303	164	238	120	99	90
107	403	NS ²	NS	84	NS	NS
1107	397	429	NS	59	56	NS
109	397	267	263	53	67	61
114	182	183	504	51	55	82
118	625	489	659	74	82	69
124	180	195	369	27	20	42
127	360	574	306	65	96	43
129	167	154	399	56	69	75
133	807	NS	1812	103	NS	117
137	340	269	411	61	64	92
148	317	380	NS	54	62	NS
149	172	156	338	47	68	86
902	221	190	211	72	72	93
905	768	365	403	86	75	70
909	197	147	289	81	42	51
916	267	274	407	63	65	88
920	168	164	185	110	299	192
921	320	337	297	112	86	87
927	410	NS	NS	69	NS	NS
932	240	216	555	53	71	108
933	354	394	293	68	80	64
937 ³	495	887	303	103	240	99
943	328	299	207	40	45	42
945	403	NS	NS	31	NS	NS
952	160	283	418	76	71	37
953	258	280	160	41	53	42
960	364	294	NS	36	44	NS
961	384	169	156	47	41	40

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² NS denotes No Sample

³ Patient 937 was originally Patient 128 at baseline visit. Data from Table 2 for Patient 128 are included in this Table for Patient 937

Figure 17**Study Nepicastat**

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma			Concentrations (pg/mL) of Dopamine in Plasma		
	Study Day ¹			Study Day		
	Day 0	Week 4	Week 12	Day 0	Week 4	Week 12
103	266	312	NS ²	53	73	NS
106	NS	565	373	NS	101	57
112	240	267	402	31	66	118
113	267	310	567	53	60	166
120	176	126	497	53	65	90
123	398	200	405	53	57	87
125	202	214	155	77	73	45
132	309	271	435	43	57	103
134	320	NS	NS	182	NS	NS
139	211	196	194	53	58	86
142	548	36 ³	751	77	36 ²	99
146	996	855	37	136	514	43
150	187	129	238	32	33	50
153	137	143	256	18	31	29

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² NS denotes No Sample

³ Hemolyzed sample – result removed from further calculations

Figure 18**Study Nepicastat**

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma			Concentrations (pg/mL) of Dopamine in Plasma		
	Study Day ¹			Study Day		
	Day 0	Week 4	Week 12	Day 0	Week 4	Week 12
904	411	361	365	70	149	87
907	719	559	NS ²	87	106	NS
911	410	363	347	100	116	75
915	399	571	227	63	68	74
917	NS	313	NS	NS	76	NS
922	245	242	476	48	107	201
926	594	415	276	72	93	63
929	331	395	208	62	81	82
934	348	NS	NS	76	NS	NS
940	277	296	279	50	80	88
942	655	113	589	78	75	124
948	1199	1192	760	134	162	118
951	230	292	78	73	65	40
955	363	399	362	53	100	68
959	227	174	NS	48	59	NS
963	286	243	231	25	38	36
965	559	330	328	48	77	68

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² NS denotes No Sample

Figure 19

Study Nepicastat

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma			Concentrations (pg/mL) of Dopamine in Plasma		
	Study Day ¹			Study Day		
	Day 0	Week 4	Week 12	Day 0	Week 4	Week 12
901	237	259	171	90	60	67
908	1182	575	531	116	119	147
912	622	331	410	62	76	70
914	NS ²	242	187	NS	86	58
923	583	NS	NS	94	NS	NS
928	303	NS	NS	78	NS	NS
930	202	189	189	47	68	84
936	1174	744	NS	74	237	NS
938	316	169	363	44	52	85
944	400	454	342	294	115	173
947	101	377	626	75	105	132
949	293	327	527	101	137	126
954	257	486	554	62	113	133
957	209	205	192	24	35	42
962	288	NS	NS	61	NS	NS

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² NS denotes No Sample

Figure 20
Study Nepicastat

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma													
	Day 0 ¹							Week 4						
	Art1 ²	Art2	Art3	Mean Art	CS ³ 1	CS2	CS3	Mean Art	CS1	CS2	CS3	Mean CS	Mean CS	CS
101	173	234	162	190	227	177	221	208	404	480	426	437	523	429
105	229	289	318	272	352	416	342	370	297	288	264	283	389	415
111	180	204	211	198	202	232	217	217	225	252	196	224	223	254
115 ⁴	NS ⁵	413	164	289	NS	NS	NS	NS	NS	NS	NS	NC ⁶	NS	NS
1115	327	381	382	363	569	595	516	560	336	284	308	309	470	540
117	650	684	577	637	1059	981	967	1002	NS	NS	NS	NC	NS	NS
121	405	420	444	423	796	888	783	822	505	505	506	505	733	839
126	658	769	734	720	1765	1518	1704	1662	599	577	555	577	1332	1627
130	353	332	388	358	648	631	594	624	NS	NS	NS	NC	NS	NS
1130	382	436	461	426	298	265	184	249	367	331	378	359	118	139
135	831	824	783	813	958	749	738	815	352	408	382	381	370	317
140	255	316	326	299	507	529	561	532	225	238	258	240	299	320
141	443	528	500	490	708	854	713	758	252	302	278	277	316	386
145	2182	2064	2186	2144	2941	3036	2916	2964	1971	2181	2279	2144	2204	1860
152	81 ⁷	768	903	836	10 ⁸	821	1300	1061	594	667	496	586	518	588

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² Art denotes Arterial

³ CS denotes Coronary Sinus

⁴ Patient 115 was reassigned as Patient 931 after baseline visit, as catheterization procedure was unsuccessful

⁵ NS denotes No Sample

⁶ NC denotes Not Calculated

⁷ Outlier value removed from further calculations

⁸ Hemolyzed sample – result removed from further calculations

Figure 21
Study Nepicastat

Patient Number	Concentrations (pg/mL) of Dopamine in Plasma									
	Day 0 ¹					Week 4				
	Art ²	Art ²	Art ³	Mean Art	Mean CS	Art ²	Art ²	Art ³	Mean Art	Mean CS
101	40	53	51	48	51	52	61	55	58	54
105	29	35	43	36	40	42	37	40	40	44
111	61	62	66	63	76	67	64	69	50	73
115 ⁵	NS	92	48	70	NS	NS	NS	NS	NC ⁶	NC
1115	40	44	44	43	71	64	59	85	39	42
117	53	53	68	58	90	85	53	69	NS	NC
121	83	85	68	79	119	106	123	116	129	113
126	72	79	69	73	92	84	85	87	72	75
130	54	67	102	74	67	84	59	63	NS	NC
1130	51	57	53	54	42	39	33	38	90	58
135	69	76	108	84	79	89	62	77	60	51
140	39	42	44	42	38	41	40	40	30	38
141	59	64	49	57	88	130	57	92	47	55
145	103	94	98	98	83	85	93	87	93	109
152	35	49	67	50	7	60	94	77	93	62

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² Art denotes Arterial

³ CS denotes Coronary Sinus

⁴ NS denotes No Sample

⁵ Patient 115 was reassigned as Patient 931 after baseline visit, as catheterization procedure was unsuccessful

⁶ NC denotes Not Calculated

⁷ Hemolyzed sample – result removed from further calculations

Figure 22

Study Nepicastat

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma															
	Day 0 ¹								Week 4							
	Art1 ²	Art2	Art3	Mean Art	CS ³ 1	CS2	CS3	Mean CS	Art1	Art2	Art3	Mean Art	CS1	CS2	CS3	Mean CS
102	270	240	190	233	498	517	457	491	411	323	370	368	521	558	516	532
108	630	475	535	547	1318 ⁴	909	926	918	530	428	426	461	563	688	702	651
110	262	318	269	283	300	342	361	334	500	533	543	525	404	364	378	382
116	341	369	410	373	527	519	472	506	396	533	487	472	461	430	434	442
119	258	228	221	236	177	205	253	212	284	285	297	289	196	324	355	292
122	174	195	237	202	312	410	288	337	420	455	457	444	362	491	370	408
128 ⁵	561	492	512	522	NS ⁶	NS	NS	NC ⁷	NS	NS	NS	NC	NS	NS	NS	NC
1128	327	261	446	345	537	465	651	551	220	191	176	196	237	304	471	337
131	128	188	195	170	186	195	256	212	NS	NS	NS	NC	NS	NS	NS	NC
1131	327	432	384	381	503	525	620	549	490	361	489	447	687	634	445	589
136	605	702	735	681	827	994	1119	980	592	479	498	523	834	736	719	763
138	229	292	310	277	275	285	278	279	275	270	301	282	264	180	238	227
143	204	247	239	230	391	416	357	388	154	254	232	213	591	756	413	587
147	423	455	472	450	632	800	1411 ⁴	716	370	435	416	407	764	719	751	745

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² Art denotes Arterial

³ CS denotes Coronary Sinus

⁴ Outlier value removed from further calculations

⁵ Patient 128 was reassigned as Patient 937 after the baseline sample was drawn as catheterization procedure was unsuccessful

⁶ NS denotes No Sample

⁷ NC denotes Not Calculated

Figure 23

Study Nepicastat

Patient Number	Concentrations (pg/mL) of Dopamine in Plasma															
	Day 0 ¹								Week 4							
	Art1 ²	Art2	Art3	Mean Art	CS1 ³	CS2	CS3	Mean CS	Art1	Art2	Art3	Mean Art	CS1	CS2	CS3	Mean CS
102	71	74	74	73	82	82	86	83	103	102	115	107	178	136	178	164
108	119	123	112	118	91	104	96	97	102	96	102	100	107	110	111	109
110	27	31	28	29	32	38	29	33	43	52	47	47	49	35	33	39
116	52	46	47	48	41	42	43	42	77	62	79	73	46	60	50	52
119	77	80	77	78	64	62	65	64	81	86	70	79	62	76	80	73
122	54	61	63	59	69	66	55	63	86	97	102	95	87	99	74	87
128 ⁴	64	64	50	59	NS ⁵	NS	NS	NC ⁶	NS	NS	NS	NC	NS	NS	NS	NC
1128	82	63	74	73	52	50	83	62	109	64	58	77	69	54	122	82
131	31	40	43	38	41	38	40	40	NS	NS	NS	NC	NS	NS	NS	NC
1131	43	55	61	53	60	58	59	59	44	34	38	39	48	51	44	48
136	59	110	87	85	80	116	149	115	93	92	105	97	100	94	96	97
138	28	37	44	36	40	41	34	38	795 ⁷	88	48	68	1272 ⁷	95	181	138
143	37	43	48	43	36	49	42	42	46	38	53	46	77	81	42	67
147	61	62	65	63	66	64	500 ⁷	65	69	96	79	81	91	86	171	116

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² Art denotes Arterial

³ CS denotes Coronary Sinus

⁴ Patient 128 was reassigned as Patient 937 after the baseline sample was drawn as catheterization procedure was unsuccessful

⁵ NS denotes No Sample

⁶ NC denotes Not Calculated

⁷ Outlier value removed from further calculations

Figure 24

Study Nepicastat

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma															
	Day 0 ¹							Week 4								
	Art1 ²	Art2	Art3	Mean Art	CS1 ³	CS2	CS3	Mean CS	Art1	Art2	Art3	Mean Art	CS1	CS2	CS3	Mean CS
104	277	290	272	280	378	534	716	643	150	152	128	143	167	157	178	167
107	495	555	402	484	374	449	478	434	NS ⁴	NS	NS	NC ⁵	NS	NS	NS	NC
1107	373	291	305	323	454	783	589	609	186 ⁶	395	352	374	696	519	719	645
109	353	260	298	303	616	796	543	652	263	332	242	279	293	428	379	366
114	231	256	247	245	316	198	200	238	213	248	256	239	294	224	264	261
118	488	486	457	470	812	980	966	919	348	348	356	351	284 ⁶	778	782	780
124	510	390	485	462	756	518	671	648	506	367	470	448	665	598	602	621
127	1189	1033	1019	1080	2314	1649	2052	2005	1227	1153	1300	1227	1194	1476	1249	1306
129	380	286	307	324	287	249	308	281	316	250	295	287	371	409	345	375
133	1003	766	813	861	679	676	750	701	776	651	868	765	NS	NS	1034	1034
137	415	396	365	392	653	653	658	655	447	483	494	475	898	675	787	787
148	236	271	339	282	299	292	319	303	388	420	457	422	262	349	349	320
149	237	222	311	257	348	311	217	292	206	177	201	195	306	325	308	313

1 All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

2 Art denotes Arterial

3 CS denotes Coronary Sinus

4 NS denotes No Sample

5 NC denotes Not Calculated

6 Outlier value removed from further calculations

Figure 25

Study Nepicastat

Patient	Concentrations (pg/mL) of Dopamine in Plasma																	
	Day 0 ¹								Week 4									
	Number	Art1 ²	Art2	Art3	Mean	CS1 ³	CS2	CS3	Mean	CS	Art1	Art2	Art3	Mean	CS1	CS2	CS3	Mean
104	95	122	106	108	181	78	346 ⁴	130	68	75	62	68	50	125	78	84		
107	62	69	72	68	90	87	45	74	NS ⁵	NS	NS	NC ⁶	NS	NS	NS	NC		
1107	89	59	57	68	55	56	60	57	89	68	83	80	120	103	118	114		
109	51	57	70	59	54	52	43	50	65	72	65	67	69	74	74	72		
114	48	52	58	53	49	46	50	48	59	73	97	76	64	65	71	67		
118	117	52	68	79	58	63	87	69	58	67	56	60	48 ⁴	103	84	94		
124	67	46	64	59	55	55	66	59	72	69	104	82	90	65	69	75		
127	108	106	113	109	139	95	124	119	151	153	175	160	168	229	190	196		
129	41	46	55	47	47	49	46	47	69	69	73	70	89	90	67	82		
133	110	71	97	93	66	68	78	71	165	160	152	159	NS	NS	133	133		
137	41	41	52	45	41	39	39	40	88	92	80	87	117	83	103	101		
148	47	51	50	49	49	45	47	47	71	67	69	69	58	55	72	61		
149	32	43	45	40	47	40	40	42	75	56	98	76	80	90	92	87		

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² Art denotes Arterial

³ CS denotes Coronary Sinus

⁴ Outlier value removed from further calculations

⁵ NS denotes No Sample

⁶ NC denotes Not Calculated

Figure 26

Study Nepicastat

Patient Number	Concentrations (pg/mL) of Norepinephrine in Plasma															
	Day 0 ¹								Week 4							
	Art1 ²	Art2	Art3	Mean Art	CS1 ³	CS2	CS3	Mean CS	Art1	Art2	Art3	Mean Art	CS1	CS2	CS3	Mean CS
103	303	319	347	323	460	248	258	322	261	263	321	282	431	411	605	482
106	409	443	341	398	350	482	509	447	431	538	362	444	774	742	667	728
112	171	178	194	181	190	173	245	203	225	218	175	206	237	254	246	246
113	509	570	534	538	563	646	415	541	337	261	216	271	290	328	382	333
120	340	385	557	427	359	438	527	441	115	112	137	121	123	139	137	133
123	332	341	411	361	492	465	536	498	230	209	180	206	261	273	345	293
125	371	403	505	426	644	635	559	613	217	205	228	217	370	399	331	367
132	618	590	618	609	1310	1422	1288	1339	428	517	454	466	440 ⁴	779	924	852
134	186	164	209	186	274	231	264	256	639	634	604	626	619	693	714	675
139	339	349	327	338	551	459	507	506	186	153	216	185	224	271	460	318
142	495	412	613	507	975	797	935	902	489	516	582	529	998	1059	1141	1066
146	376	506	545	476	609	772	773	718	402	442	394	413	492	606	696	598
150	343	393	359	365	756	790	793	780	217	285	264	255	469	533	447	483
153	0 ⁵	0 ⁵	3 ⁵	NC ⁶	11 ⁵	6 ⁵	12 ⁵	NC	232	251	301	261	228	275	260	254

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² Art denotes Arterial

³ CS denotes Coronary Sinus

⁴ Outlier value removed from further calculations

⁵ Hemolyzed sample – result removed from further calculations

⁶ NC denotes Not Calculated

Figure 27

Study Nopicastat														
Concentrations (pg/mL) of Dopamine in Plasma														
Patient Number	Day 0 ¹					Week 4								
	Art1 ²	Art2	Art3	Mean Art	CS	Art1	Art2	Art3	Mean Art	CS1	CS2	CS3	Mean CS	
103	71	115	81	89	69	107	107	120	111	128	124	163	138	
106	76	76	93	82	52	103	114	84	100	134	119	134	129	
112	35	40	38	38	28	75	68	66	70	71	81	84	79	
113	56	56	58	57	50	89	49	49	82	57	51	74	61	
120	45	57	62	55	43	41	45	52	46	53	50	58	54	
123	66	64	80	70	76	55	64	54	58	55	60	68	61	
125	59	78	80	72	81	66	62	71	66	103	100	80	94	
132	59	64	64	62	87	92	85	82	86	80 ⁴	155	151	153	
134	46	40	48	45	31 ⁴	108	97	99	101	100	111	106	106	
139	63	68	69	67	70	89	75	72	79	76	87	124	96	
142	35	36	51	41	37	96	113	100	103	113	135	145	131	
146	45	45	52	47	42	87	108	85	93	88	115	108	104	
150	44	41	49	45	54	47	80	73	67	66	88	65	73	
153	4 ⁵	5 ⁵	4 ⁵	NC ⁶	11 ⁵	55	60	64	60	55	56	58	56	

¹ All samples were collected at a nominal time of 2 hr post-dose. Samples collected on Day 0 were collected at a clock time equivalent to 2 hr post-dose

² Art denotes Arterial

³ CS denotes Coronary Sinus

⁴ Outlier value removed from further calculations

⁵ Hemolyzed sample – result removed from further calculations

⁶ NC denotes Not Calculated

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Figure 28

Patient #	Visit #	Sample	PNE/pg/ml	DA/pg/ml	Comment
104	2	CS-3	-	346	Outlier
1107	6	AO-1	186	-	Outlier
108	2	CS-1	1318	-	Outlier
118	6	CS-1	264	48	Outlier
132	6	CS-1	440	80	Outlier
134	2	CS-1	-	31	Outlier
138	6	CS-1	-	1272	Outlier
138	6	AO-1	-	795	Outlier
142	6	BASELINE	36	36	Hemolyzed
147	2	CS-3	1411	500	Outlier
152	2	CS-1	10	7	Hemolyzed
152	2	AO-1	81	-	Outlier
153	2	ALL TM	ALL	ALL	Hemolyzed

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Figure 29

Parameter	Dose Rate and Sex			
	10 mg/kg (Male)	30 mg/kg (Male)	100 mg/kg (Male)	30 mg/kg (Female)
Plasma				
C _{max} (µg/ml)	1.95	4.74	7.92	7.66
T _{max} (hr)	0.50	0.50	1.00	0.50
T 1/2 (hr)	1.70	2.09	3.88	2.60
AUC (0 - 8 hr)	3.88	13.9	36.1	20.8
AUC (total)	4.04	15.0	54.1	26.6
Brain				
C _{max} (µg/g)	0.987	ND	ND	ND
T _{max} (hr)	2.00	ND	ND	ND
T 1/2 (hr)	3.45	ND	ND	ND
AUC (0 - 8 hr)	5.03	ND	ND	ND
AUC (total)	6.39	ND	ND	ND

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Figure 30

Concentration in Plasma (µg/ml)				
Time (hr)	Individual Values			Mean ± S.D.
0	BQL	BQL	BQL	0 ± 0
0.25	1.59	1.79	1.28	1.55 ± 0.26
0.5	1.77	2.13	1.94	1.95 ± 0.18
1	1.43	1.05	1.24	1.24 ± 0.19
2	0.823	0.767	0.921	0.837 ± 0.078
3	0.468	0.259	0.308	0.345 ± 0.110
4	0.240	0.129	0.169	0.179 ± 0.056
6	0.175	0.111	0.187	0.158 ± 0.041
8	0.0827	0.0415	0.0689	0.0644 ± 0.0210
24	BQL	BQL	BQL	0 ± 0
Cmax (µg/ml)				1.95
Tmax (hr)				0.50
AUC (0 - 8 hr)				3.88
AUC (total)				4.04
β (1/hr)				0.409
T 1/2 (hr)				1.70

Concentrations are expressed as free base.

BQL = Below quantification limit of 0.01 µg/ml.

AUC values calculated by the trapezoidal rule. Units are µg·hr/ml.

β calculated from plasma concentrations between 1 and 8 hr.

Figure 31

Concentration in Plasma (µg/ml)				
Time (hr)	Individual Values			Mean ± S.D.
0	BQL	BQL	BQL	0 ± 0
0.25	3.35	3.41	2.95	3.24 ± 0.25
0.5	3.18	6.75	4.29	4.74 ± 1.83
1	4.47	5.19	2.59	4.08 ± 1.34
2	1.86	3.27	1.85	2.33 ± 0.82
3	2.71	2.16	NS	2.44 ± 0.39
4	1.39	1.08	0.585	1.02 ± 0.41
6	1.00	0.773	0.546	0.774 ± 0.228
8	0.291	0.619	0.203	0.371 ± 0.219
24	BQL	BQL	BQL	0 ± 0
Cmax (µg/ml)				4.74
Tmax (hr)				0.50
AUC (0 - 8 hr)				13.9
AUC (total)				15.0
β (1/hr)				0.332
T 1/2 (hr)				2.09

Concentrations are expressed as free base.

BQL = Below quantification limit of 0.01 µg/ml.

NS = No sample.

AUC values calculated by the trapezoidal rule. Units are µg·hr/ml.

β calculated from plasma concentrations between 1 and 8 hr.

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Figure 32

Time (hr)	Concentration in Plasma (µg/ml)			Mean ± S.D.
	Individual Values			
0	BQL	BQL	BQL	0 ± 0
0.25	1.70	4.45	3.43	3.19 ± 1.39
0.5	3.74	11.3	7.01	7.34 ± 3.77
1	7.80	7.29	8.67	7.92 ± 0.70
2	6.23	7.46	6.44	6.71 ± 0.66
3	4.52	6.52	5.81	5.62 ± 1.01
4	3.83	2.91	5.61	4.11 ± 1.37
6	4.05	2.24	2.78	3.02 ± 0.93
8	1.56	3.38	1.17	2.04 ± 1.18
24	0.0839	0.159	0.144	0.129 ± 0.040
Cmax (µg/ml)				7.92
Tmax (hr)				1.00
AUC (0 - 8 hr)				36.1
AUC (total)				54.1
B (1/hr)				0.179
T 1/2 (hr)				3.88

Concentrations are expressed as free base.

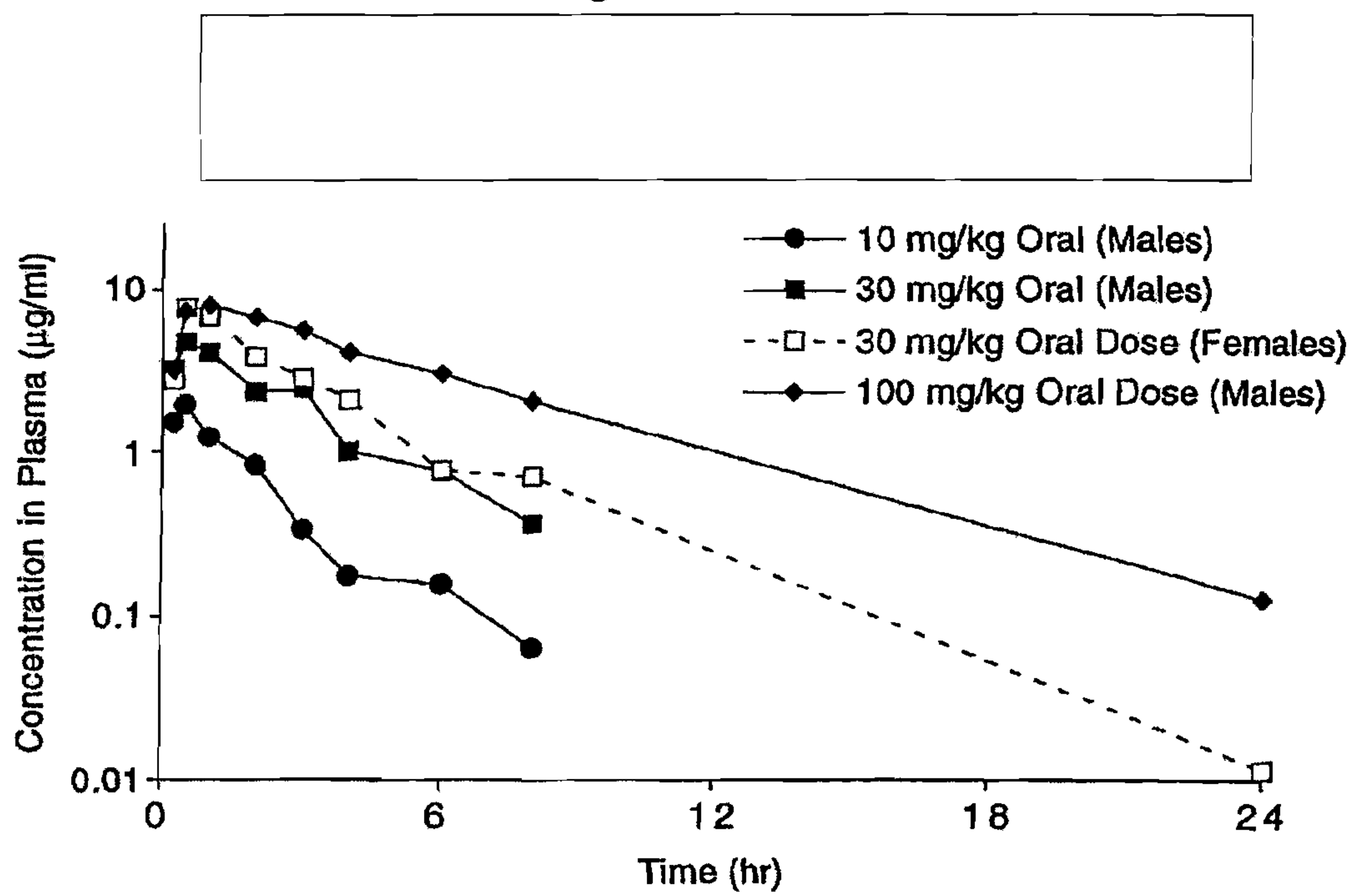
BQL = Below quantification limit of 0.01 µg/ml.

AUC values calculated by the trapezoidal rule. Units are µg•hr/ml.

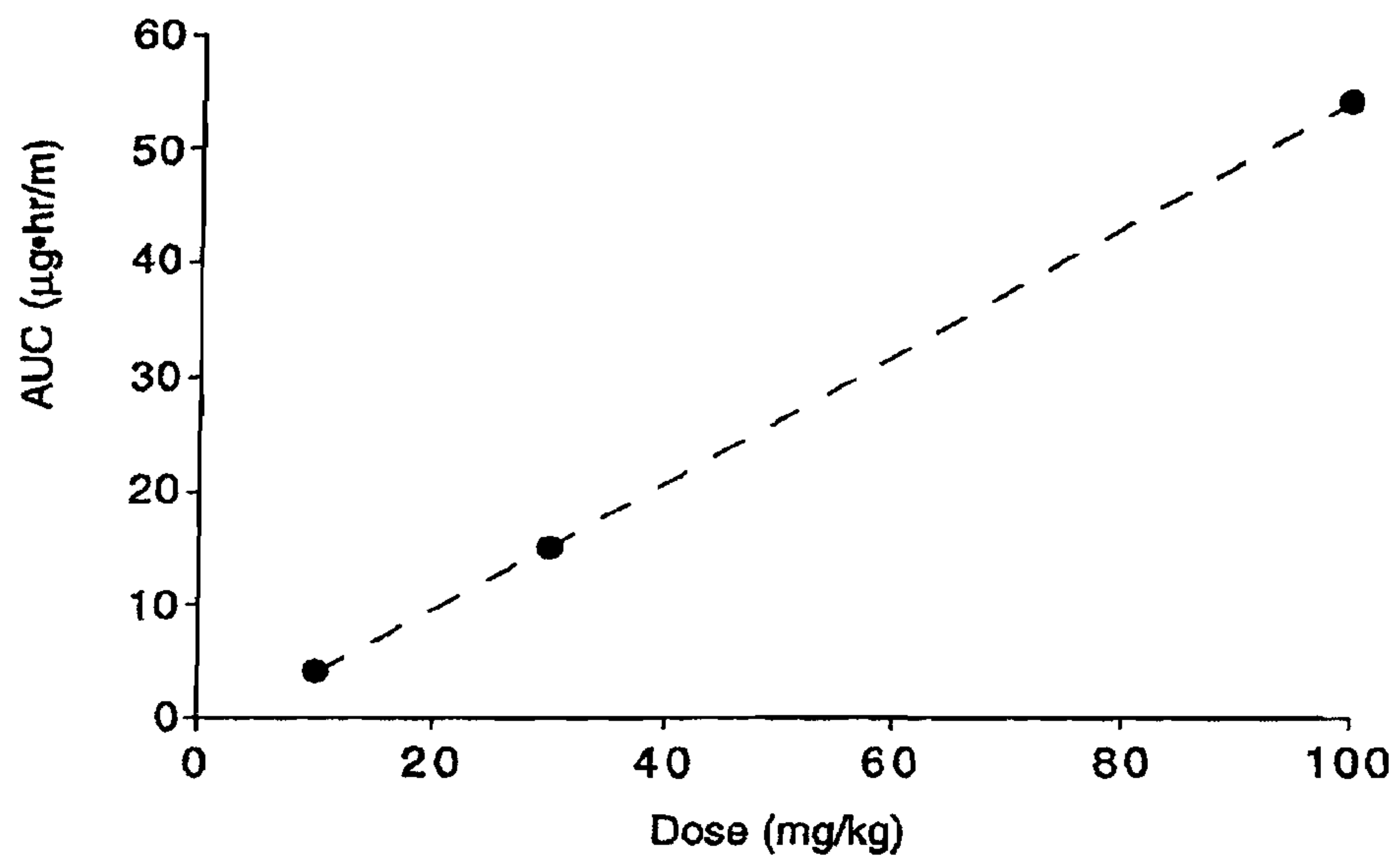
B calculated from plasma concentrations between 1 and 24 hr.

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Figure 33



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Figure 34

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Figure 35

Time (hr)	Concentration in Plasma (µg/ml)			
	Individual Values			Mean ± S.D.
0	BQL	BQL	BQL	0 ± 0
0.25	2.51	2.99	2.81	2.77 ± 0.24
0.5	8.29	6.19	8.51	7.66 ± 1.28
1	7.60	6.43	6.37	6.80 ± 0.69
2	4.59	2.95	3.99	3.84 ± 0.83
3	3.19	2.24	3.11	2.85 ± 0.53
4	2.11	2.56	1.63	2.10 ± 0.46
6	0.687	0.848	0.820	0.785 ± 0.086
8	0.455	0.637	1.05	0.715 ± 0.308
24	0.0131	BQL	0.0218	0.0116 ± 0.0110
C _{max} (µg/ml)				7.66
T _{max} (hr)				0.50
AUC (0 - 8 hr)				20.8
AUC (total)				26.6
β (1/hr)				0.267
T _{1/2} (hr)				2.60

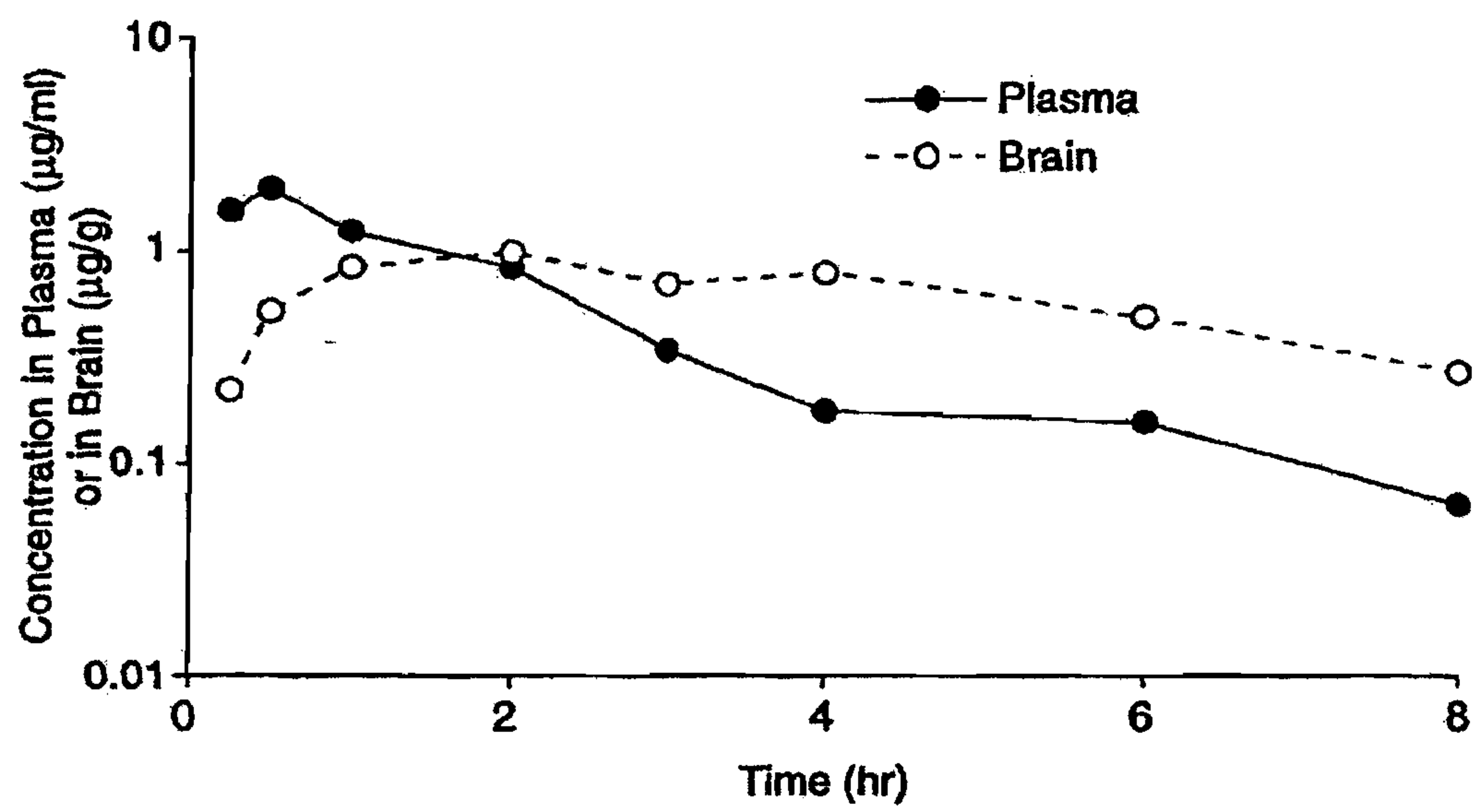
Concentrations are expressed as free base.

BQL = Below quantification limit of 0.01 µg/ml.

AUC values calculated by the trapezoidal rule. Units are µg•hr/ml.

β calculated from plasma concentrations between 1 and 24 hr.

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Figure 36

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Figure 37

Concentration in Brain (μg/g)				
Time (hr)	Individual Values			Mean ± S.D.
0	BQL	BQL	BQL	0 ± 0
0.25	0.210	0.225	0.236	0.224 ± 0.013
0.5	0.565	0.519	0.490	0.524 ± 0.038
1	1.05	0.698	0.783	0.843 ± 0.183
2	1.06	0.735	1.16	0.987 ± 0.224
3	0.800	0.554	0.758	0.704 ± 0.132
4	1.05	0.568	0.766	0.796 ± 0.245
6	0.499	0.477	0.508	0.494 ± 0.016
8	0.309	0.223	0.289	0.274 ± 0.045
24	BQL	BQL	BQL	0 ± 0
Cmax (μg/g)				0.987
Tmax (hr)				2.00
AUC (0 - 8 hr)				5.03
AUC (total)				6.39
B (1/hr)				0.201
T 1/2 (hr)				3.45

Concentrations are expressed as free base.

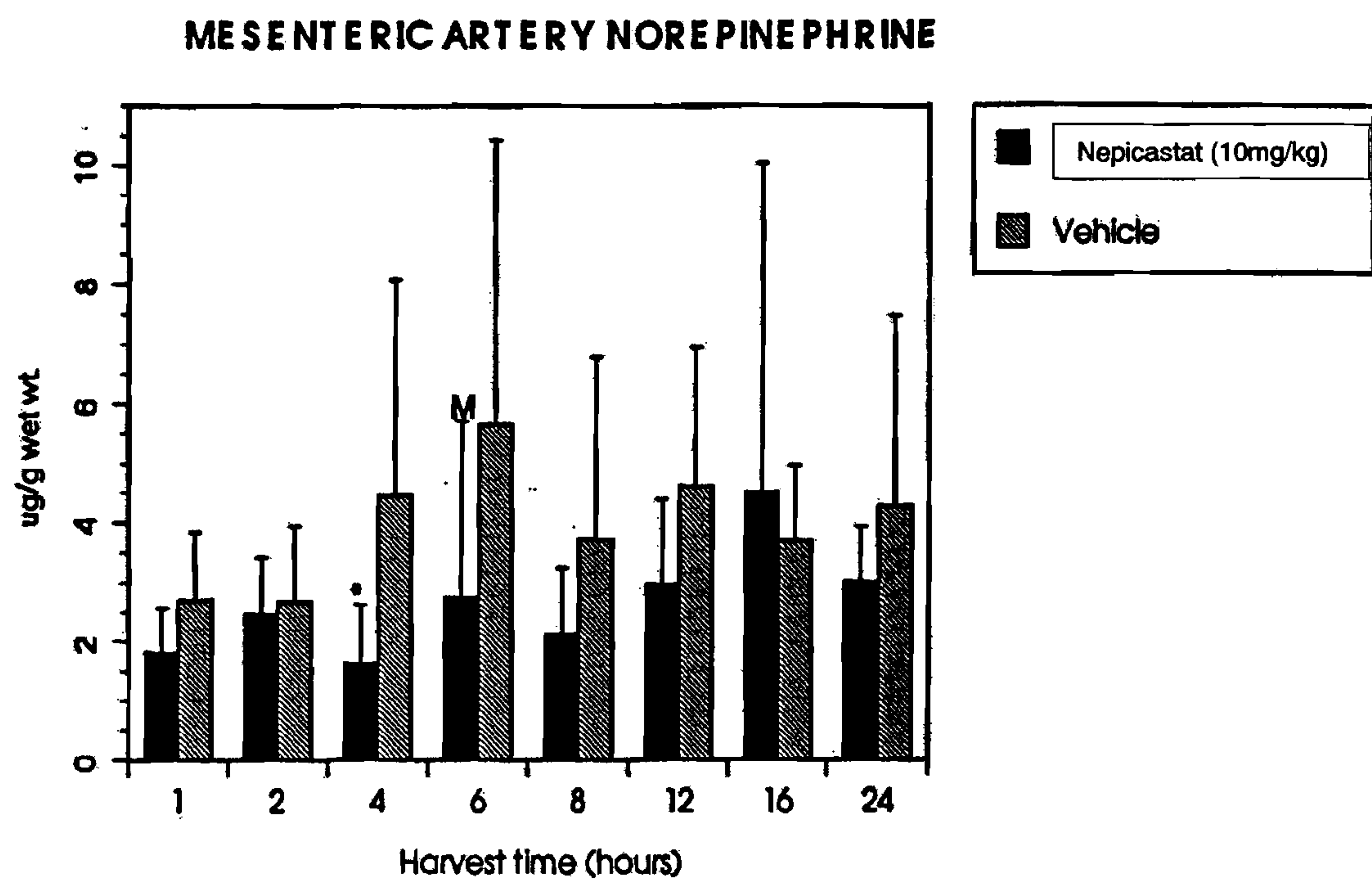
BQL = Below quantification limit of 0.02 $\mu\text{g/g}$.

AUC values calculated by the trapezoidal rule. Units are $\mu\text{g}\cdot\text{hr/g}$.

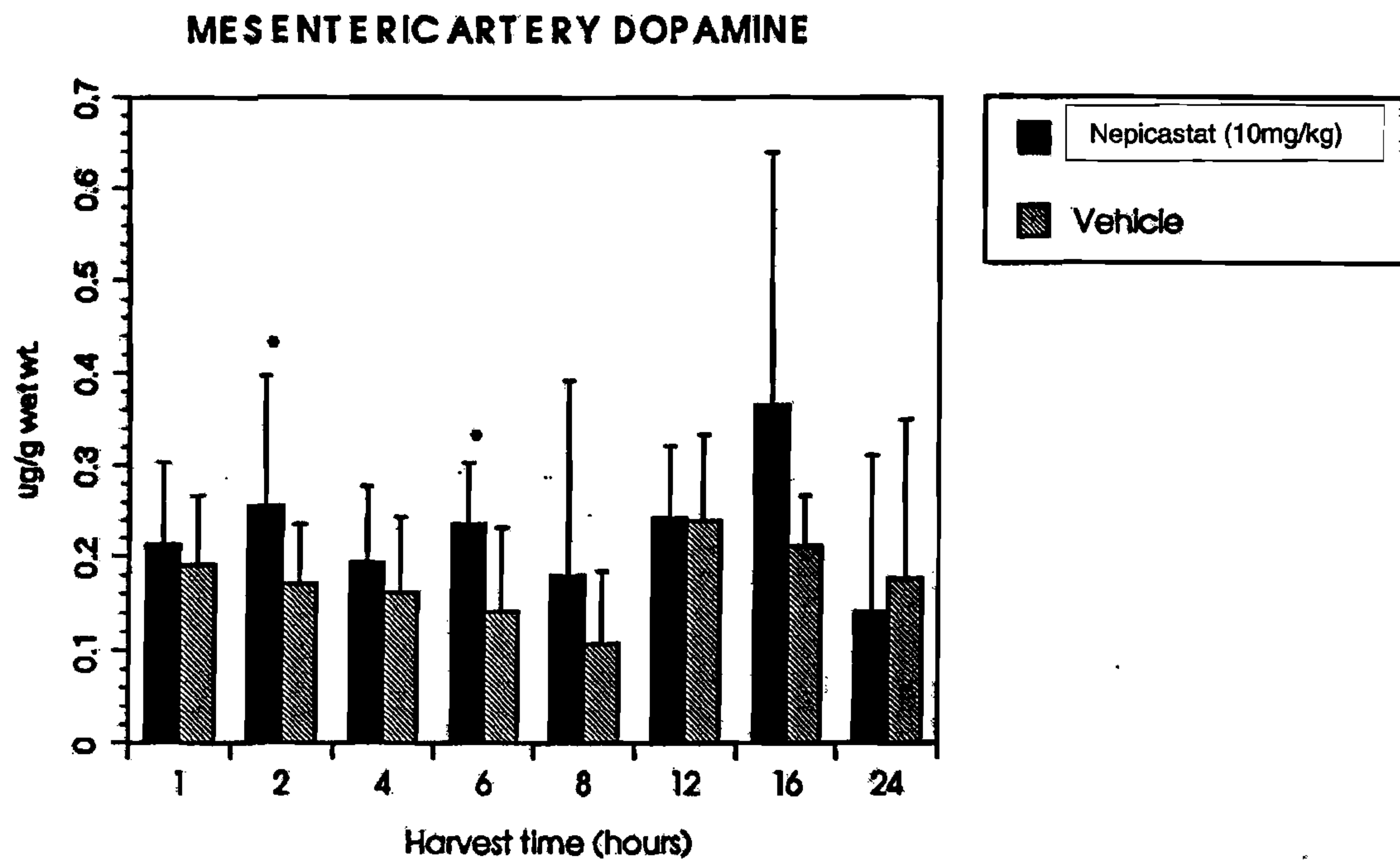
β calculated from brain concentrations between 2 and 8 hr.

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Figure 38

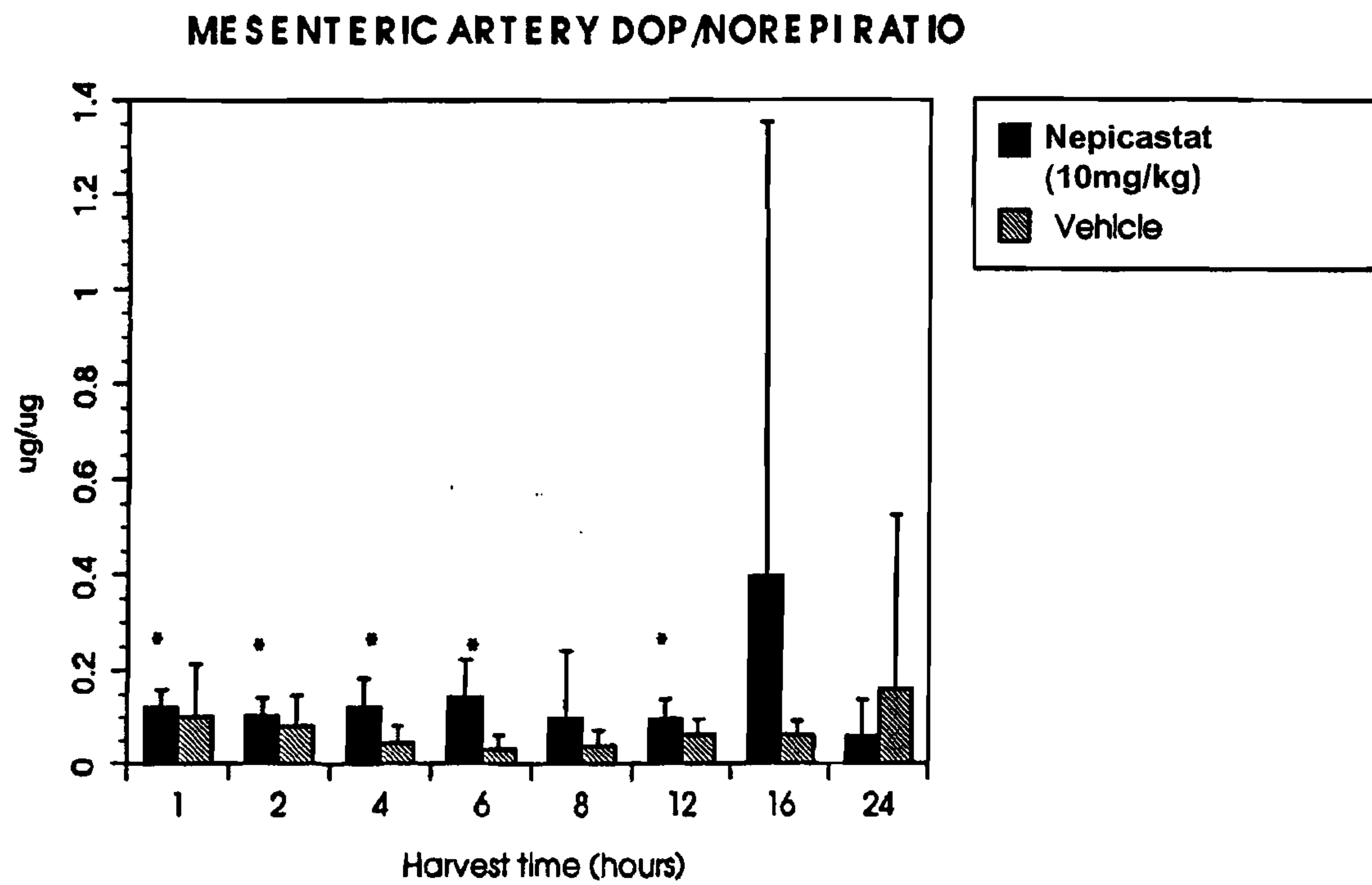


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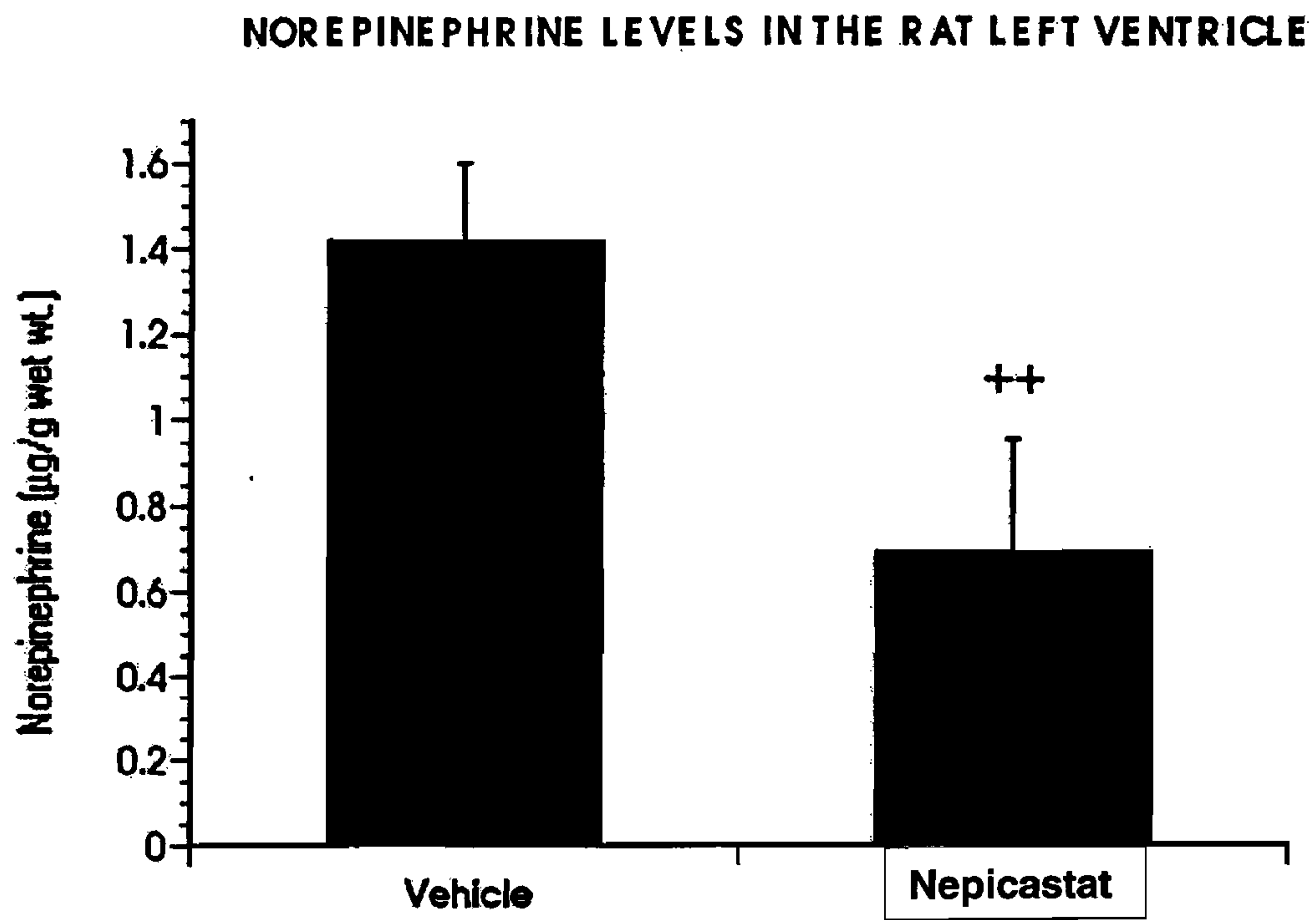
Figure 39

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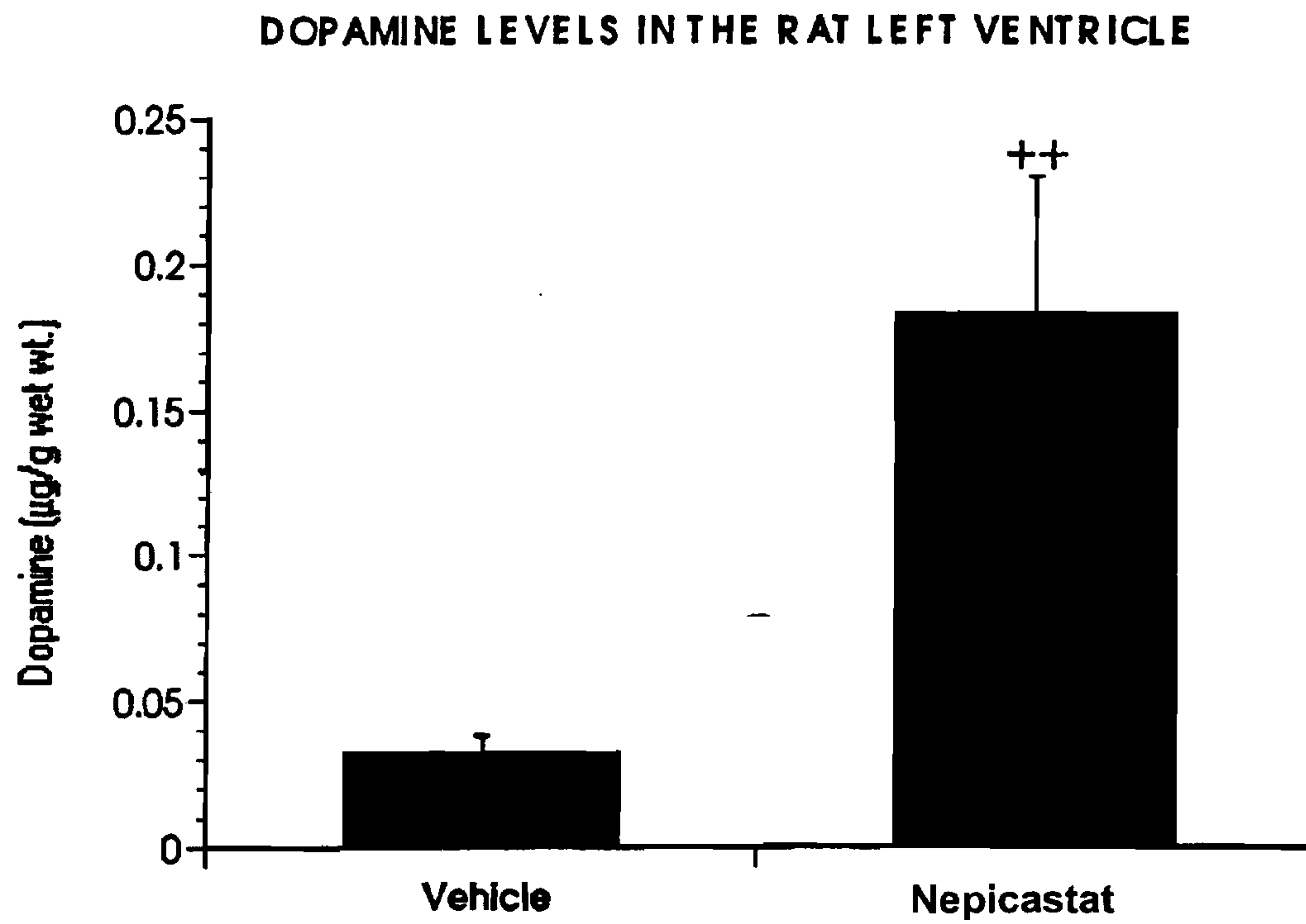
Figure 40



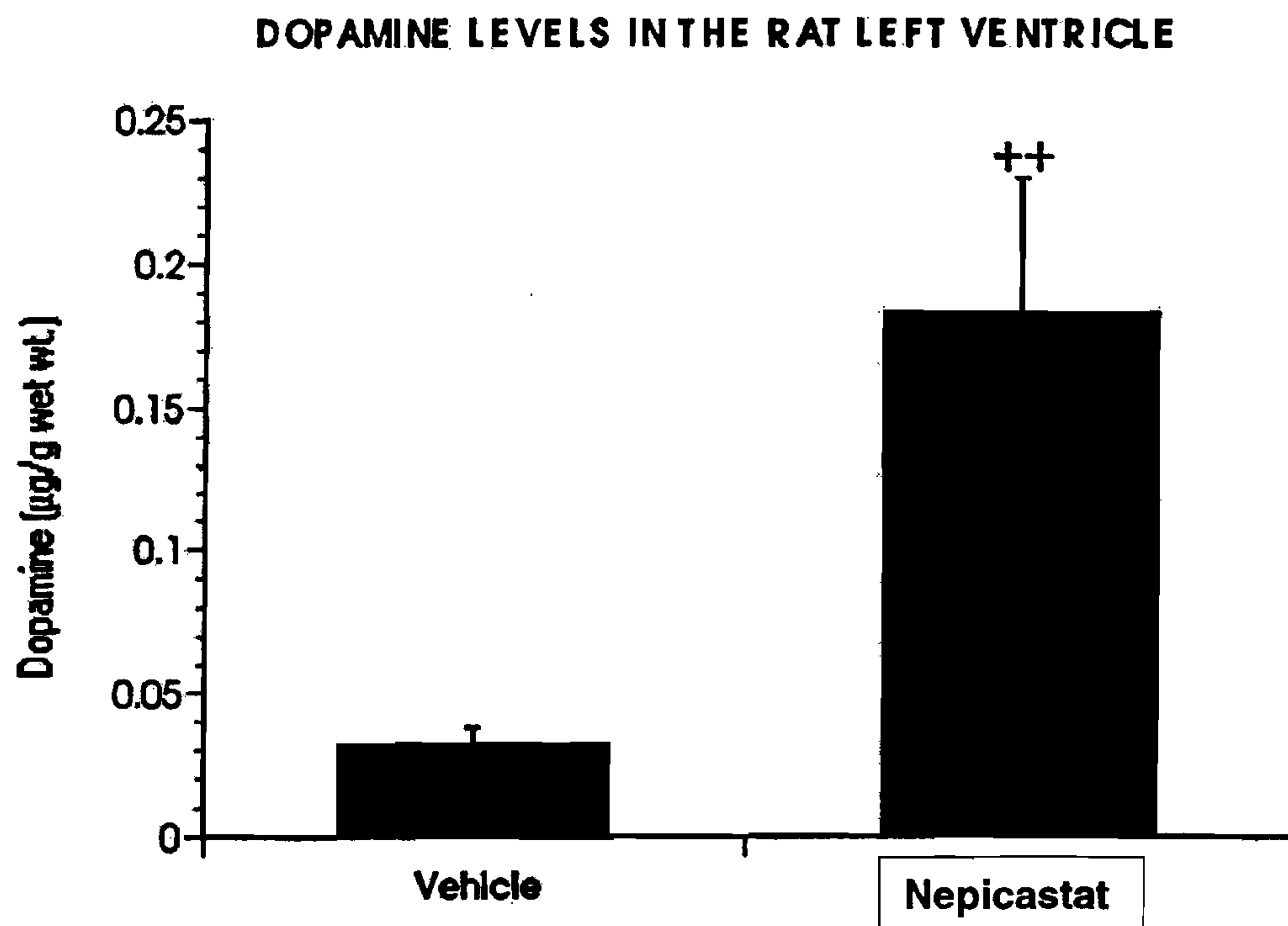
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Figure 41

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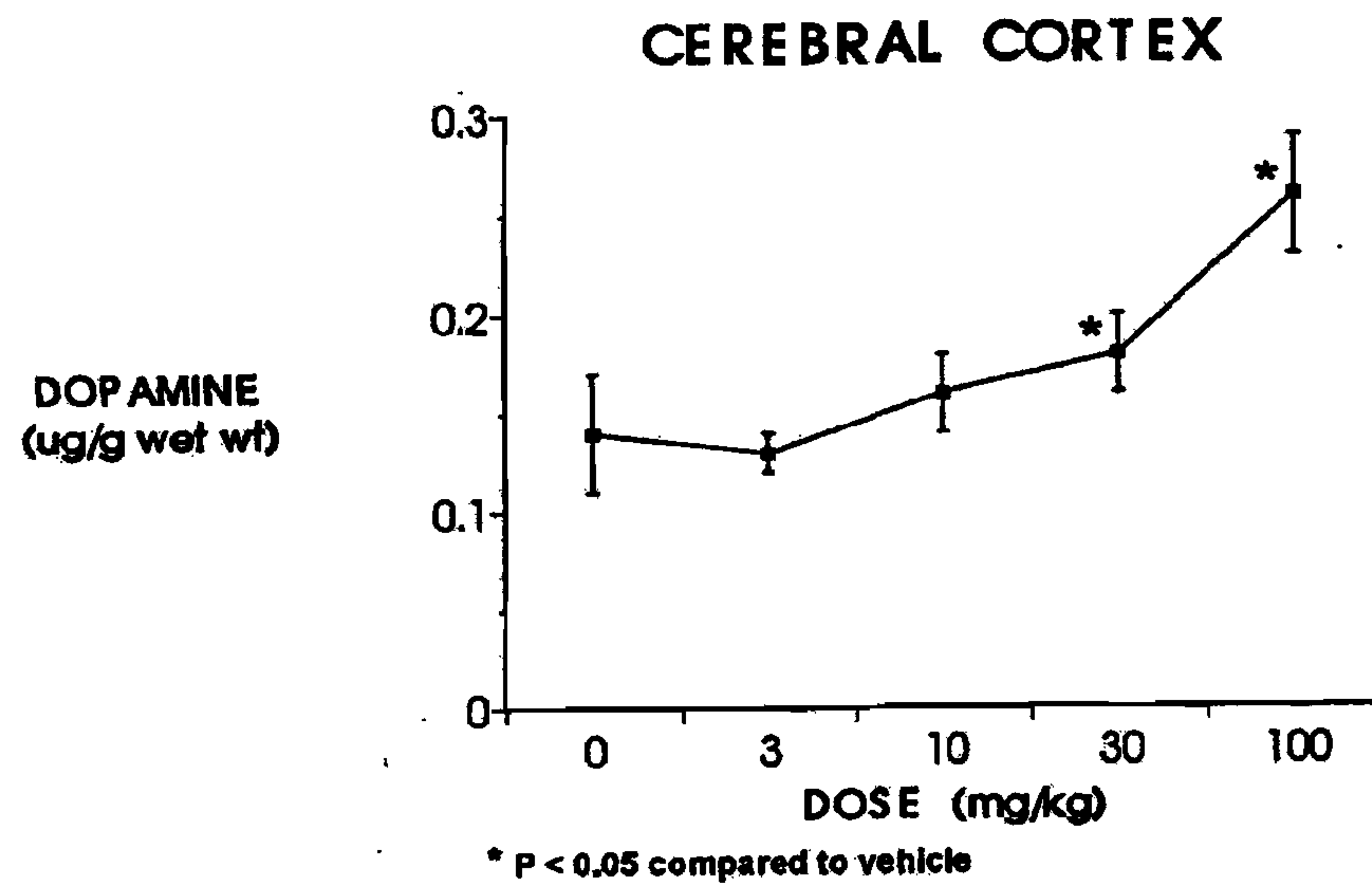
Figure 42

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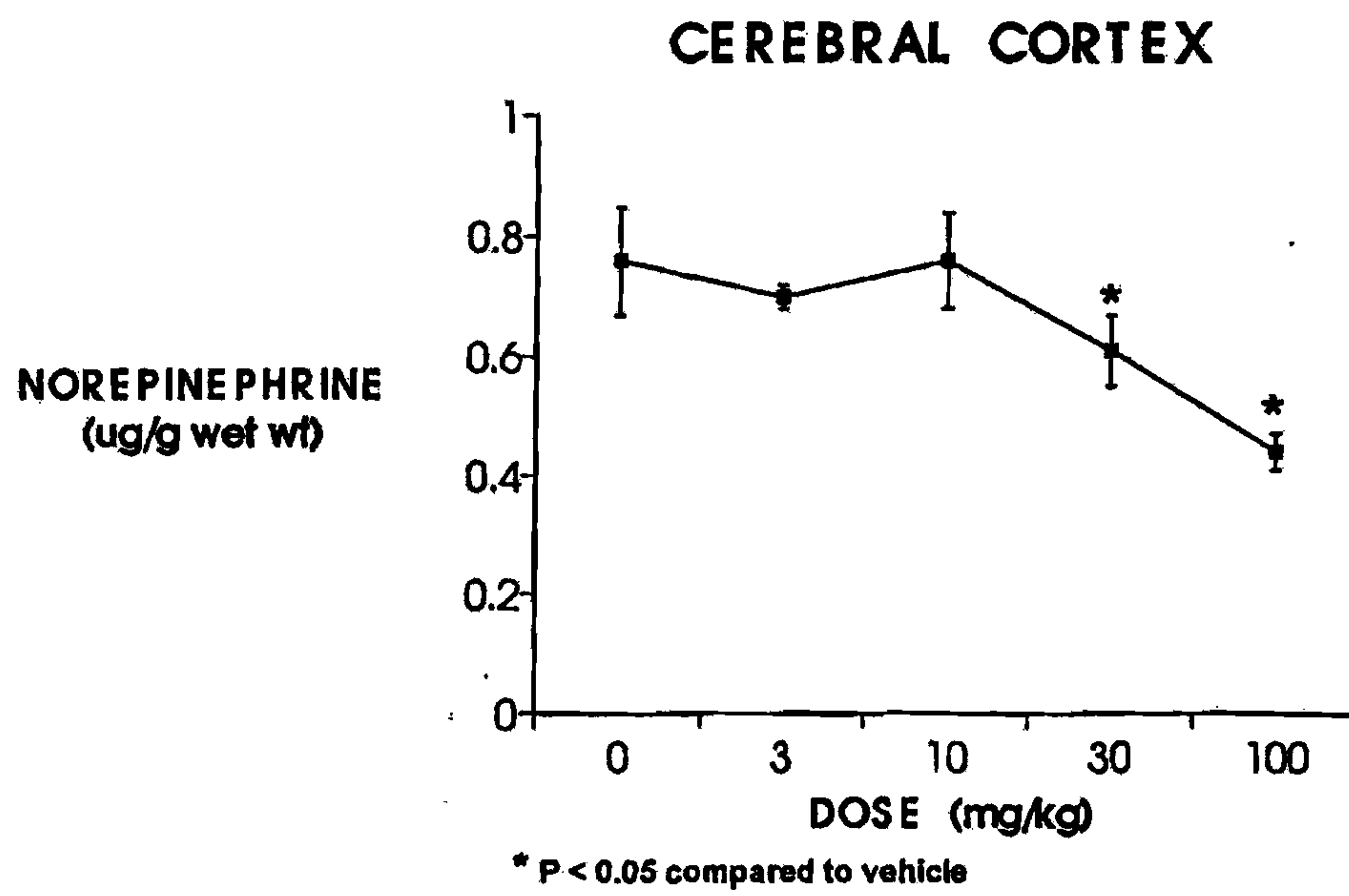
Figure 43

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Figure 44

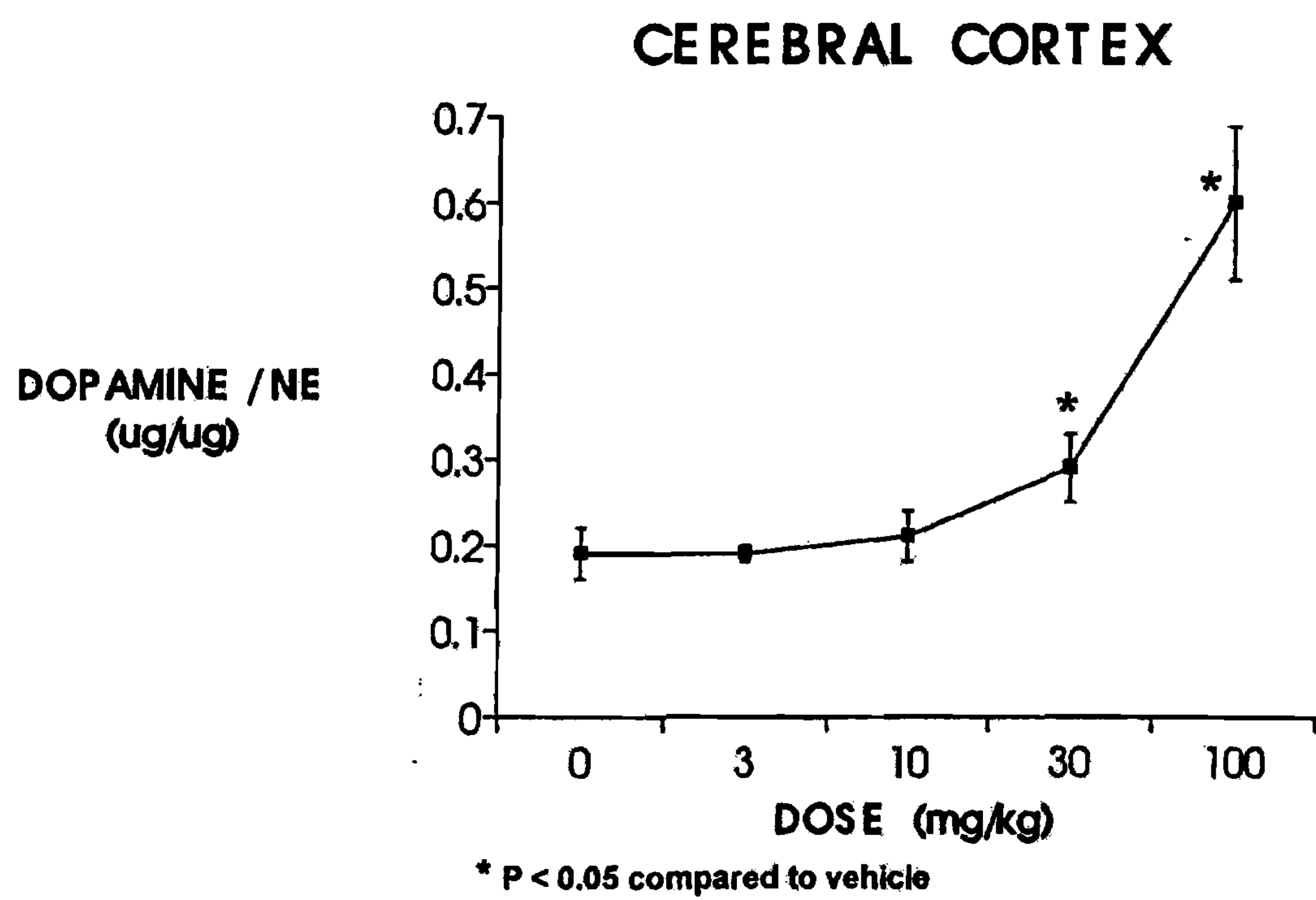


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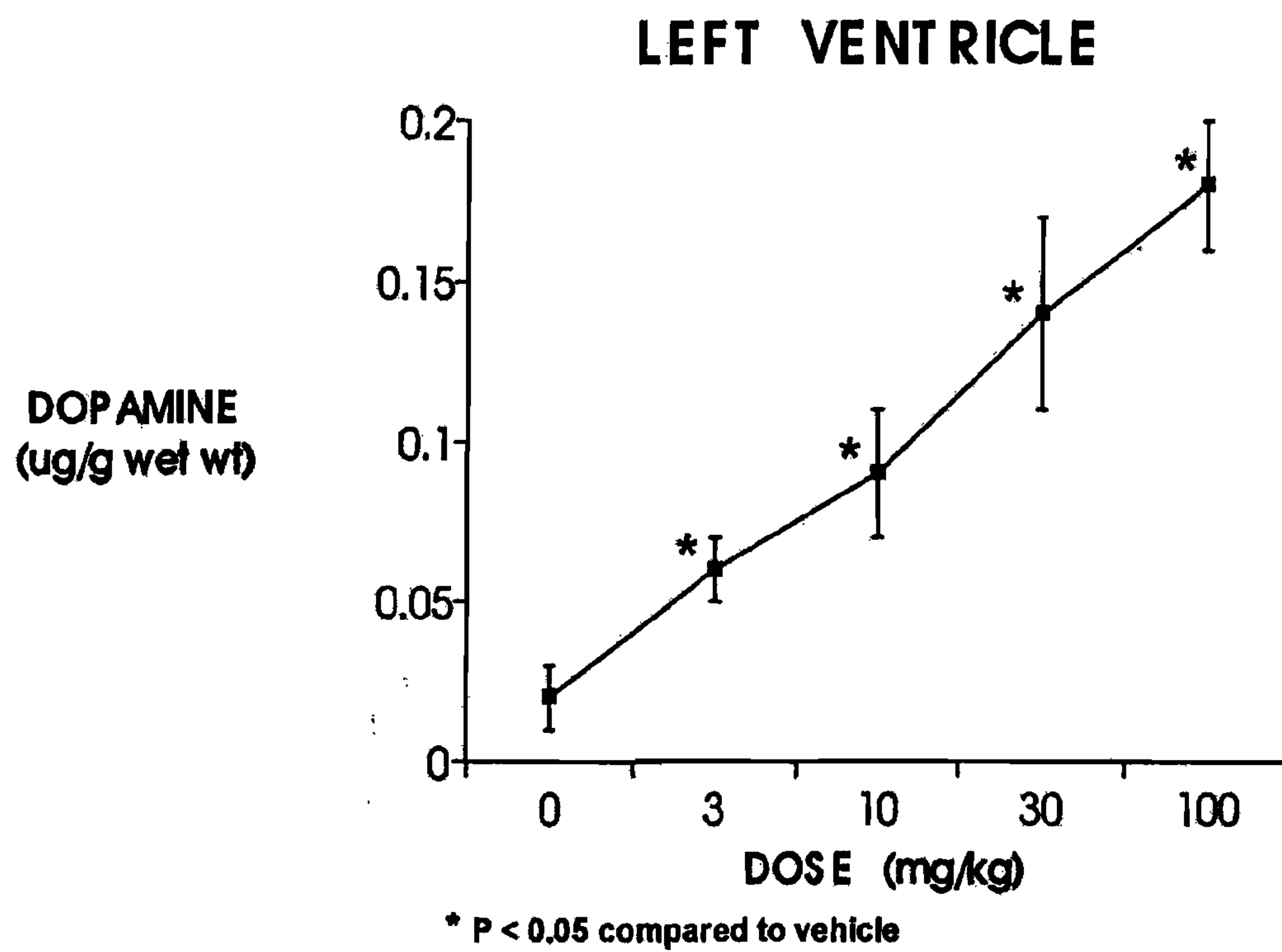
Figure 45

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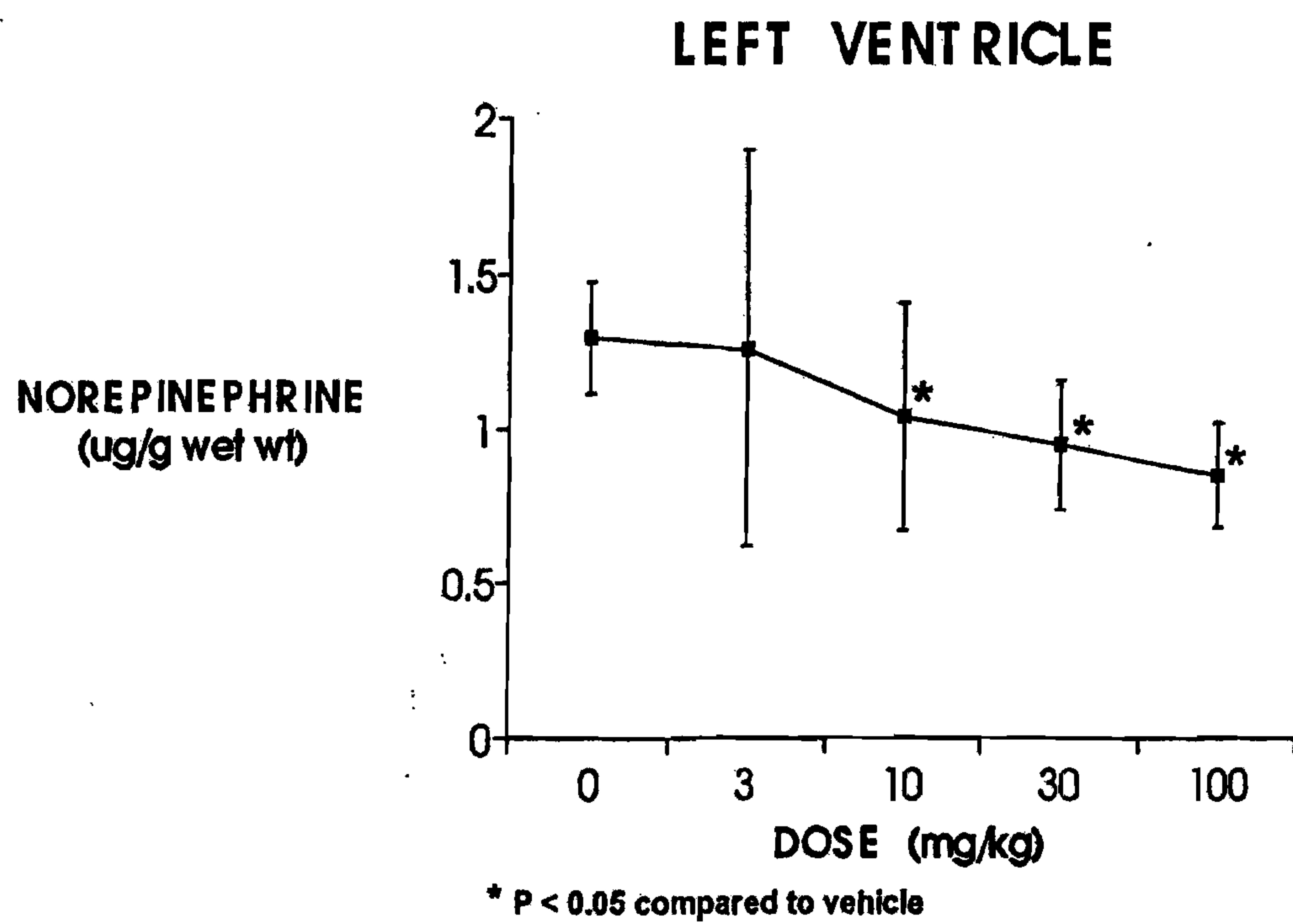
Figure 46



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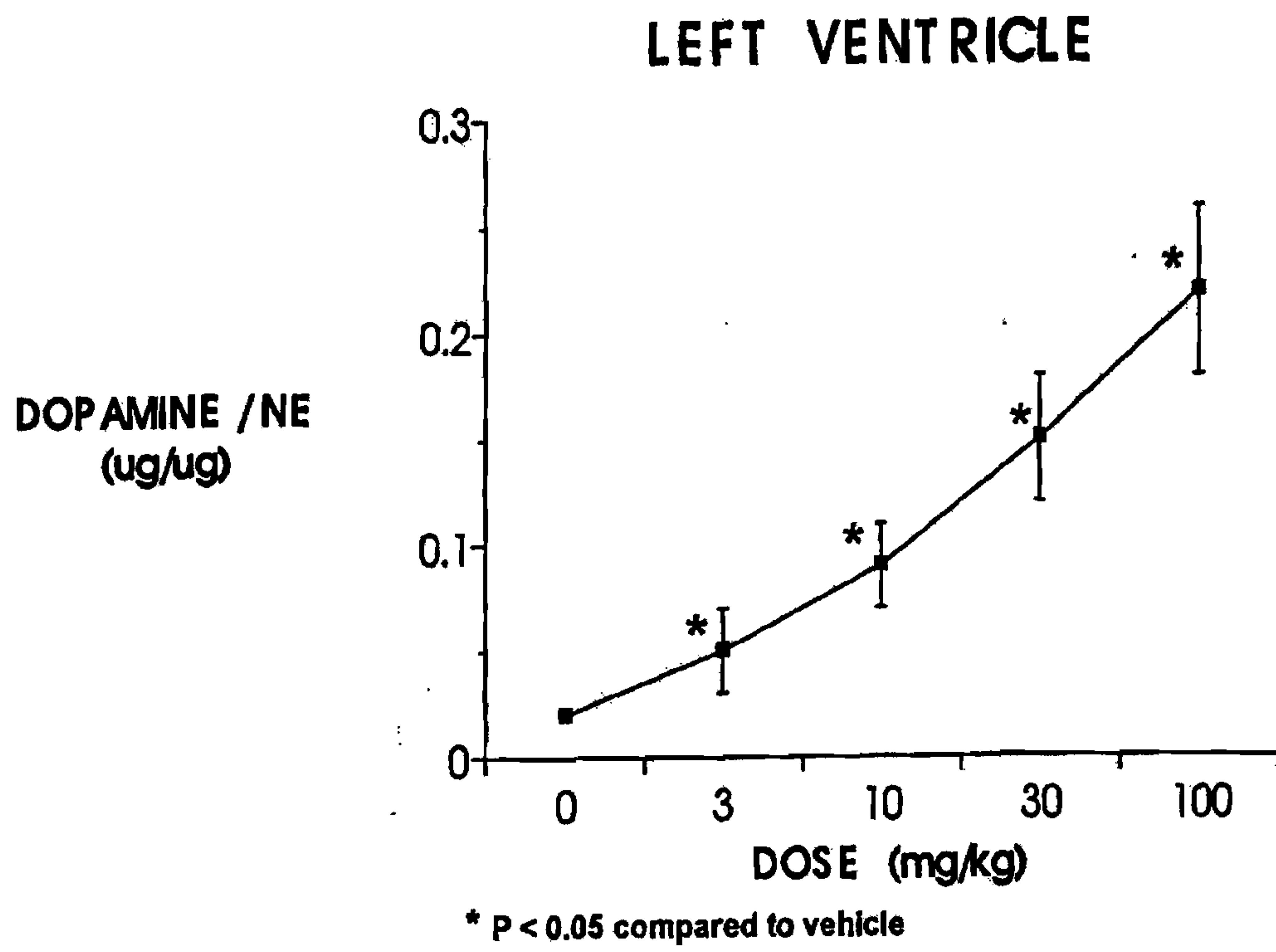
Figure 47

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Figure 48

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Figure 49



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Figure 50

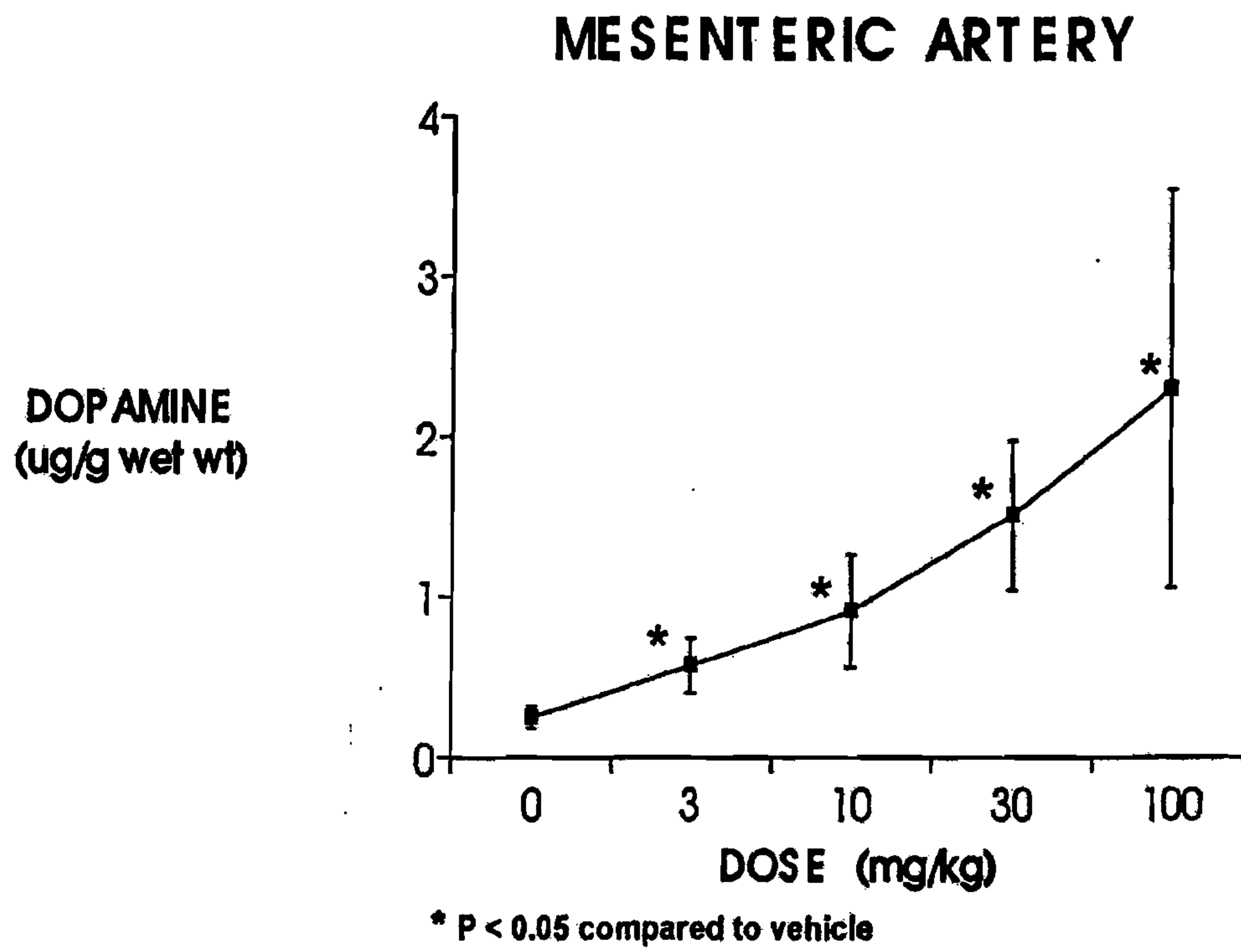
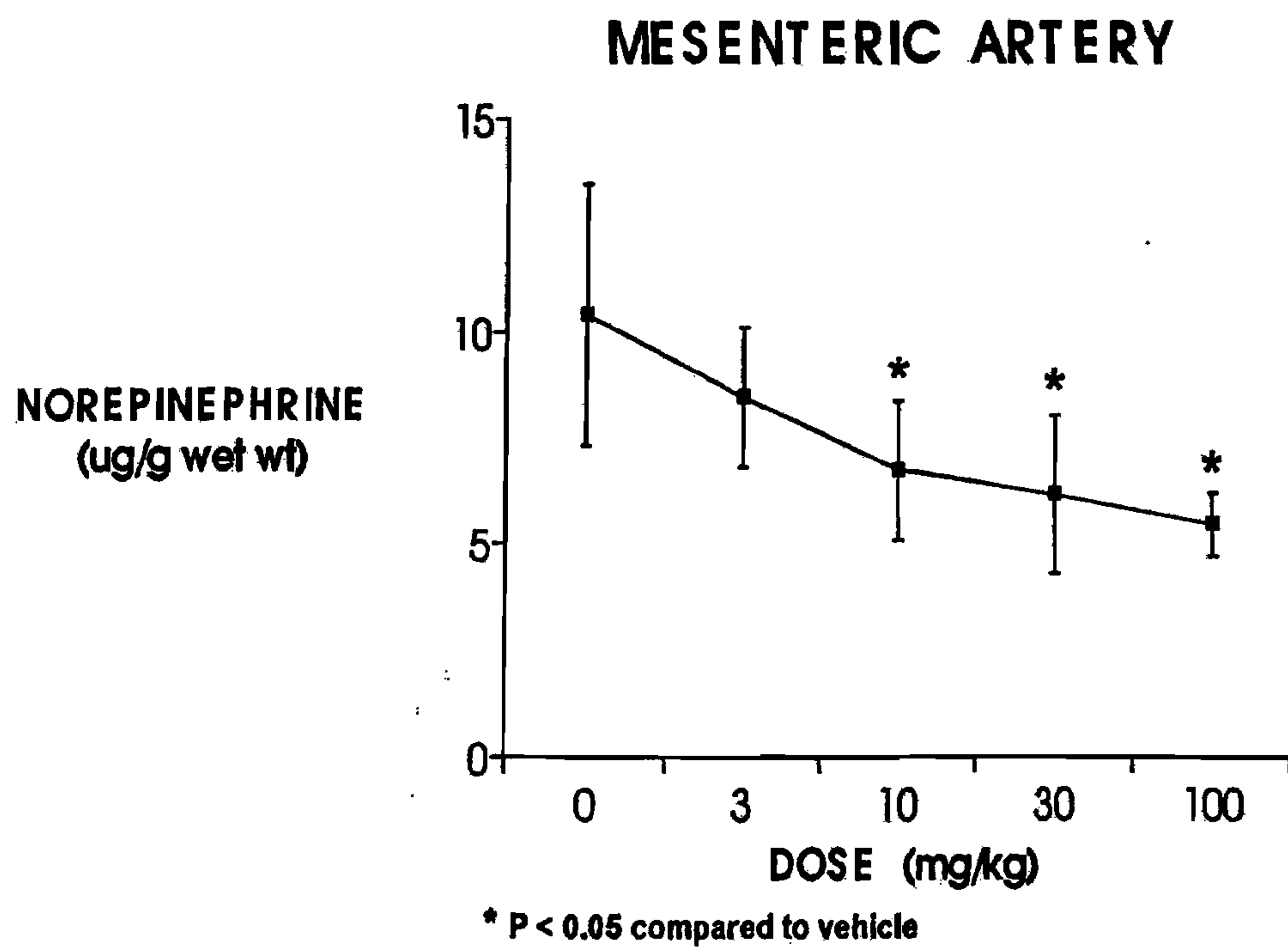
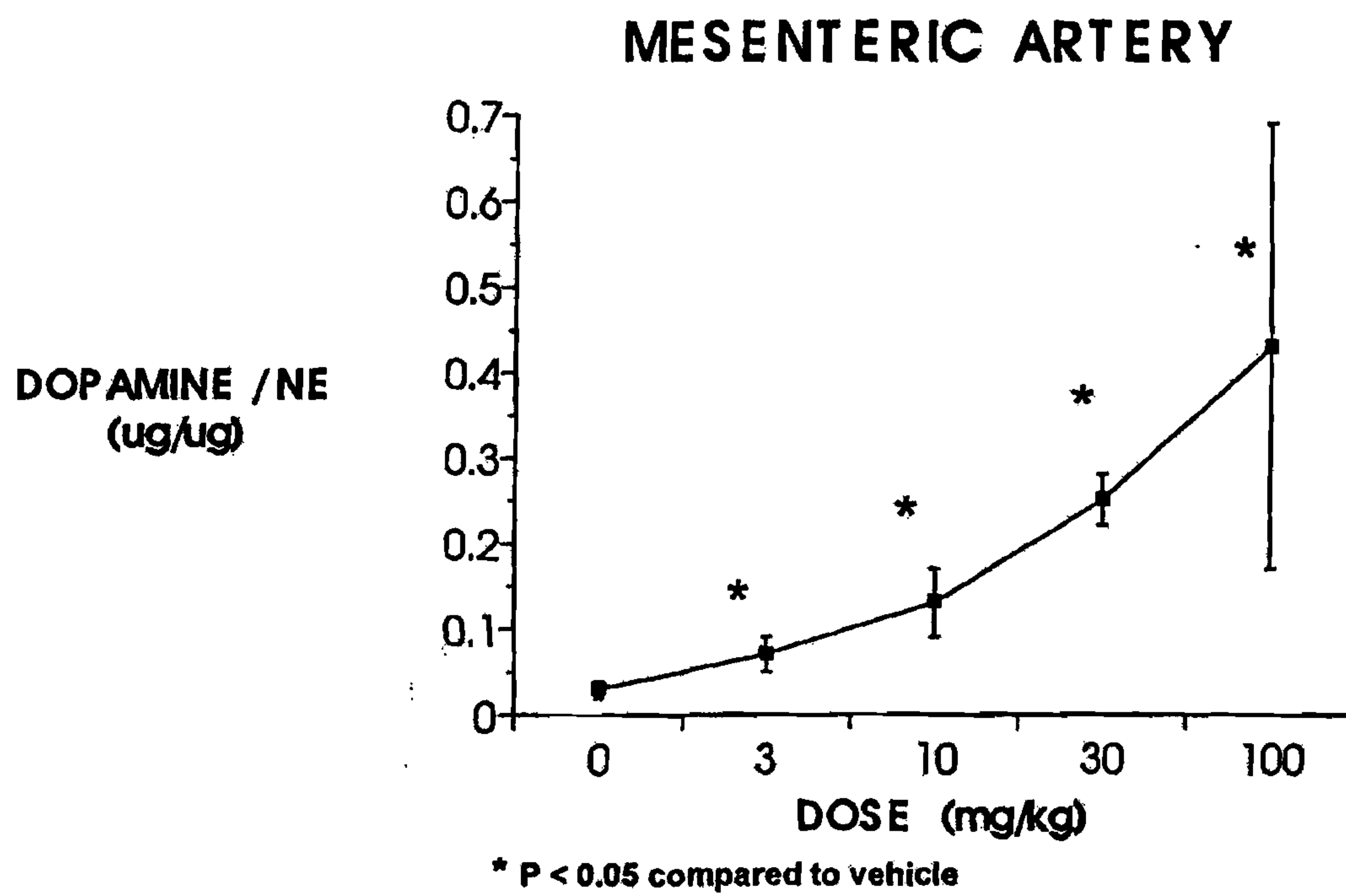


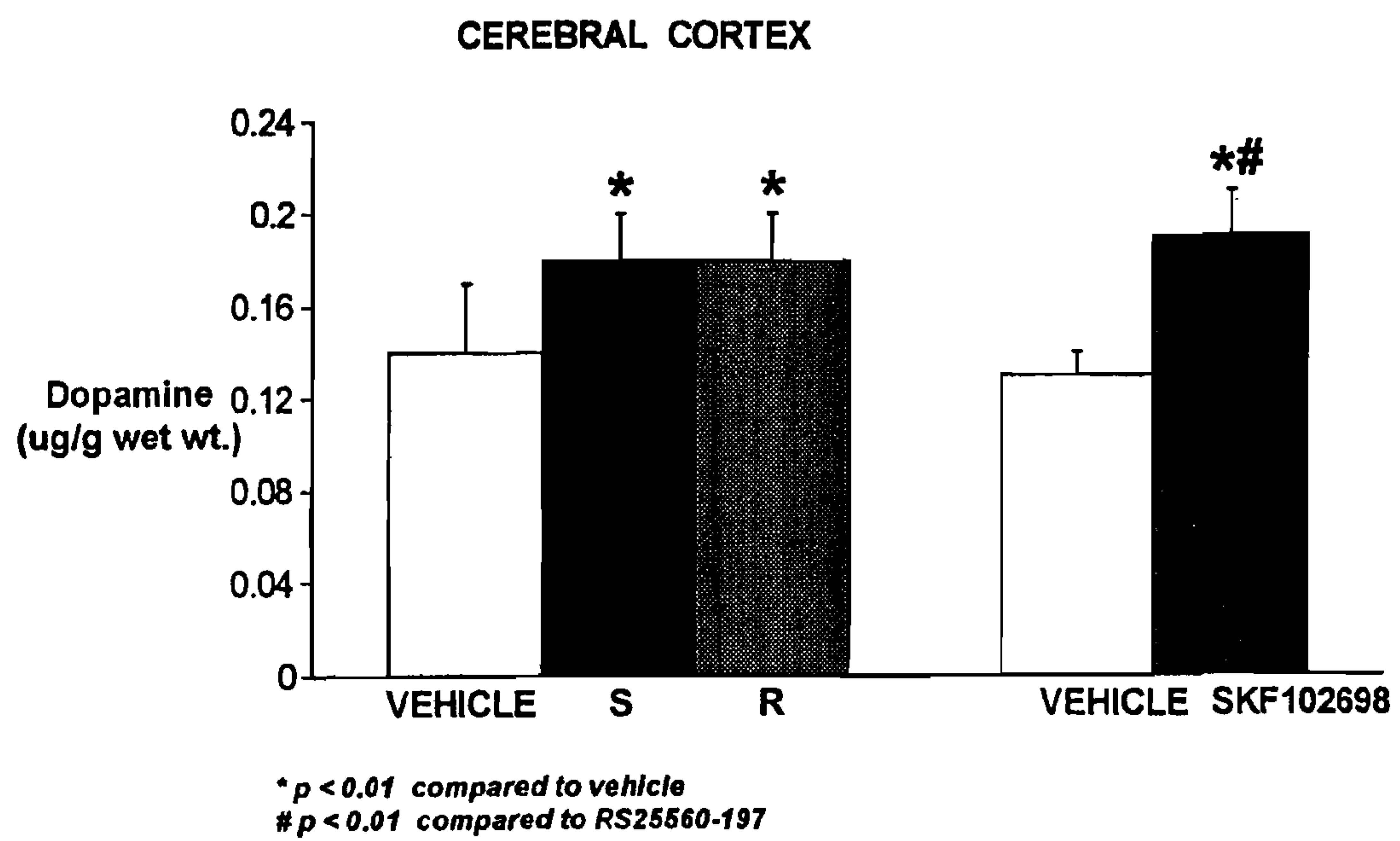
Figure 51



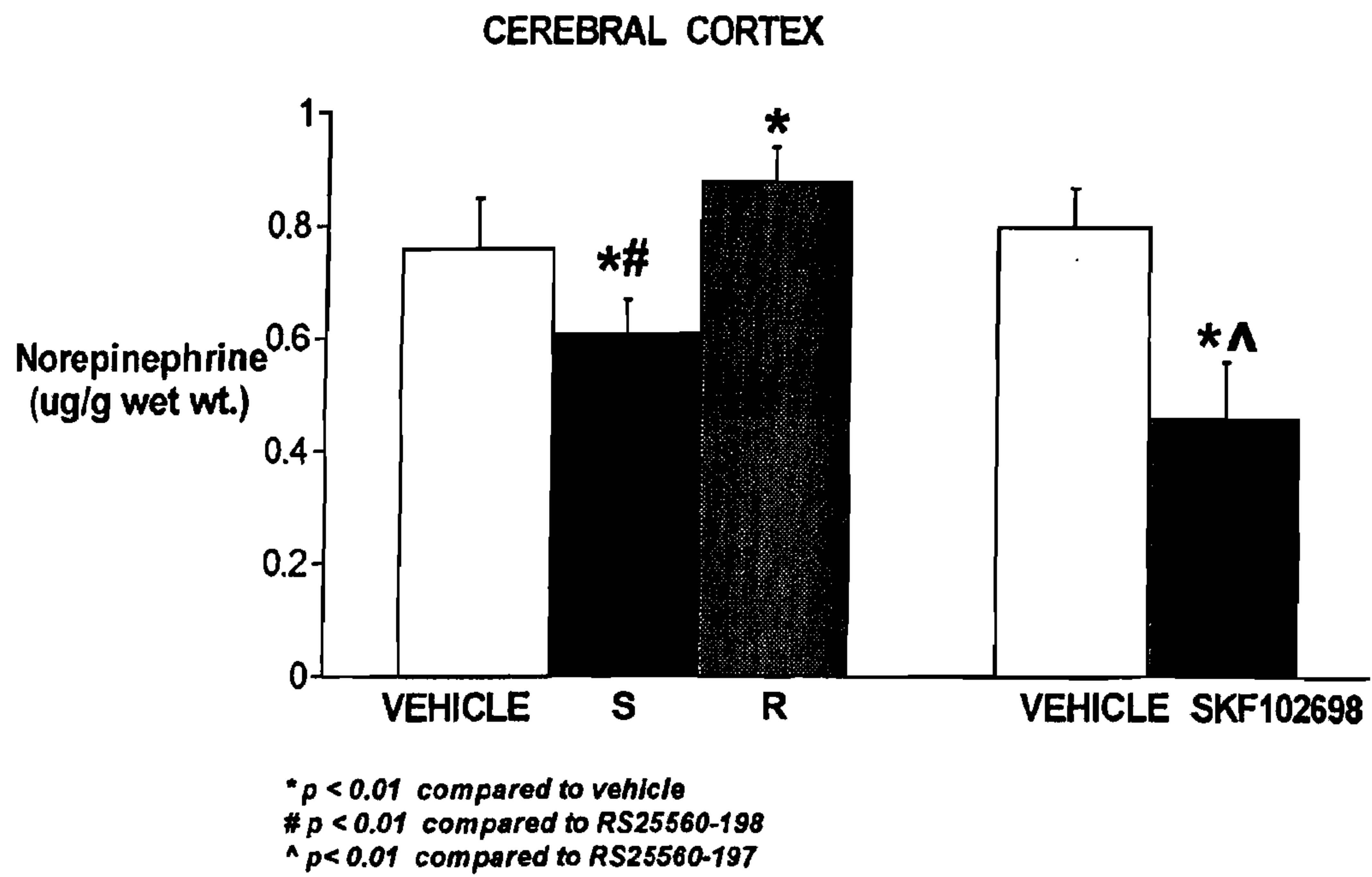
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Figure 52

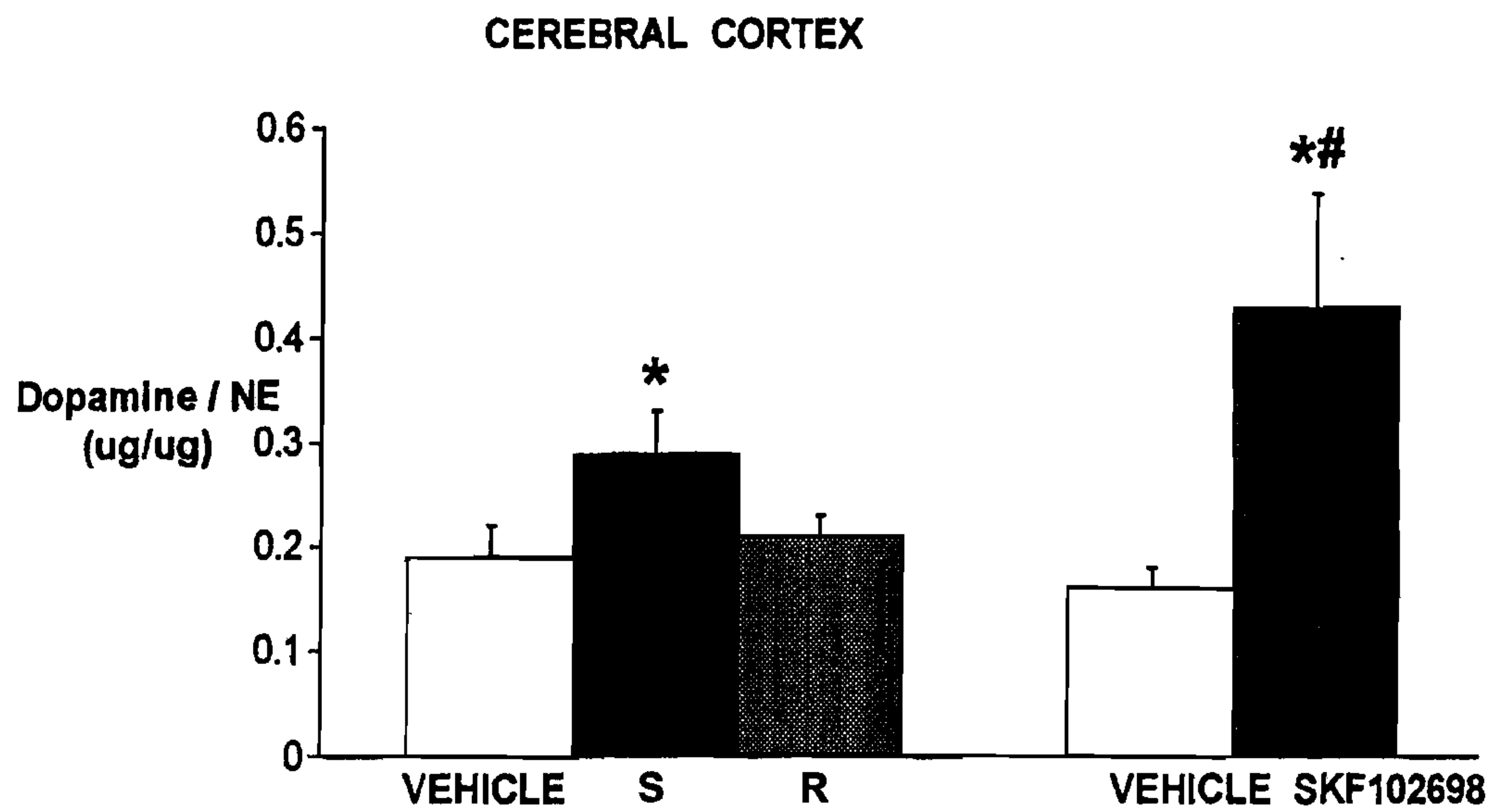
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Figure 53

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Figure 54

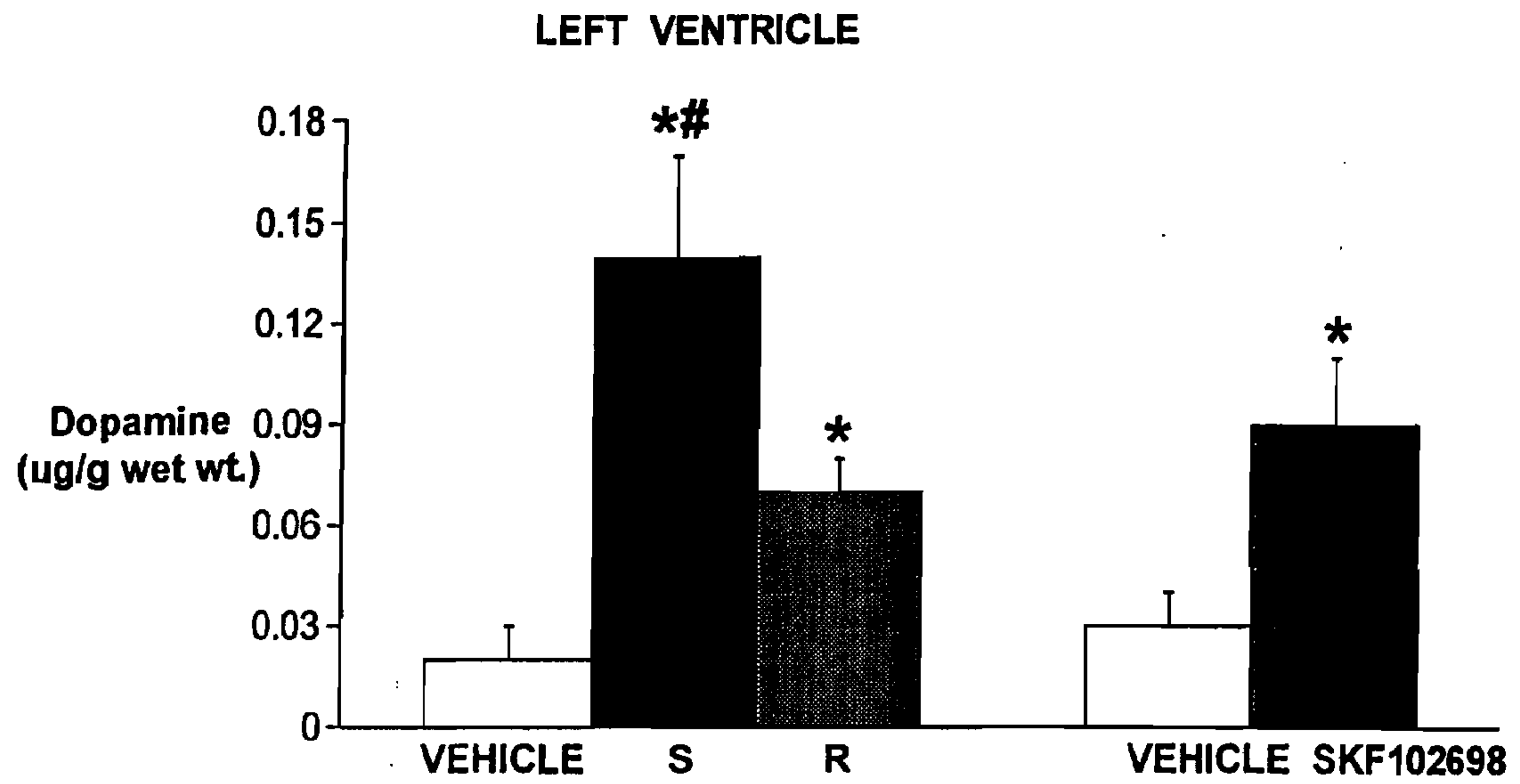
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Figure 55

* $p < 0.01$ compared to vehicle
$p < 0.01$ compared to RS25560-197

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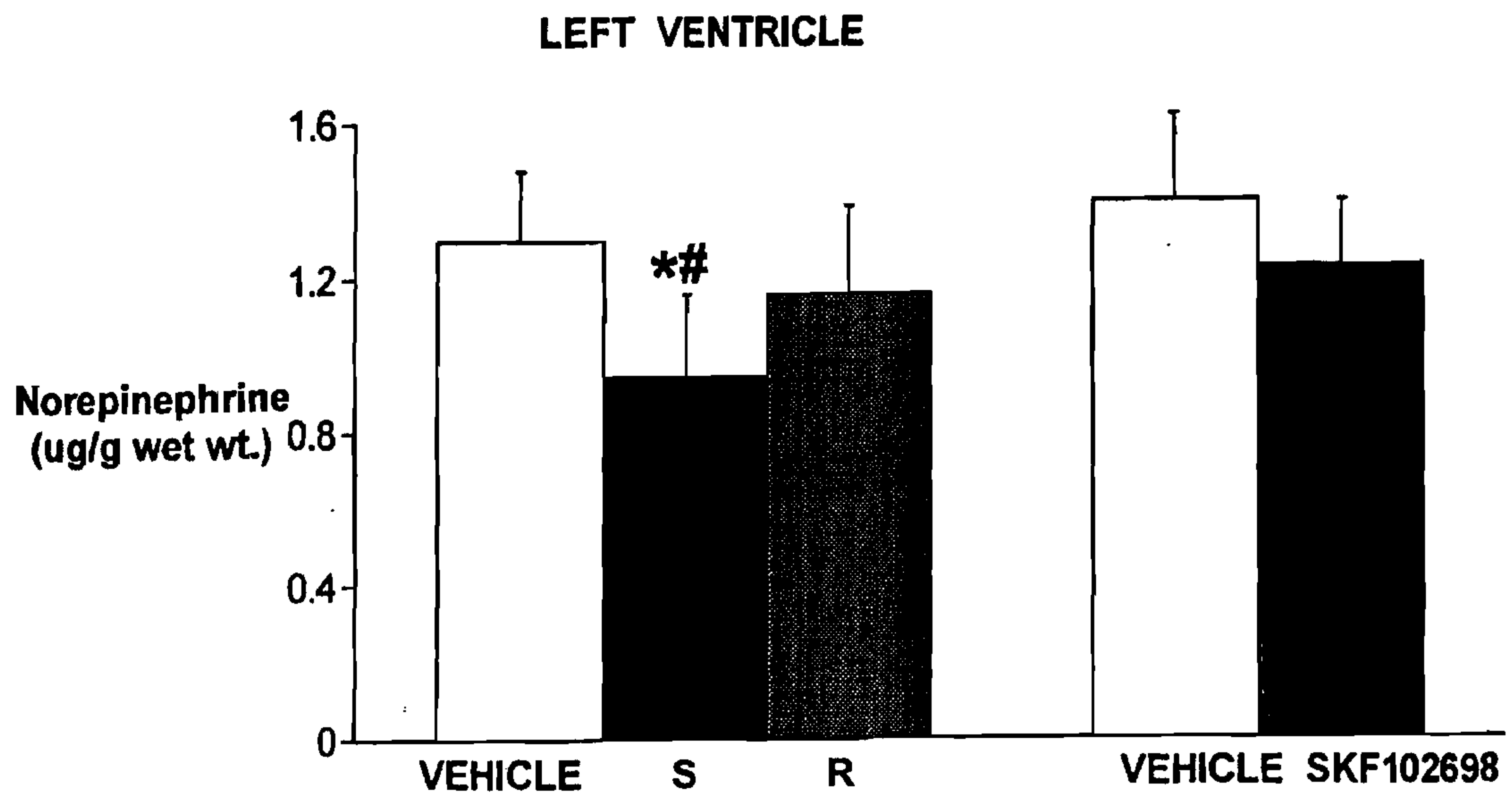
Figure 56



* $p < 0.01$ compared to vehicle

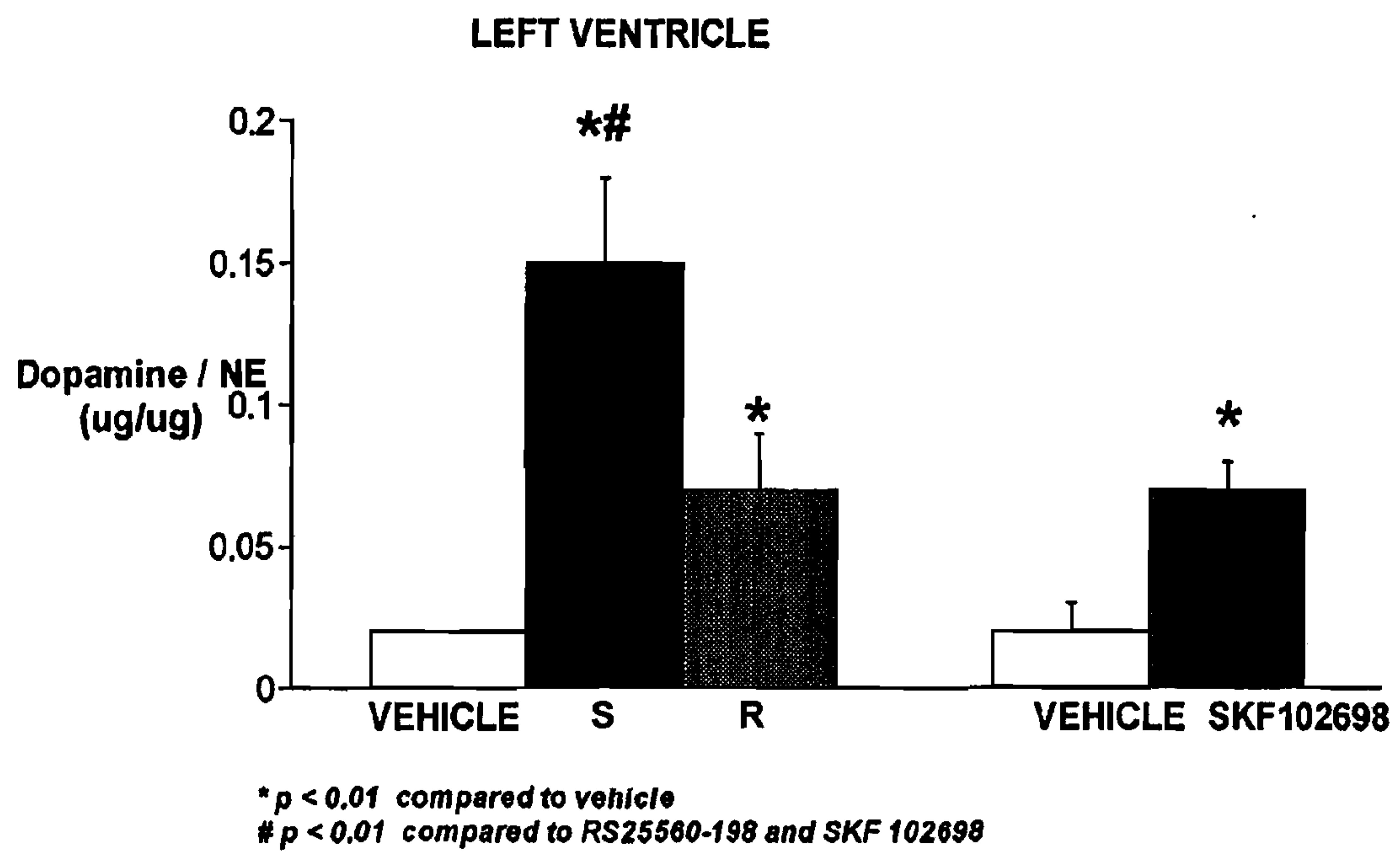
$p < 0.01$ compared to RS25560-198 and SKF102698

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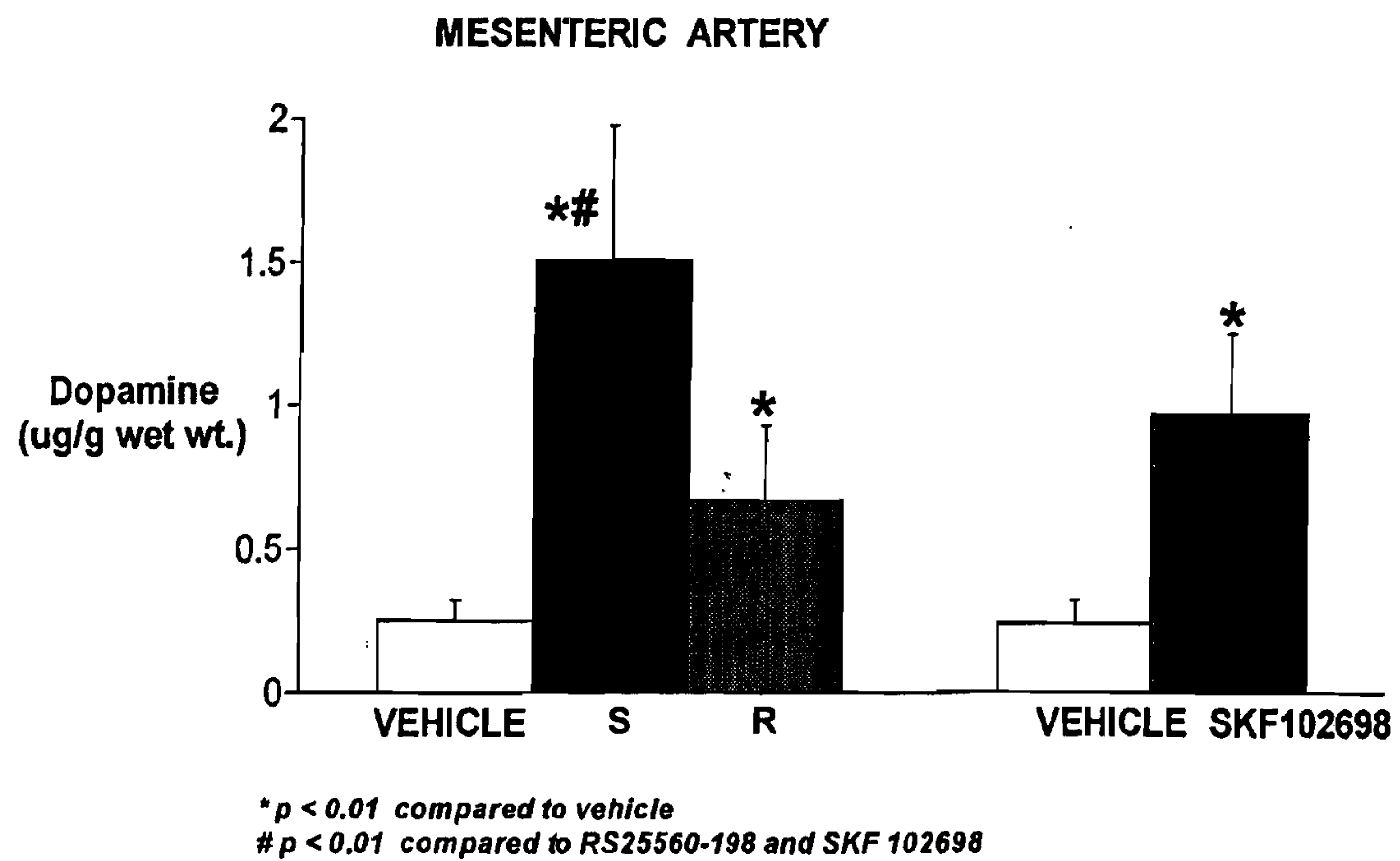
Figure 57

* $p < 0.01$ compared to vehicle
$p < 0.01$ compared to RS25560-198

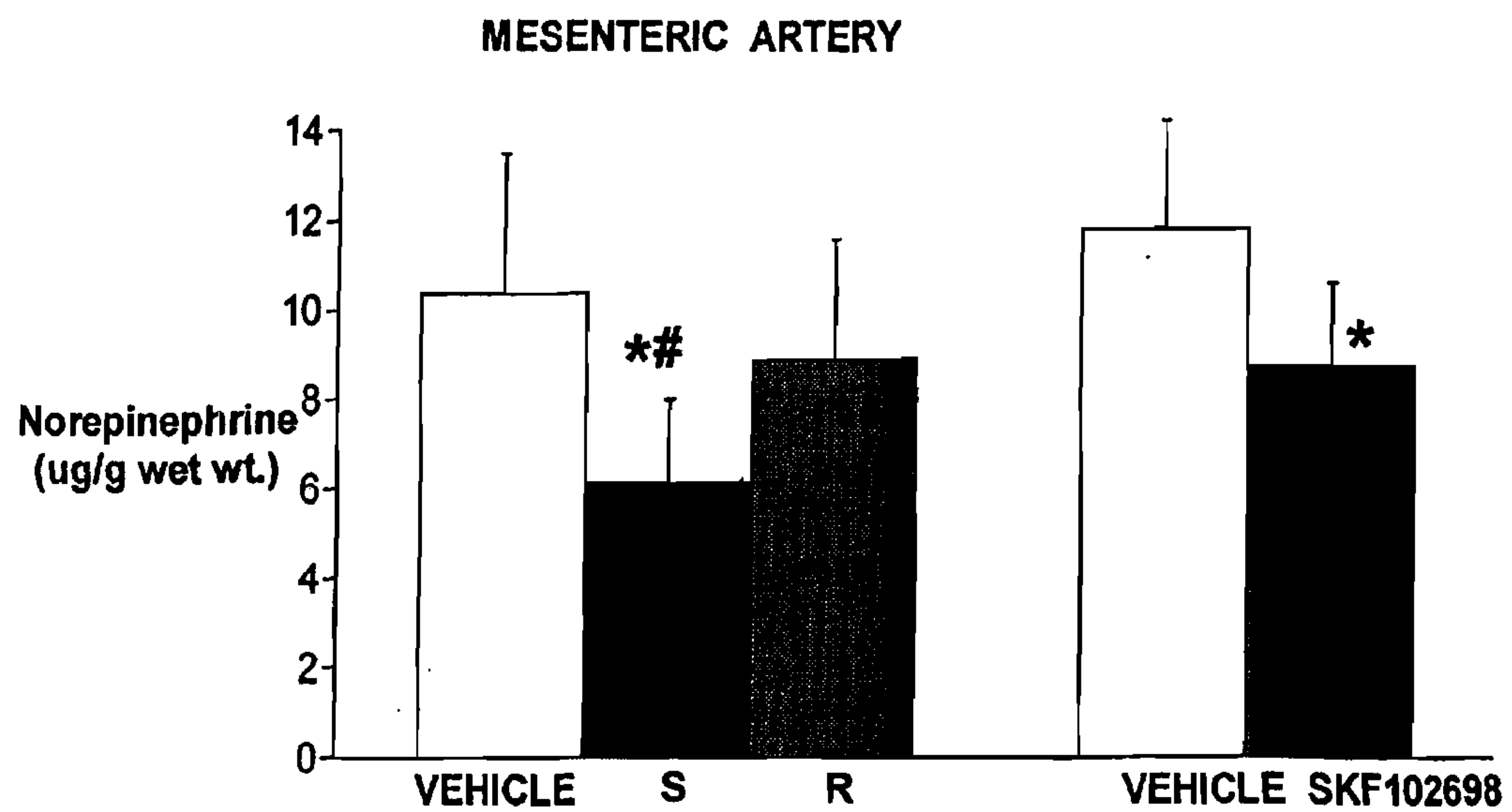
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Figure 58

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Figure 59

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Figure 60** p < 0.01 compared to vehicle**# p < 0.01 compared to RS25560-198*

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Figure 61

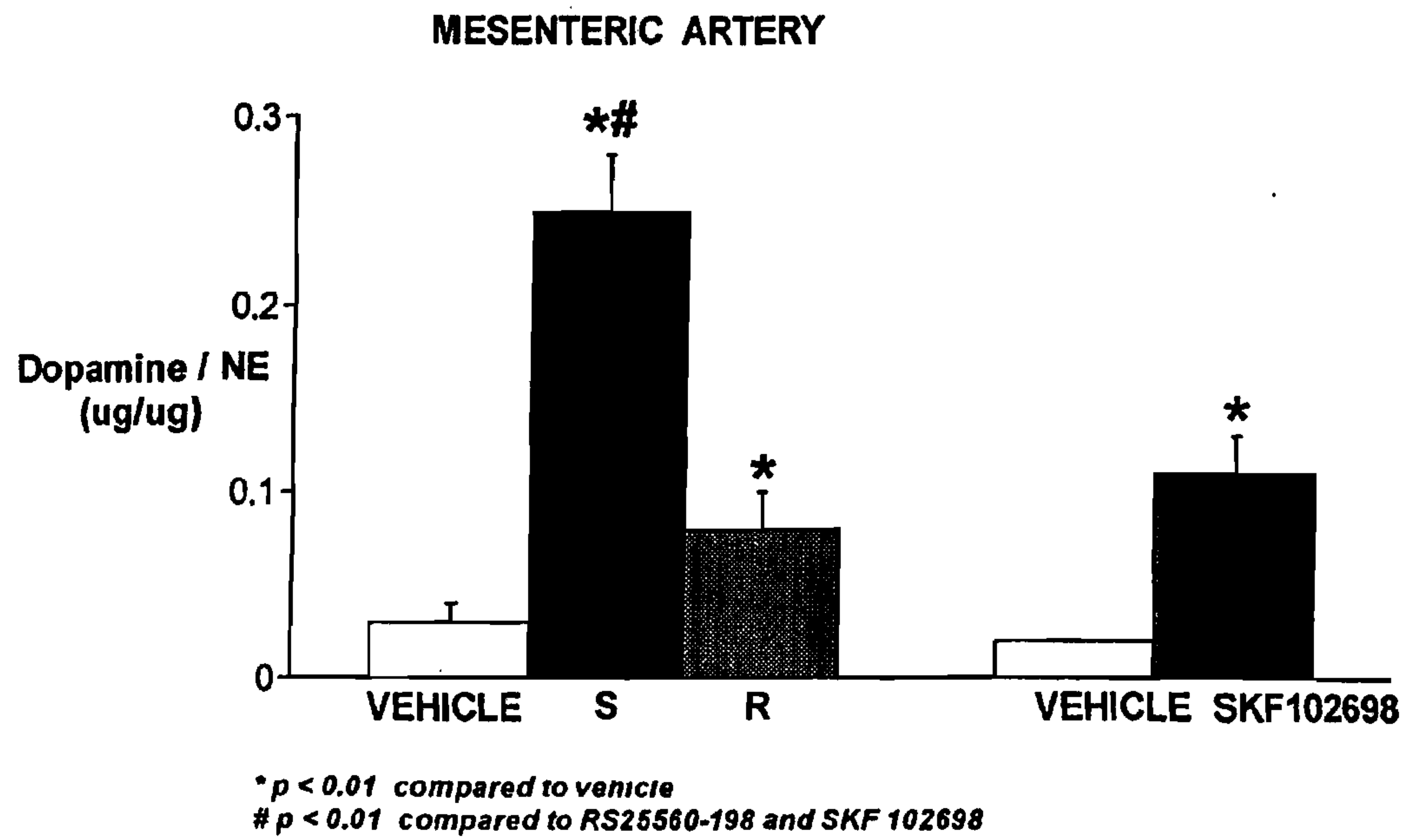


Figure 62

NOREPINEPHRINE:

Dose (mg/kg)	Cortex	Striatum	Mesenteric Artery
0	1.528 ± 0.282	1.059 ± 0.271	15.517 ± 4.116
2.0	1.471 ± 0.249	0.868 ± 0.279	11.666 ± 4.141 *
6.2	1.657 ± 0.181	0.766 ± 0.341	11.191 ± 2.793 *

DOPAMINE:

Dose (mg/kg)	Cortex	Striatum	Mesenteric Artery
0	0.120 ± 0.037	14.027 ± 2.533	1.105 ± 0.979
2.0	0.167 ± 0.110	14.026 ± 1.643	1.075 ± 0.669
6.2	0.161 ± 0.072	14.056 ± 2.483	1.516 ± 0.441

DOPAMINE/NOREPINEPHRINE RATIO:

Dose (mg/kg)	Cortex	Striatum	Mesenteric Artery
0	0.081 ± 0.025	14.159 ± 4.493	0.072 ± 0.064
2.0	0.111 ± 0.060	17.415 ± 5.046	0.090 ± 0.032 **
6.2	0.098 ± 0.046	22.385 ± 12.029	0.140 ± 0.041 **

*p<0.05, **p<0.01

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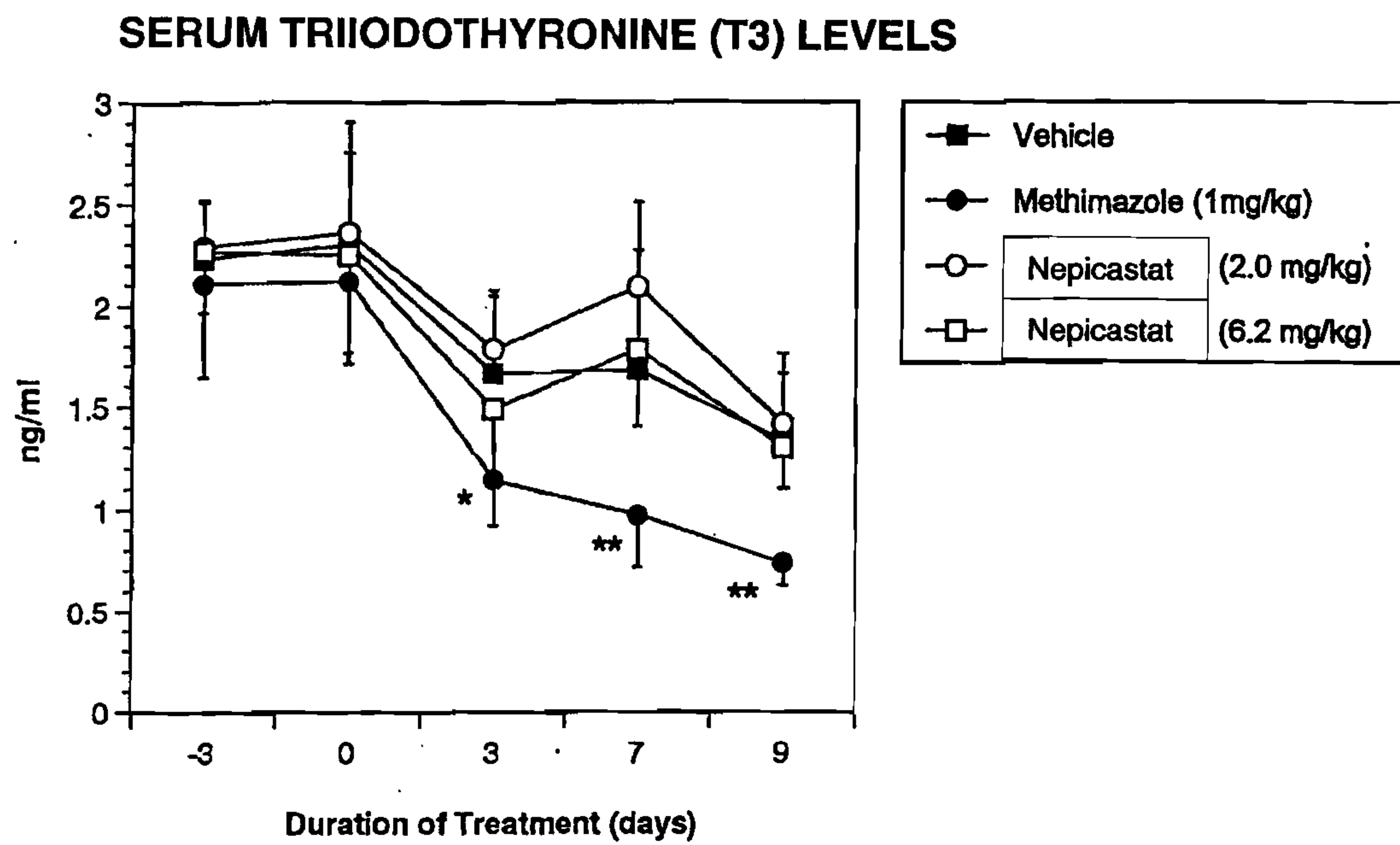
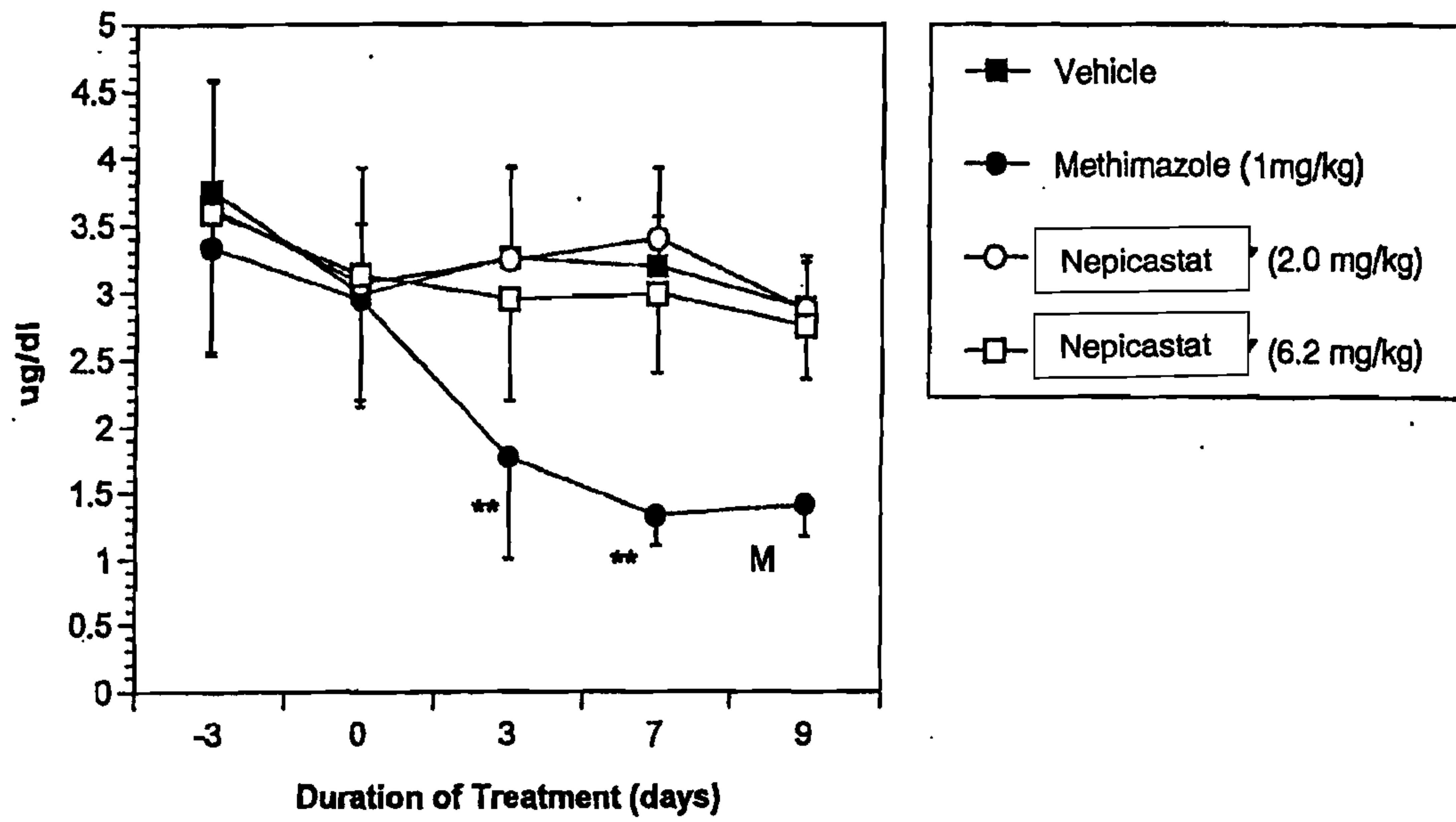
Figure 63

Figure 64**SERUM THYROXINE (T4) LEVELS**

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Figure 65

Compound	Dog	Dose mg/kg	Norepinephrine		Dopamine		D/NE Ratio	
			$\mu\text{g/g}$	Mean $\pm$ Std.Dev	$\mu\text{g/g}$	Mean $\pm$ Std.Dev	Per dog	Mean $\pm$ Std.Dev
Vehicle	4	-	0.16	1.04 $\pm$ 0.76	0.08	0.18 $\pm$ 0.22	0.47	0.22 $\pm$ 0.17
	8		1.78		0.68		0.38	
	9		0.34		0.08		0.17	
	11		0.89		0.07		0.08	
	17		1.08		BQL		BQL	
	18		2.33		0.13		0.05	
	26		1.39		0.11		0.08	
	28		0.38		0.11		0.30	
Nepicastat	1	10	0.85	0.29 $\pm$ 0.24	1.51	0.45 $\pm$ 0.45	1.79	1.44 $\pm$ 0.43
	6		0.07		0.09		1.27	
	13		0.23		0.44		1.86	
	16		0.20		0.23		1.12	
	19		0.20		0.21		1.05	
	21		0.42		0.54		1.31	
	25		0.17		0.37		2.14	
	32		0.20		0.20		1.00	
Nepicastat	3	30	0.58	0.79 $\pm$ 1.05	1.04	1.12 $\pm$ 1.63	1.81	1.62 $\pm$ 0.67
	5		BQL		0.08		BQL	
	12		3.12		4.94		1.68	
	14		0.26		0.25		0.97	
	20		0.10		0.19		1.87	
	24		0.21		0.48		2.28	
	27		0.53		0.27		0.52	
	29		0.72		1.68		2.32	
Nepicastat	2	60	0.20	0.32 $\pm$ 0.20	0.46	0.46 $\pm$ 0.27	2.35	1.71 $\pm$ 0.58
	7		0.39		0.65		1.67	
	10		BQL		0.08		BQL	
	15		0.47		0.73		1.56	
	22		0.19		0.22		1.17	
	23		0.10		0.16		1.50	
	30		0.24		0.64		2.65	
	31		0.68		0.74		1.08	

* BQL: Below the Quantitation Limit of the assay. For NE, BQL<2.0 ng/ml

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Figure 66

Compound	Dog	Dose mg/kg	Norepinephrine		Dopamine		D/NE Ratio	
			$\mu\text{g/g}$	Mean $\pm$ Std.Dev	$\mu\text{g/g}$	Mean $\pm$ Std.Dev	Per dog	Mean $\pm$ Std.Dev
Vehicle	4	-	1.50	3.80	0.15	0.41	0.10	0.15
	8		1.07	± 3.74	0.47	± 0.40	0.44	± 0.16
	9		1.78		0.11		0.06	
	11		2.65		0.24		0.09	
	17		4.00		0.19		0.05	
	18		12.70		0.63		0.05	
	26		3.15		0.19		0.06	
	28		3.55		1.30		0.36	
Nepicastat	1	10	0.94	0.54	1.83	1.08	1.95	2.63
	6		1.31	± 0.43	1.48	± 0.61	1.13	± 1.28
	13		0.27		0.58		2.19	
	16		0.12		0.44		3.58	
	19		0.28		0.68		2.41	
	21		0.65		1.17		1.79	
	25		0.08		0.40		5.24	
	32		0.66		1.85		2.79	
Nepicastat	3	30	0.60	1.27	1.32	2.47	2.20	2.31
	5		2.87	± 1.15	3.93	± 1.88	1.47	± 0.65
	12		3.25		5.99		1.84	
	14		1.33		2.42		1.82	
	20		0.16		0.37		2.32	
	24		0.22		0.76		3.51	
	27		1.38		3.37		2.45	
	29		0.56		1.63		2.91	
Nepicastat	2	60	0.31	0.59	0.83	1.58	2.65	2.69
	7		0.19	± 0.31	0.66	± 1.09	3.55	± 0.74
	10		0.55		1.08		1.91	
	15		0.93		2.29		2.46	
	22		0.70		1.04		1.49	
	23		0.30		0.90		2.98	
	30		1.08		3.89		3.61	
	31		0.62		1.61		2.89	

Figure 67

DAY	Placebo					Steady State ?	Nepicastat			
	N	Mean	Std Dev	Std Error of Mean	Statistical comparison		N	Mean	StdDev	StdErr of Mean
0	8	34.38	33.87	11.97			8	41.13	24.79	8.77
1	8	23.50	7.05	2.49	***		8	92.63	24.60	8.70
2	8	20.13	2.42	0.85	***		8	284.63	306.81	108.47
3	8	34.25	30.26	10.70	***		8	271.88	73.98	26.16
4	8	29.75	21.43	7.58	***		8	246.75	98.90	34.97
5	8	31.25	19.41	6.86	***		8	277.63	97.46	34.46
6	8	22.13	4.19	1.48	***		8	228.38	123.95	43.82
7	8	28.13	12.46	4.41	***		8	307.00	205.83	72.77
8	8	25.38	8.72	3.08	***	SS	8	205.63	81.65	28.87
9	8	22.50	7.43	2.63	***	SS	8	199.38	47.78	16.89
10	8	17.00	8.02	2.84	***	SS	8	200.88	57.88	20.47
11	8	23.25	5.57	1.97	***	SS	8	190.38	62.03	21.93
12	8	42.25	39.20	13.86	***	SS	8	182.38	50.17	17.74
13	8	35.88	18.94	6.70	***	SS	8	163.25	58.87	20.81
14	8	35.88	24.09	8.52	***	SS	8	217.13	105.10	37.16
15	8	24.75	6.71	2.37	***	SS	8	202.38	143.44	50.71

* Statistically significant at the 5% level vs placebo.
** Statistically significant at the 1% level vs placebo.
*** Statistically significant at the 0.1% level vs placebo.
SS = Steady state

Figure 68

DAY	Placebo					Statistical comparison	Steady State ?	Nepicastat			
	N	Mean	Std Dev	Std Error of Mean	N			Mean	StdDev	StdErr of Mean	
0	8	460.25	168.50	59.58				8	401.88	80.68	28.52
1	8	397.13	69.56	24.59				8	403.63	135.70	47.98
2	8	316.25	79.88	28.24				8	419.63	297.97	105.35
3	8	273.75	64.83	22.92				8	320.13	112.41	39.74
4	8	376.38	203.63	72.00	**		SS	8	230.13	79.13	27.98
5	8	367.00	87.83	31.05	**		SS	8	251.63	107.60	38.04
6	8	286.50	75.11	26.56	*		SS	8	191.50	53.89	19.05
7	8	334.38	71.17	25.16	*		SS	8	249.00	113.34	40.07
8	8	274.50	81.65	28.87	*		SS	8	193.38	30.81	10.89
9	8	291.13	64.72	22.88	*		SS	8	198.50	58.62	20.73
10	8	243.50	84.27	29.79			SS	8	203.75	49.70	17.57
11	8	275.25	75.08	26.54	*		SS	8	194.00	43.03	15.21
12	8	353.25	113.72	40.21	**		SS	8	207.63	58.55	20.70
13	8	307.50	81.58	28.84	**		SS	8	210.38	86.38	30.54
14	8	287.88	116.63	41.24			SS	8	250.00	91.26	32.27
15	8	299.00	69.16	24.45			SS	8	233.63	80.48	28.45

* Statistically significant at the 5% level vs placebo.
** Statistically significant at the 1% level vs placebo.
*** Statistically significant at the 0.1% level vs placebo.
SS = Steady state

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Figure 69

DAY	Placebo				N	Nepicastat			
	Mean	Std Dev	Std Error of Mean	Statistical comparison		Steady State ?	Mean	StdDev	StdErr of Mean
0	0.07	0.04	0.01	*	8	0.10	0.06	0.02	
1	0.06	0.01	0.00	***	8	0.24	0.06	0.02	
2	0.07	0.02	0.01	***	8	0.61	0.17	0.06	
3	0.12	0.09	0.03	***	8	0.90	0.24	0.09	
4	0.08	0.02	0.01	***	8	1.13	0.47	0.17	
5	0.09	0.06	0.02	***	8	1.22	0.59	0.21	
6	0.08	0.02	0.01	***	8	1.27	0.77	0.27	
7	0.08	0.02	0.01	***	8	1.36	0.77	0.27	
8	0.10	0.04	0.01	***	8	1.06	0.35	0.12	
9	0.08	0.04	0.01	***	8	1.08	0.40	0.14	
10	0.07	0.04	0.01	***	8	1.03	0.35	0.13	
11	0.09	0.04	0.01	***	8	1.04	0.43	0.15	
12	0.11	0.07	0.03	***	8	0.94	0.37	0.13	
13	0.12	0.06	0.02	***	8	0.82	0.27	0.09	
14	0.12	0.04	0.02	***	8	0.87	0.28	0.10	
15	0.09	0.02	0.01	***	8	0.89	0.51	0.18	

* Statistically significant at the 5% level vs placebo.

** Statistically significant at the 1% level vs placebo.

*** Statistically significant at the 0.1% level vs placebo.

SS = Steady state

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Figure 70

DAY	Placebo				Steady State ?	Statistical comparison	Nepicastat			
	N	Mean	Std Dev	Std Error of Mean			N	Mean	StdDev	StdErr of Mean
0	8	246.00	71.32	25.22			8	167.98	73.86	26.11
1	8	181.88	64.62	22.85			8	181.00	121.23	42.86
2	8	167.75	52.94	18.72			8	179.50	114.51	40.48
3	8	148.38	37.22	13.16			8	137.63	77.04	27.24
4	8	176.38	97.85	34.60			8	133.63	82.98	29.34
5	8	163.13	51.80	18.31			8	123.63	61.06	21.59
6	8	129.50	33.59	11.88			8	93.63	61.49	21.74
7	8	193.13	43.60	15.41		*	8	111.50	66.77	23.61
8	8	165.25	73.46	25.97		*	8	87.63	54.06	19.11
9	8	136.63	36.34	12.85		*	8	93.38	73.43	25.96
10	8	119.50	49.04	17.34			8	110.38	81.86	28.94
11	8	121.88	46.71	16.51			8	108.50	72.77	25.73
12	8	196.25	86.38	30.54		**	8	103.13	84.95	30.03
13	8	133.63	30.19	10.67			8	116.00	122.85	43.44
14	8	163.00	152.69	53.99			8	105.00	96.03	33.95
15	8	188.38	75.32	26.63			8	145.00	103.90	36.73

* Statistically significant at the 5% level vs placebo.

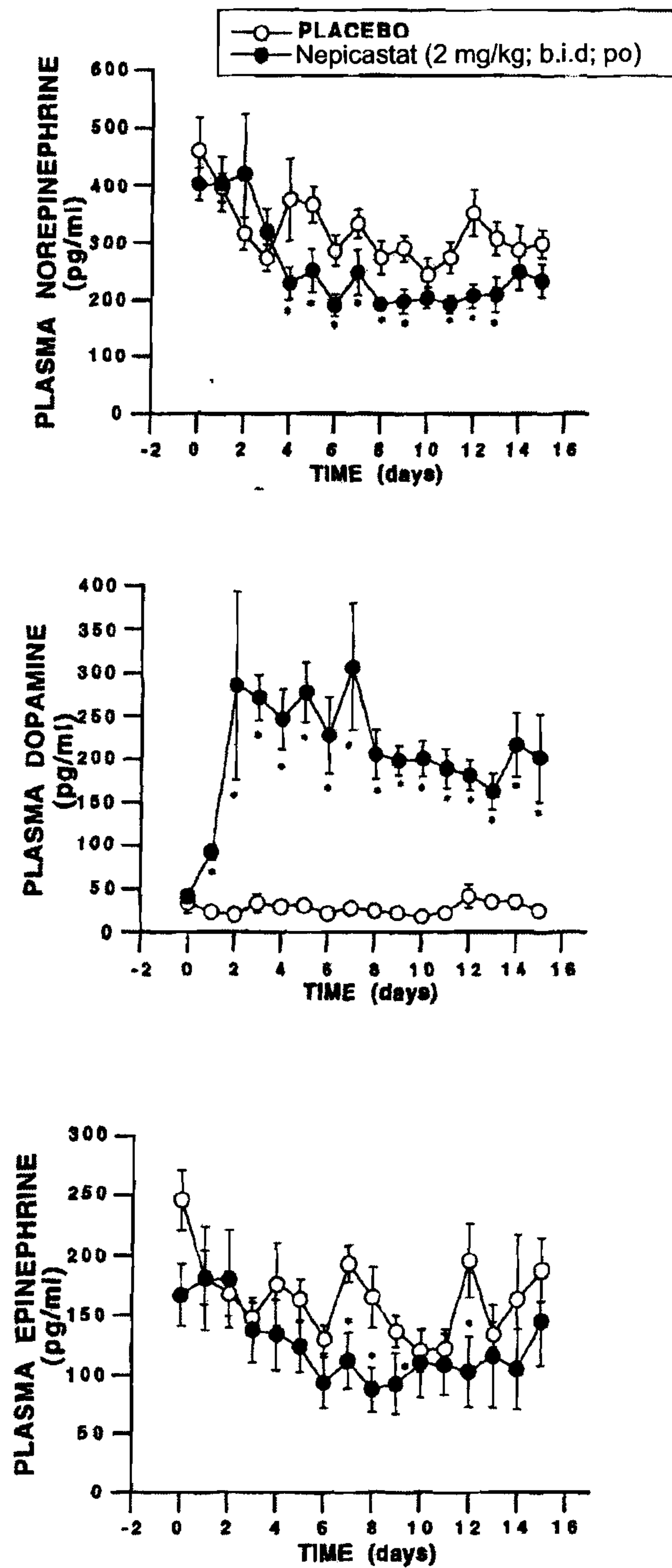
** Statistically significant at the 1% level vs placebo.

*** Statistically significant at the 0.1% level vs placebo.

SS = Steady state

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Figure 71



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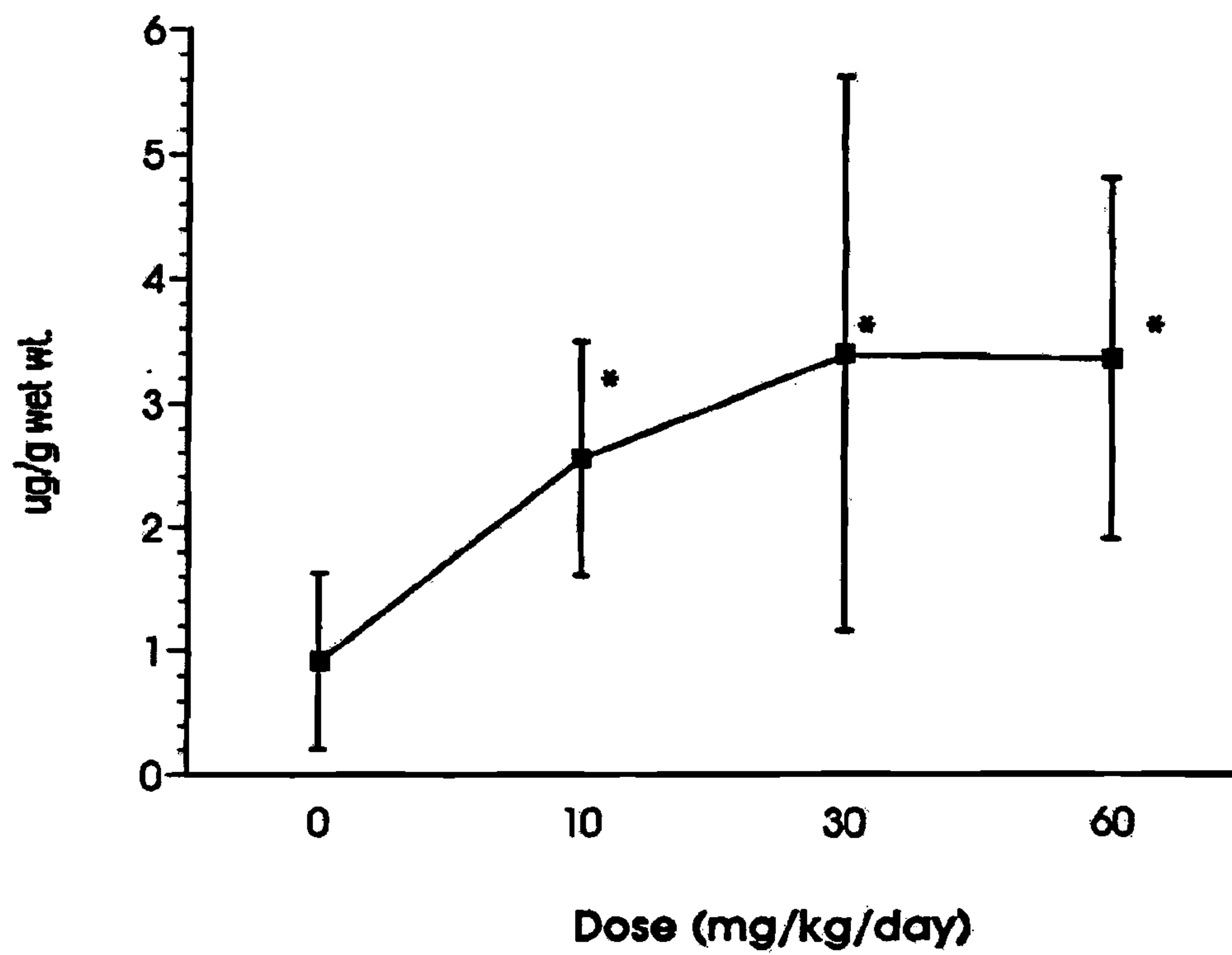
Figure 72

DAY	Nepicastat	ng/ml)	Std.dev	RS 47831 (ng/ml)	Std.Dev
0	BQL		NV	BQL	NV
1	300		57.5	BQL	4.80
2	361		114	BQL	8.87
3	351		88.6	BQL	12.6
4	298		135	BQL	NV
5	322		74.4	BQL	NV
6	327		85.2	BQL	NV
7	319		104	BQL	NV
8	327		100	BQL	NV
9	332		109	BQL	NV
10	348		105	BQL	NV
11	326		83.2	BQL	NV
12	347		104	BQL	NV
13	330		112	BQL	NV
14	313		97.0	BQL	NV
15	316		105	BQL	NV

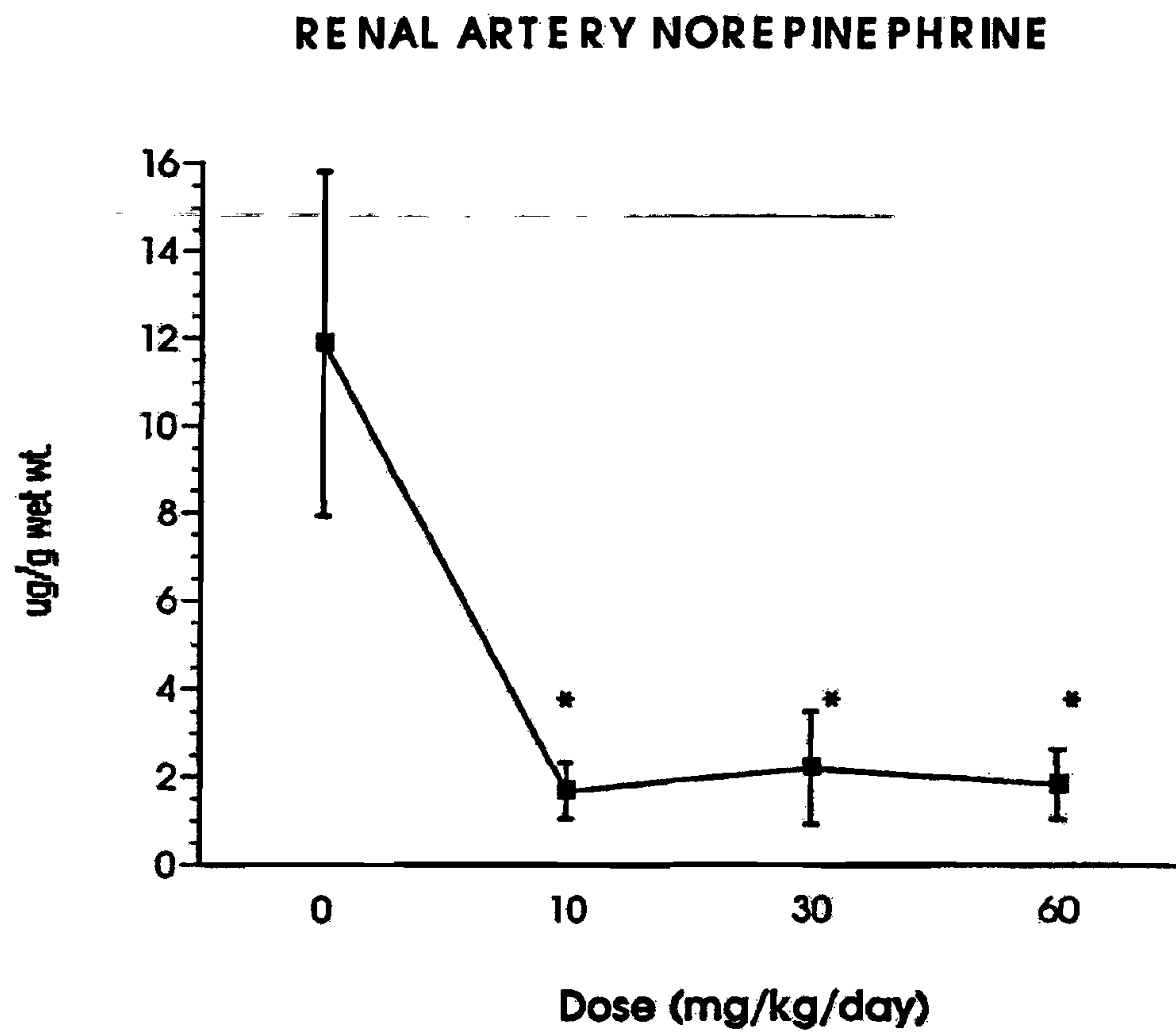
BQL = Below quantification level.

NV = No value.

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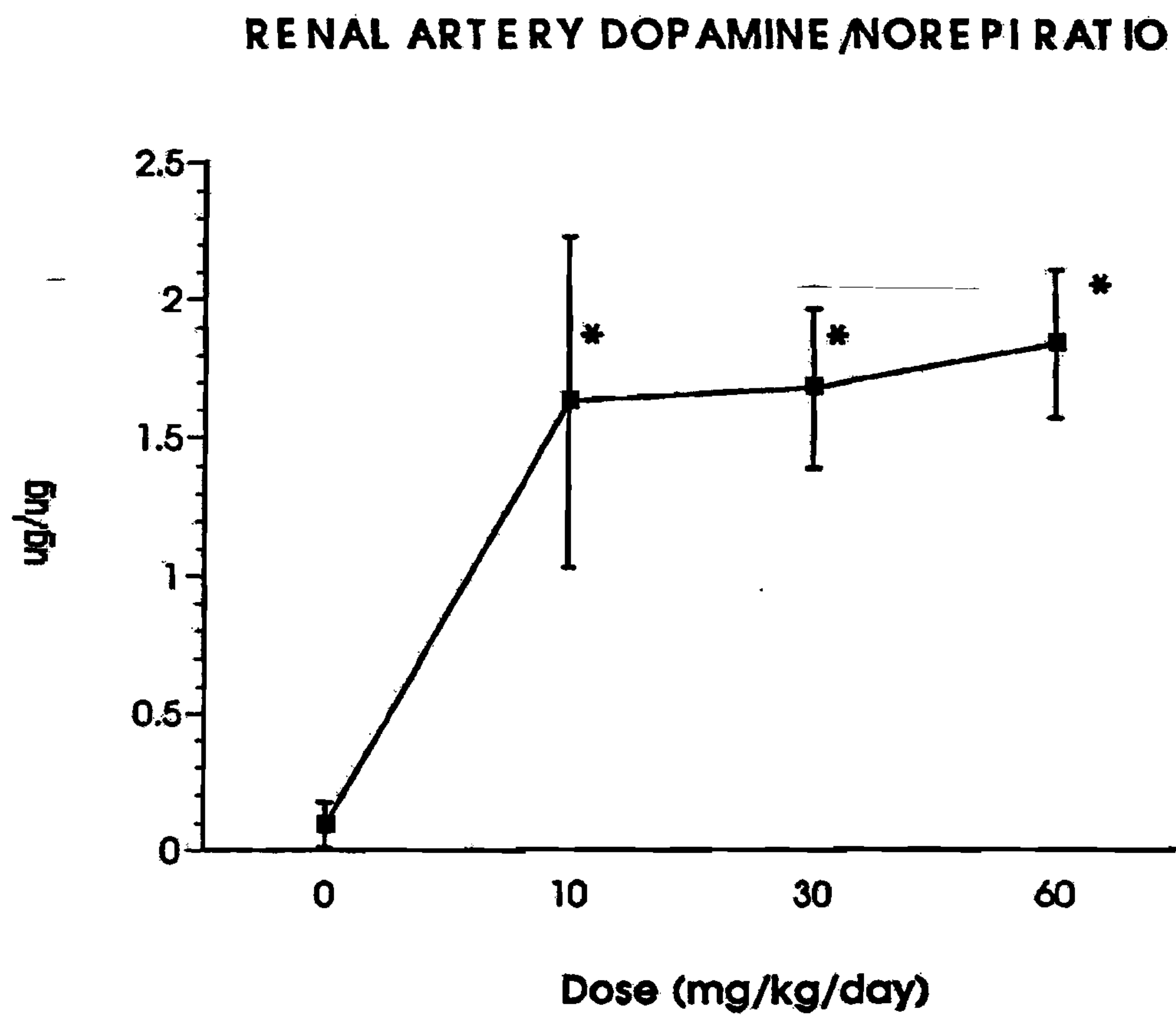
Figure 73**RENAL ARTERY DOPAMINE**

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Figure 74

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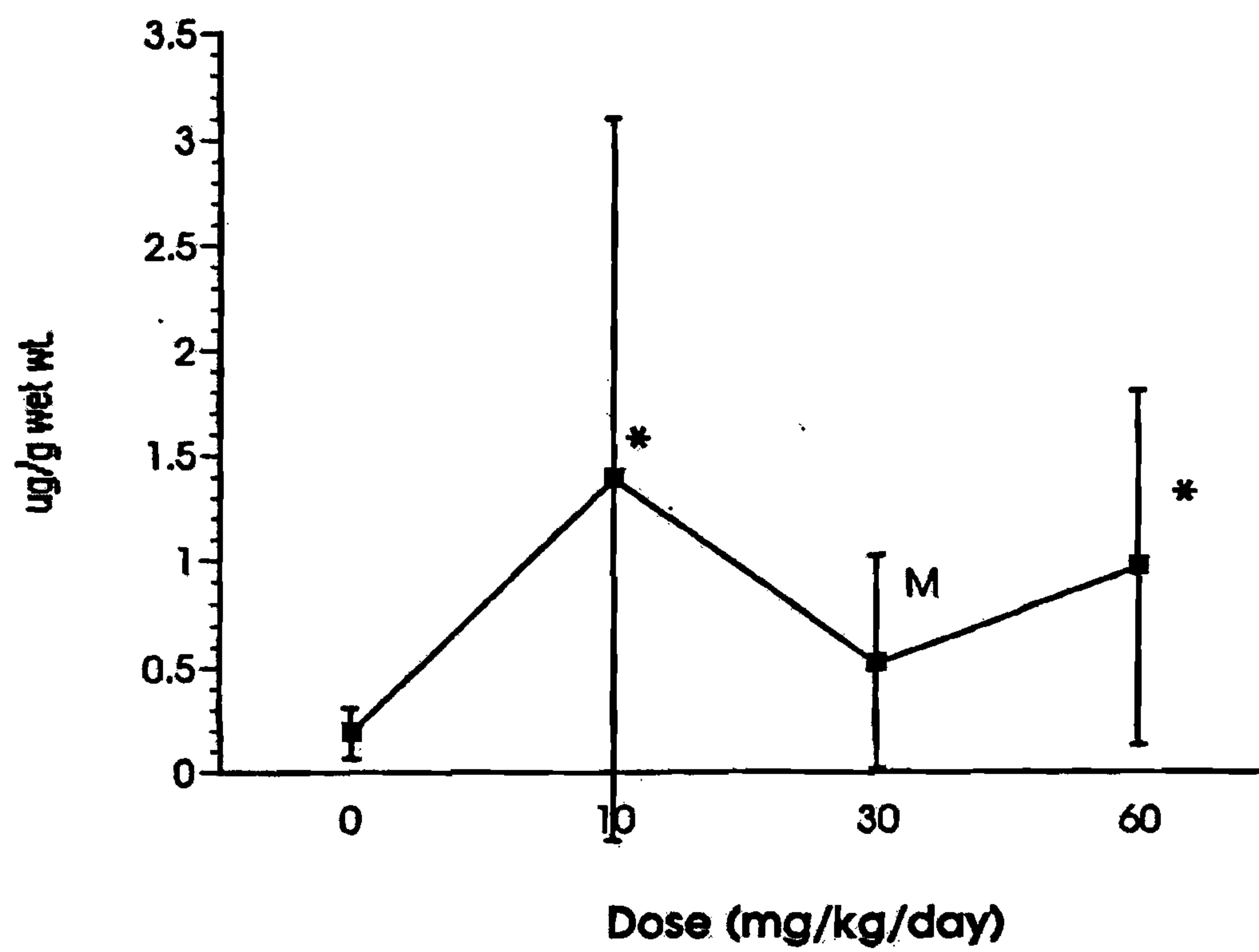
Figure 75



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Figure 76

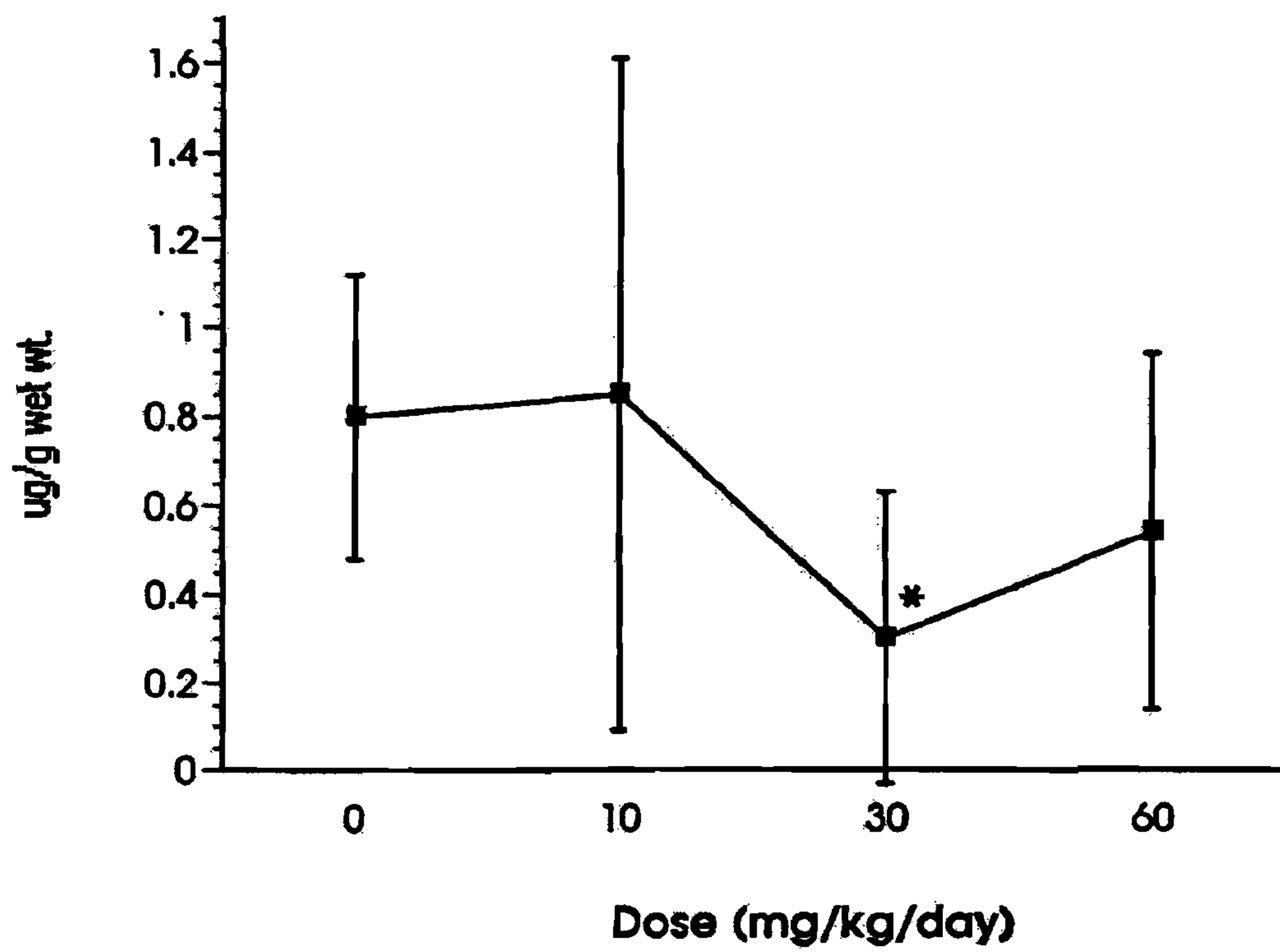
CEREBRAL CORTEX DOPAMINE



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Figure 77

CEREBRAL CORTEX NOREPINEPHRINE



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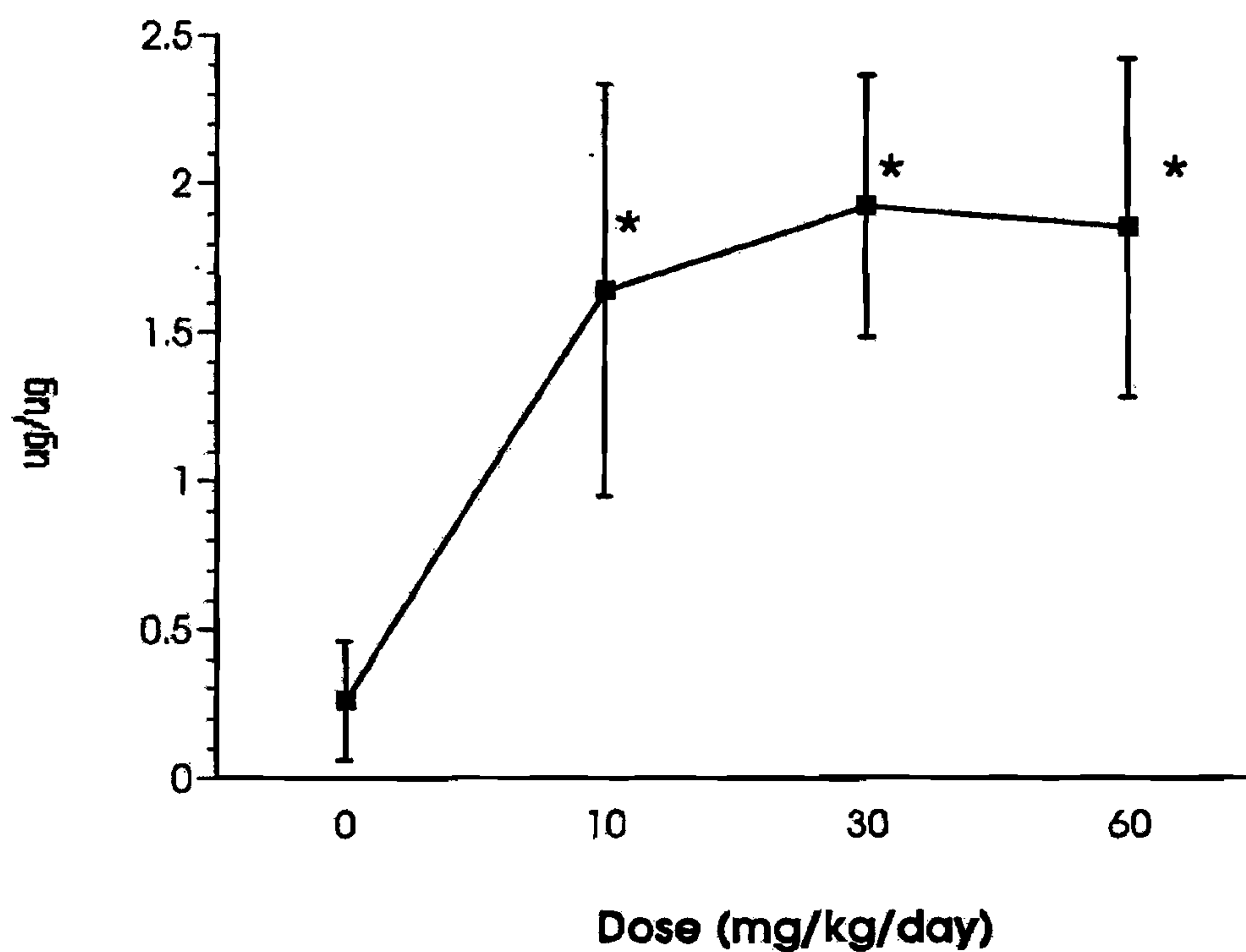
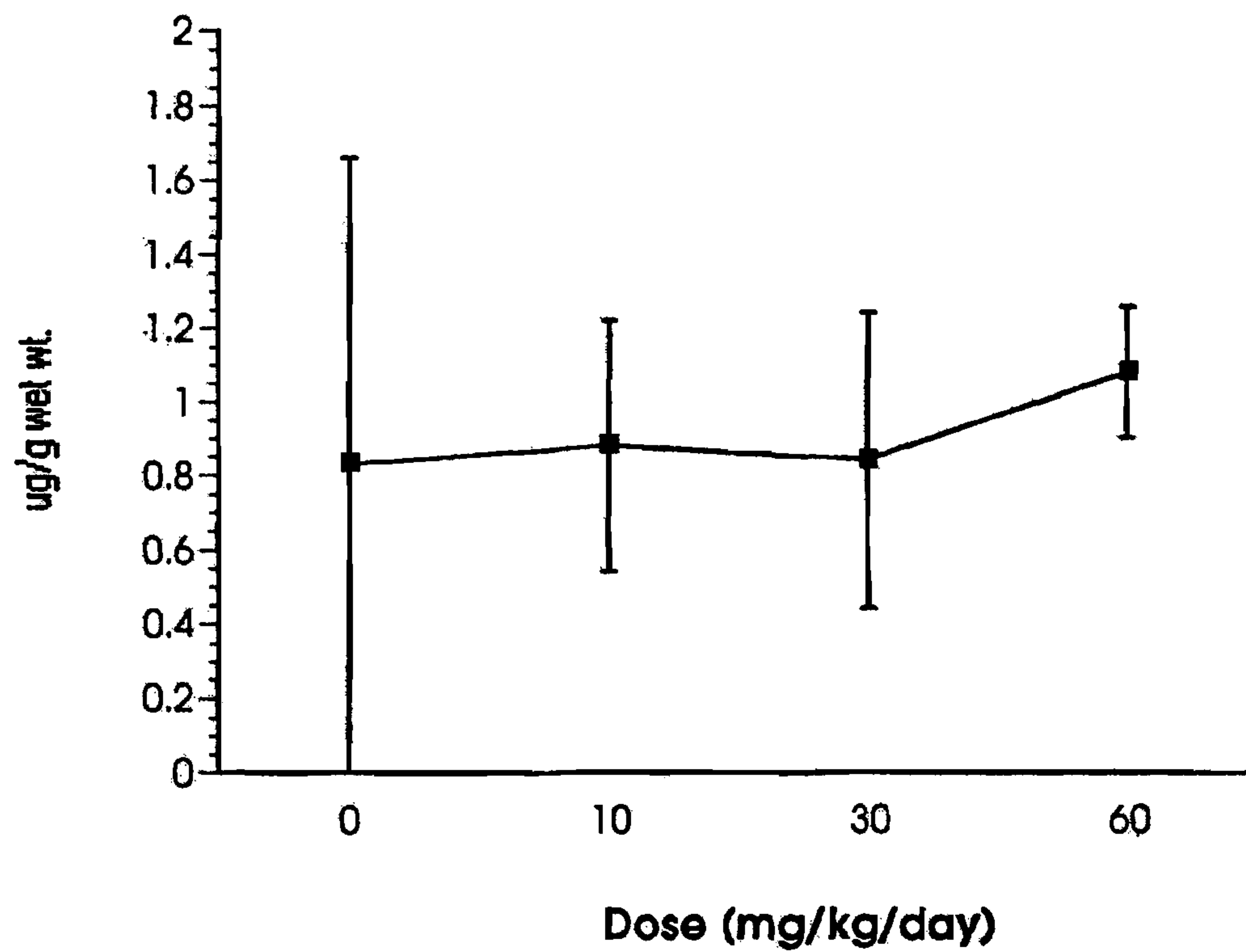
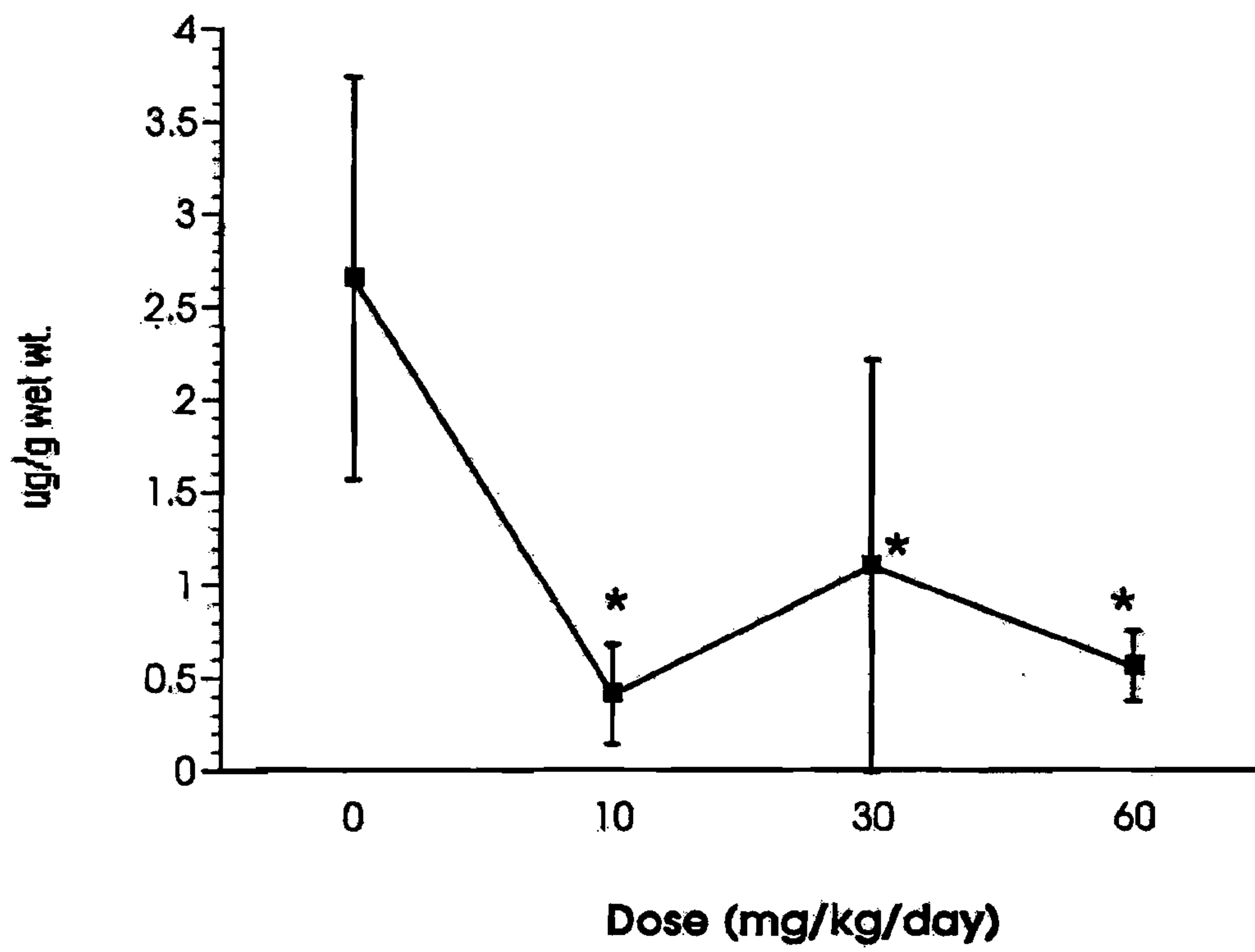
Figure 78**CEREBRAL CORTEX DOPAMINE/NOREPI RATIO**

Figure 6. Dopamine/norepi ratio in the cerebral cortex in dogs. Dogs were orally administered 0, 5, 15, or 30 mg/kg capsules b.i.d. for 4.5 days and tissue was harvested 6 hr after the final administration. N=8, *p<0.01 vs. placebo (mean $\pm$ SD).

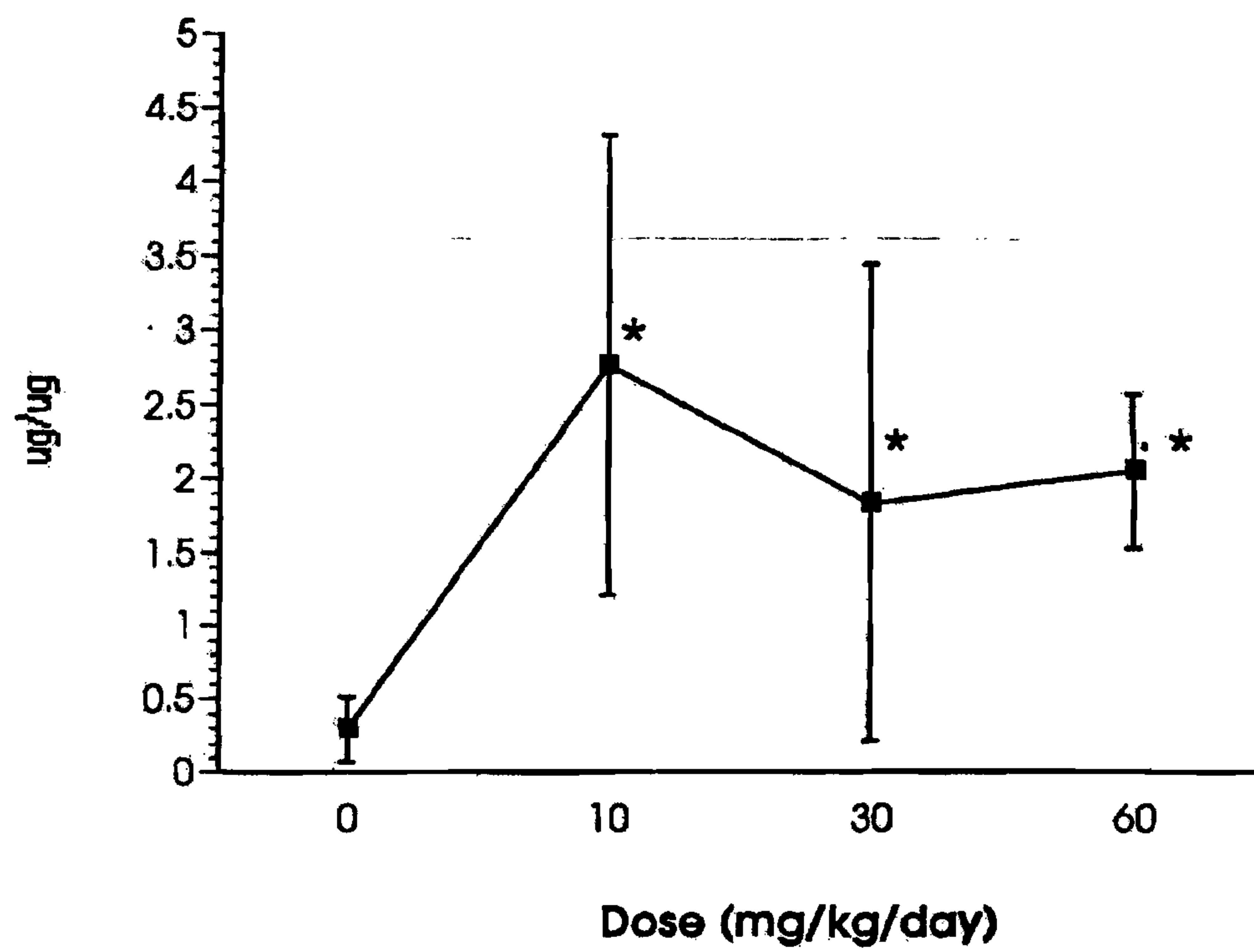
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Figure 79**LEFT VENTRICLE DOPAMINE**

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Figure 80**LEFT VENTRICLE NOREPINEPHRINE**

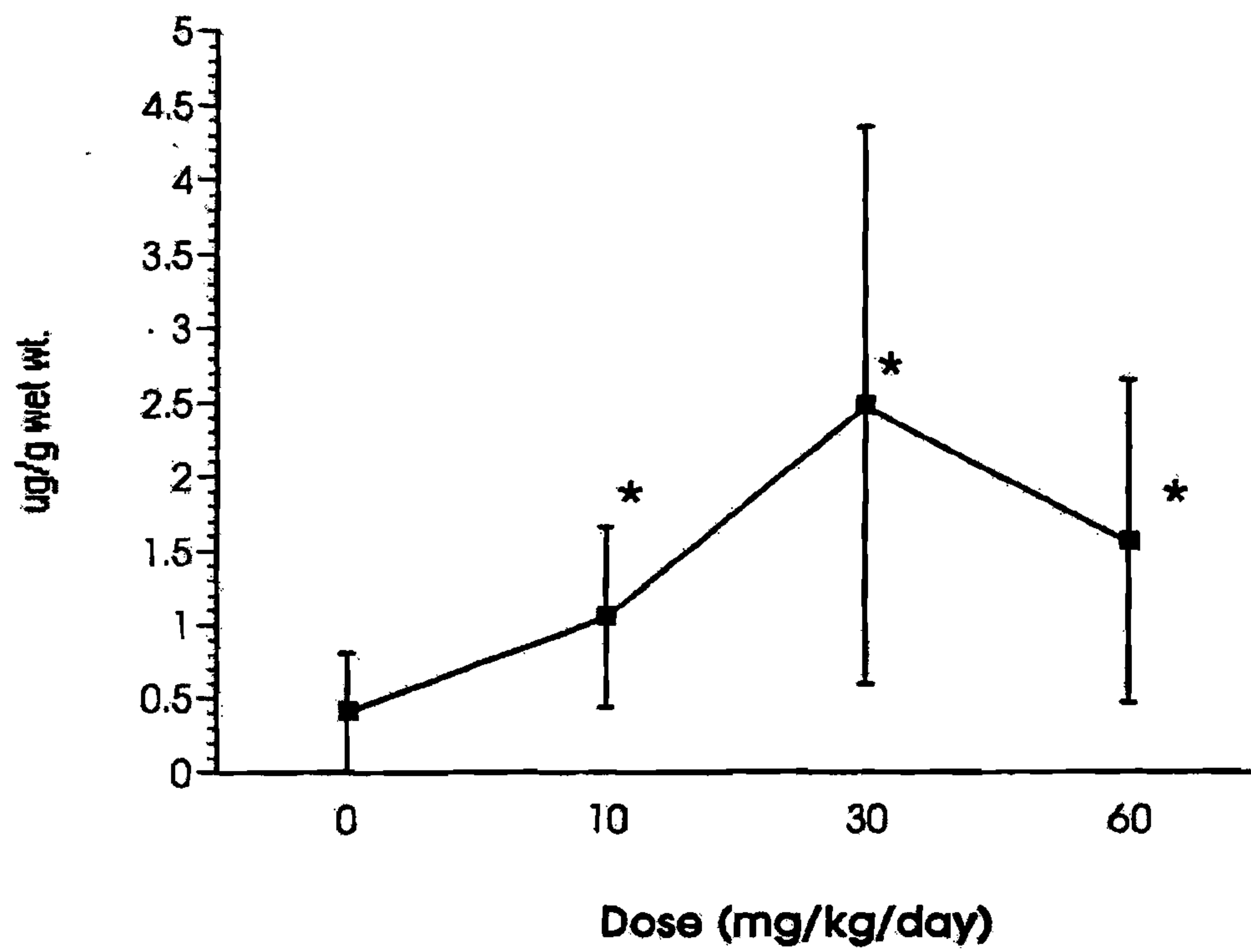
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Figure 81**LEFT VENTRICLE DOPAMINE/NOREPI RATIO**

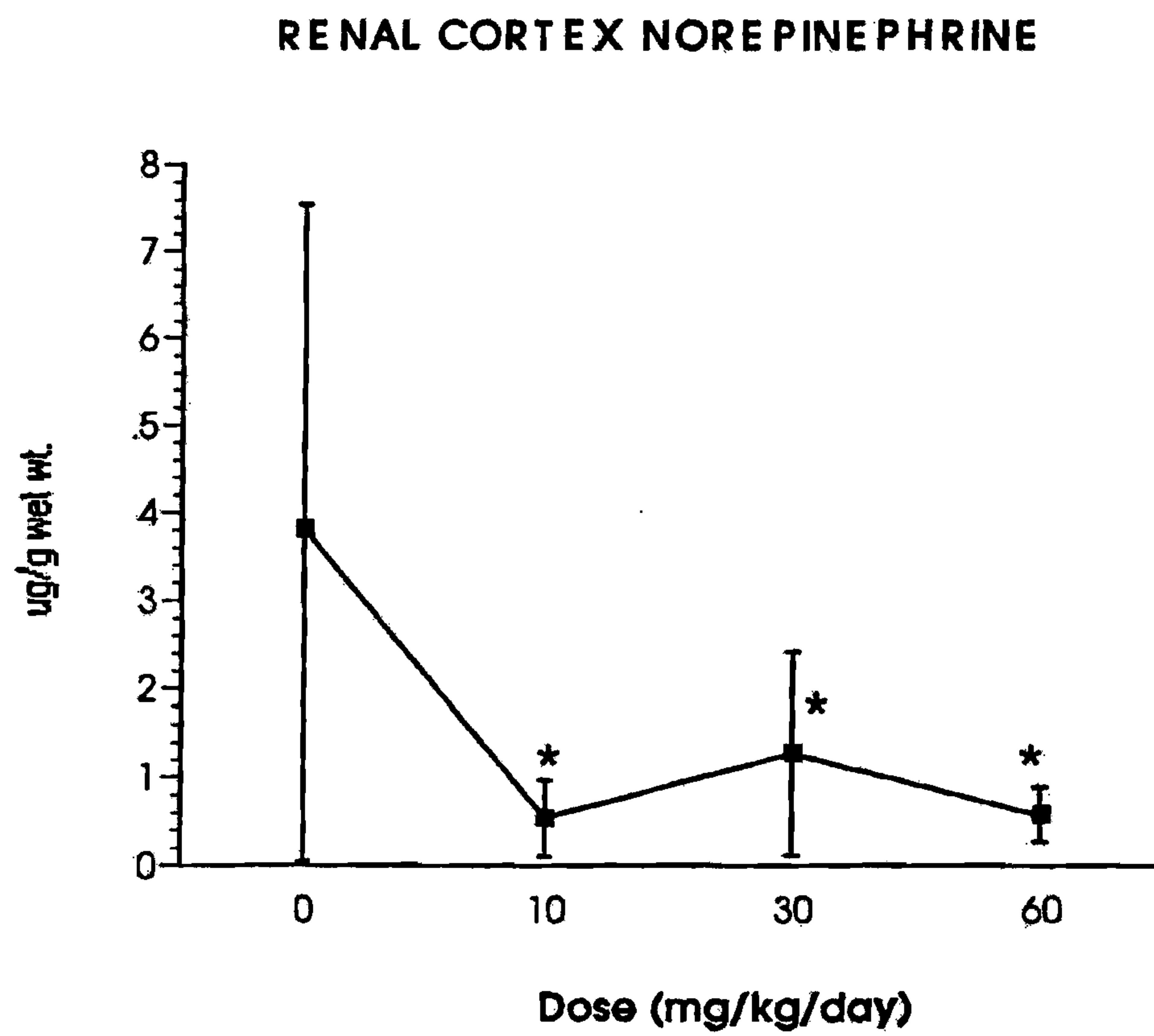
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Figure 82

RENAL CORTX DOPAMINE

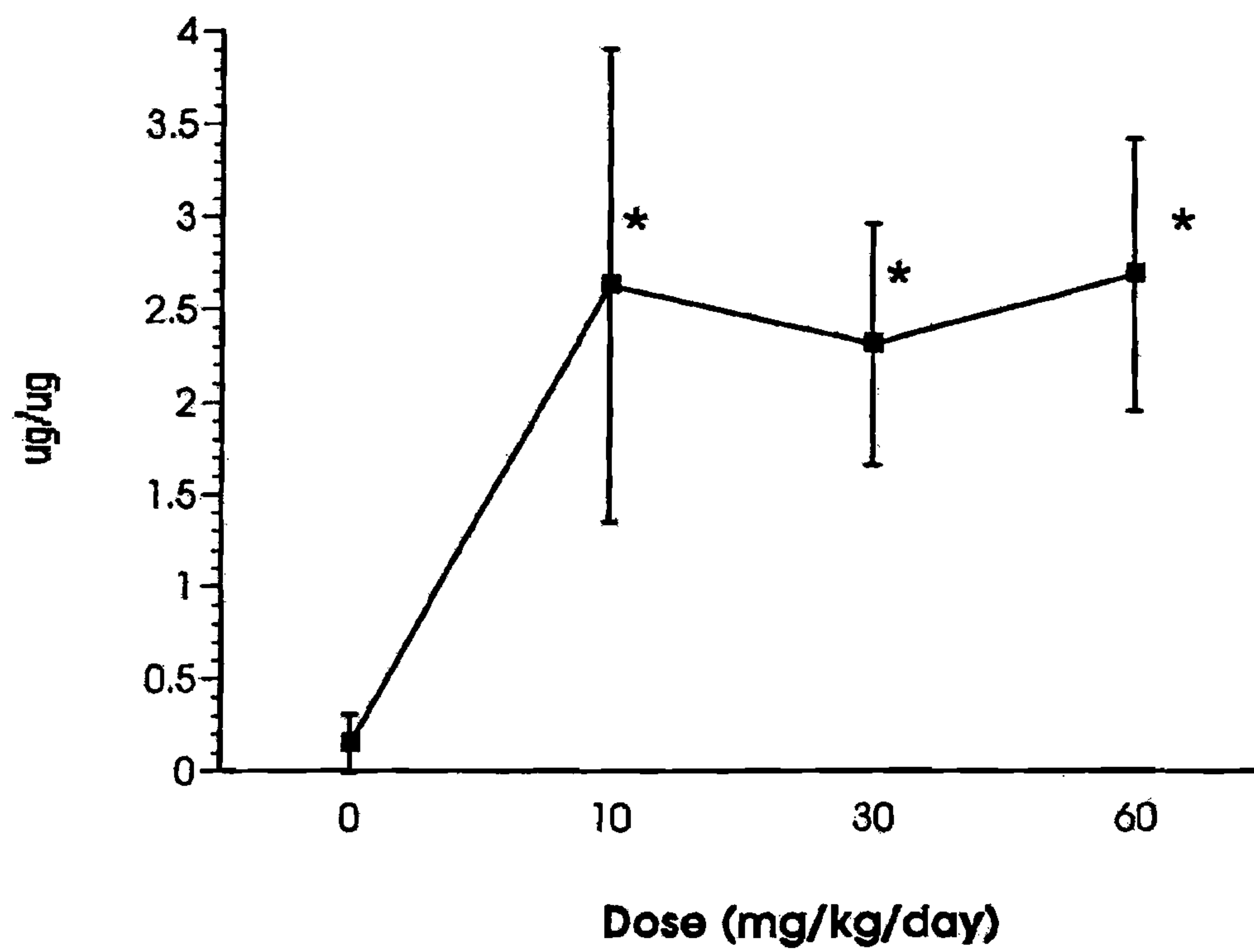


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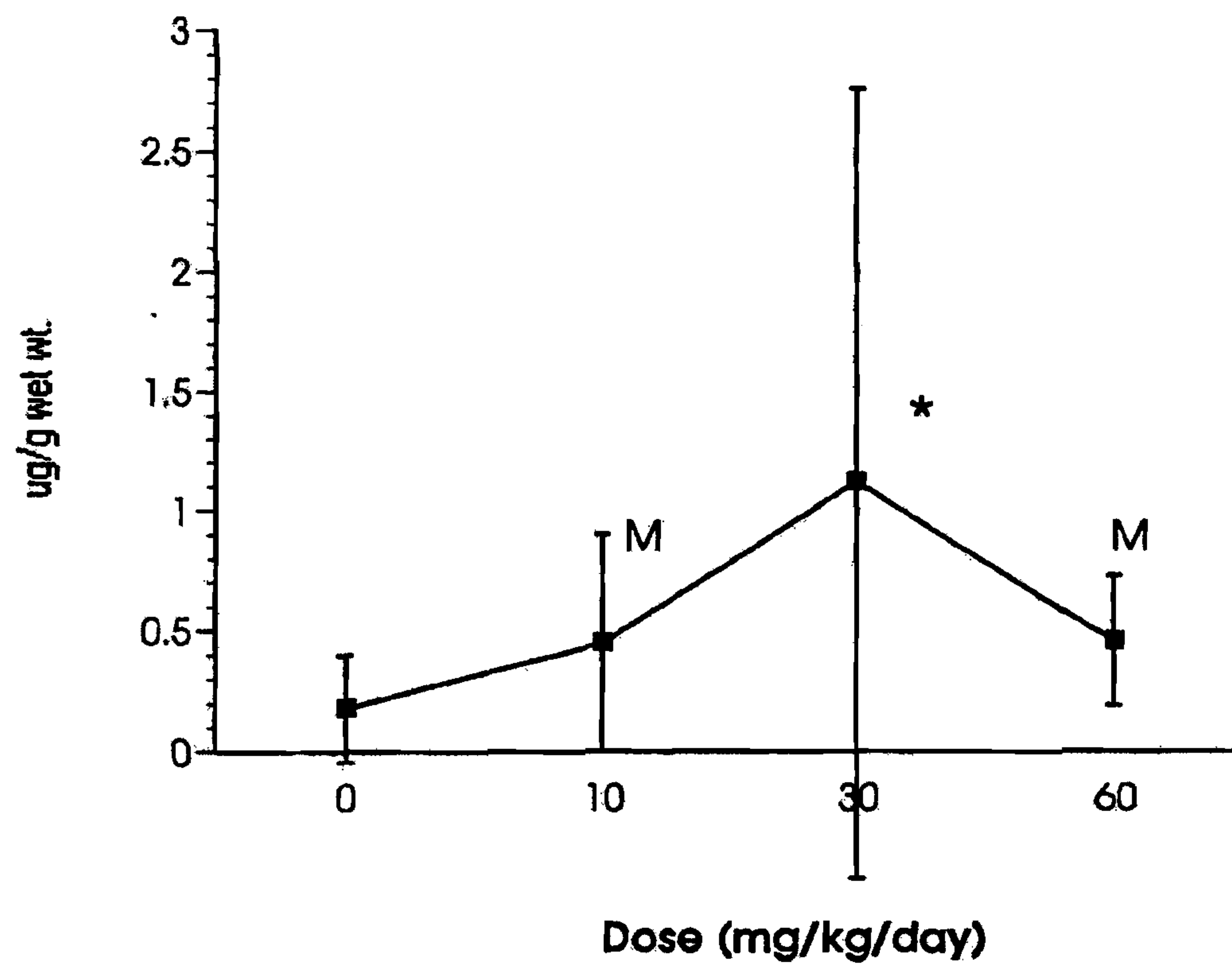
Figure 83

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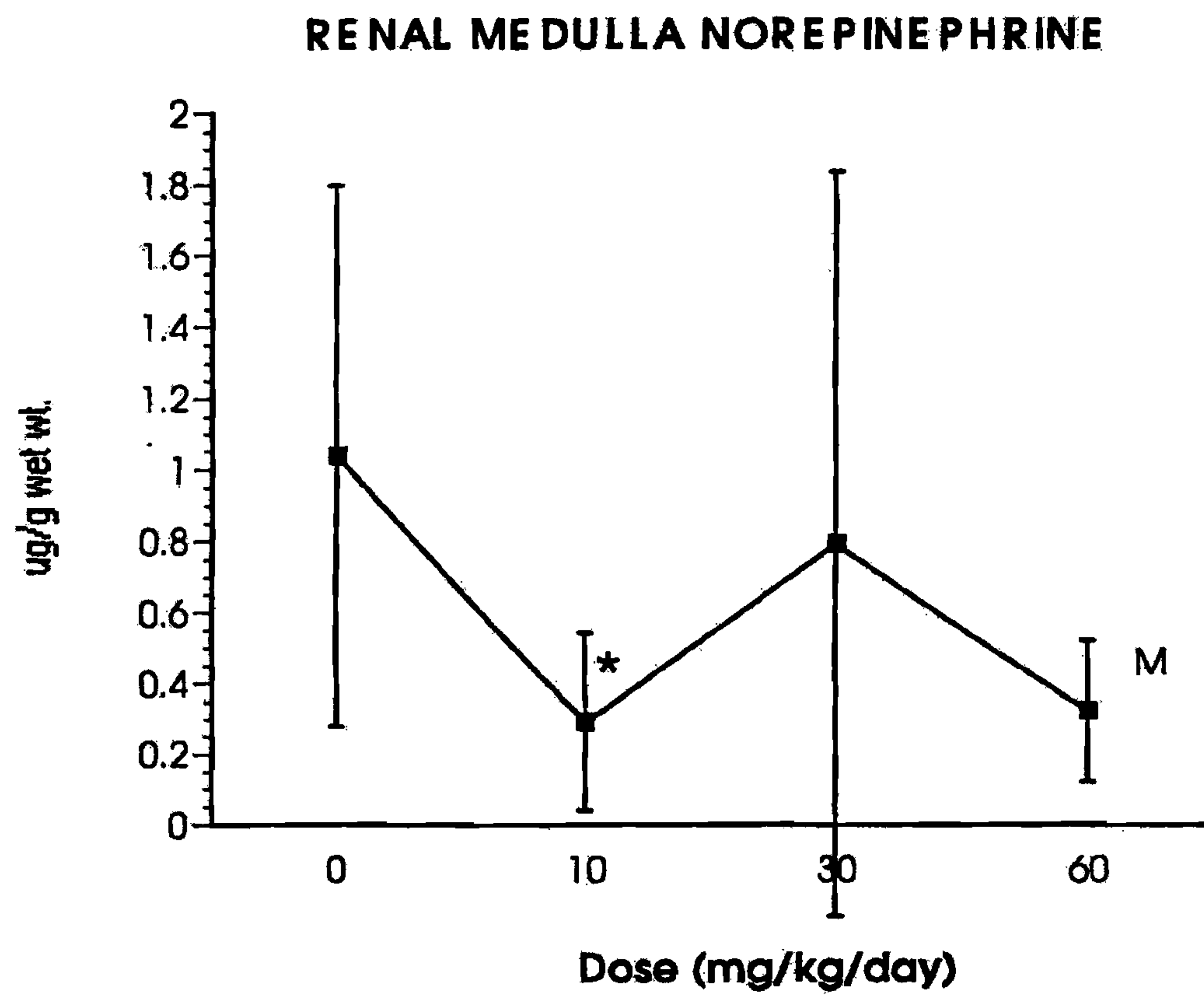
Figure 84
RENAL CORTX DOPAMINE/NOREPI RATIO



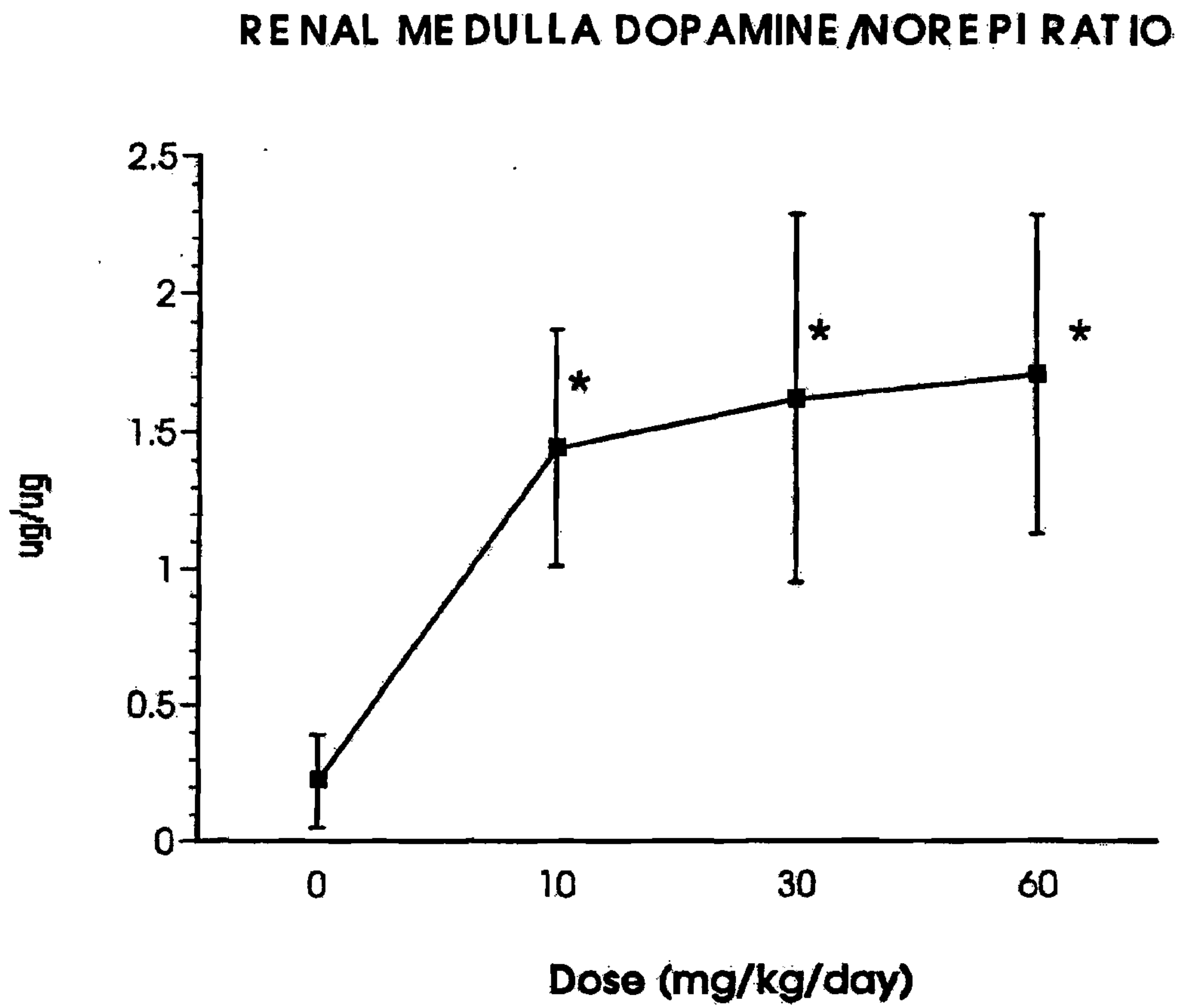
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Figure 85**RENAL MEDULLA DOPAMINE**

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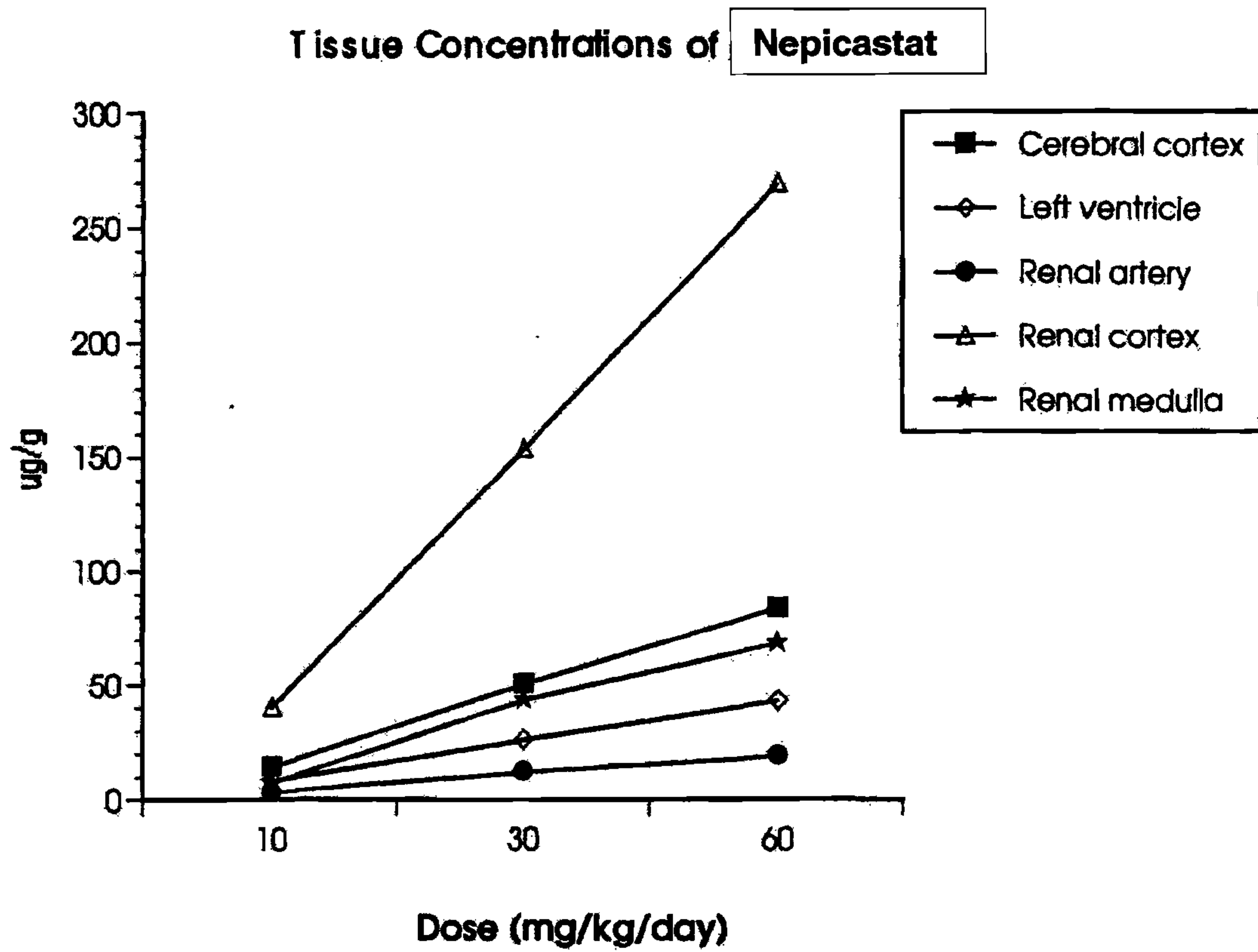
Figure 86

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Figure 87

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Figure 88



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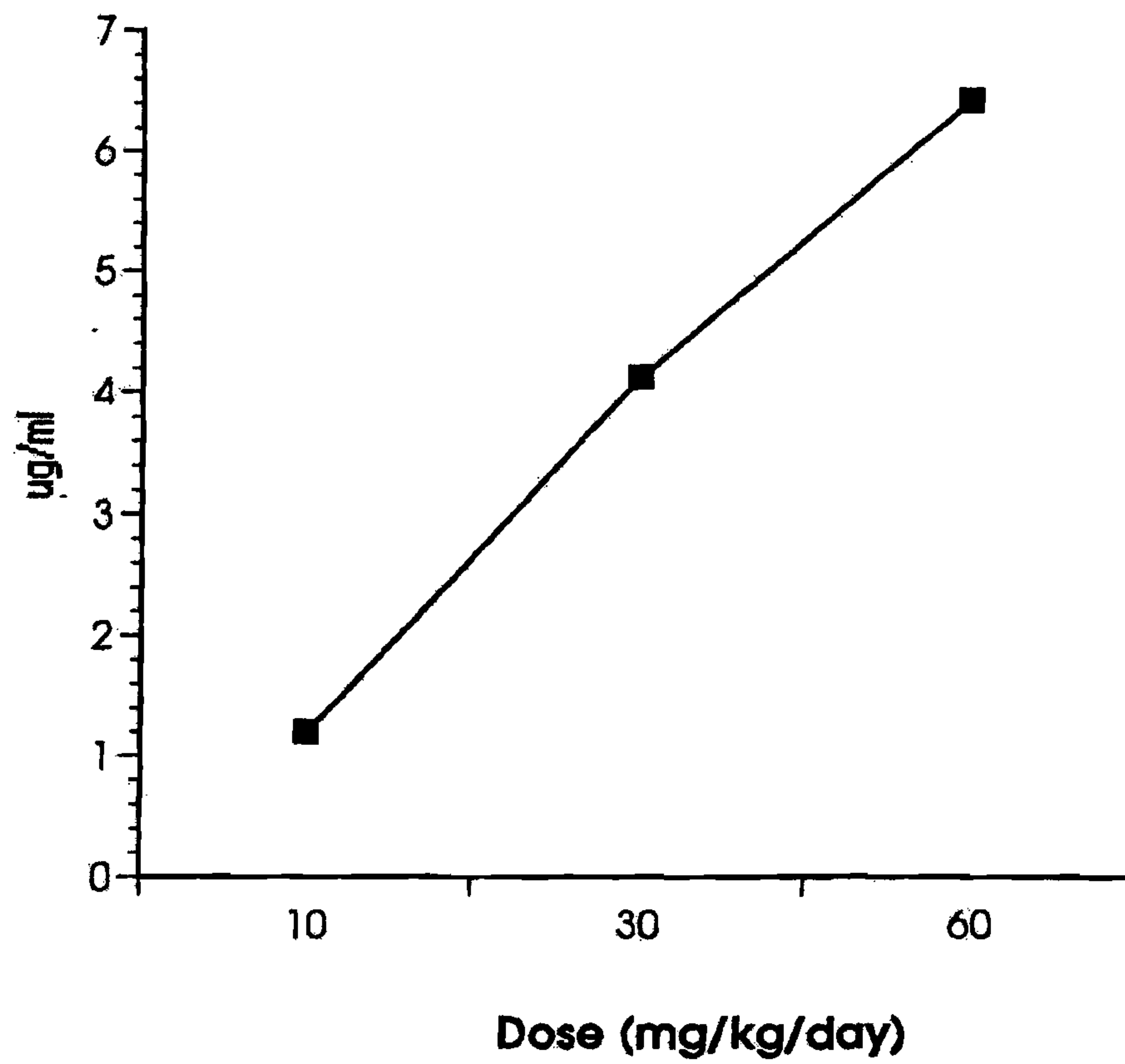
Figure 89**Day 5 Plasma Concentrations of Nepicastat**

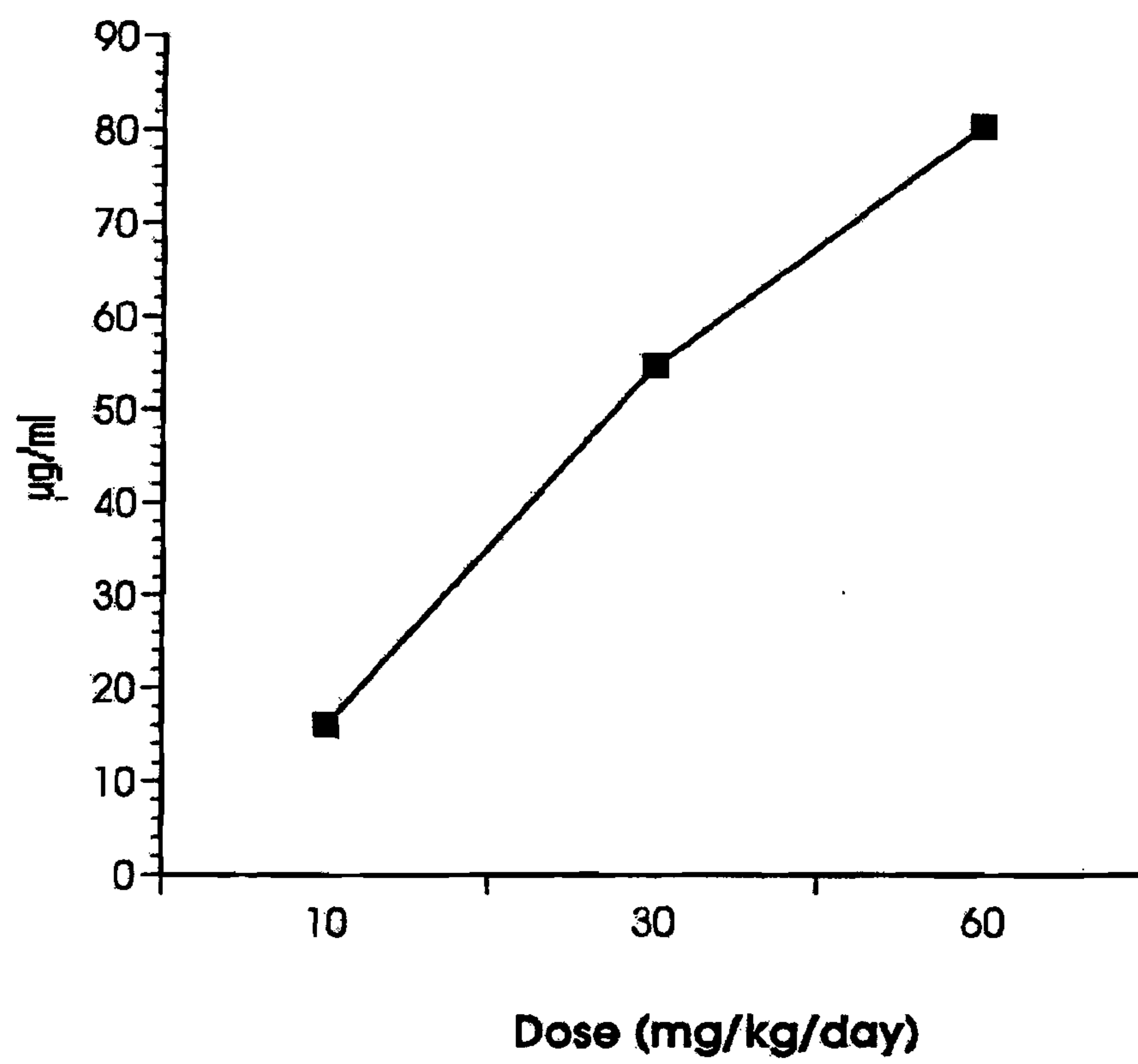
Figure 90**AUC of Day 4 Plasma Concentrations of****Nepicastat**

Figure 91

Unknown #	Dose		Kd (nM)	Bmax (fmoles/mg protein)
	(mg/kg/day)	Nepicastat		
18	0		0.38	35.49
8	0		0.36	33.39
9	0		0.35	65.41
28	0		0.37	48.22
17	0		0.36	29.96
26	0		0.43	21.50
4	0		0.35	41.58
11	0		0.38	31.99
	MEAN		0.37	38.44
	SEM		0.01	4.76
19	10		0.36	37.06
1	10		0.46	23.49
13	10		0.34	39.39
32	10		0.32	25.67
16	10		0.39	38.92
25	10		0.39	50.44
21	10		0.30	31.11
	MEAN		0.37	35.15
	SEM		0.02	3.49

Figure 92

ENZYME	N	CONCENTRATION OF Nepicastat (μ M)	% INHIBITION OF ENZYME	IC ₅₀ * (μ M)
Tyrosine hydroxylase	2	100	11	>100
NO synthase (constitutive)	2	10	6	>10
NO synthase (inducible)	2	10	-1	>10
Phosphodiesterase III	2	100	-9	>100
Phospholipase A ₂	2	300	3	>300
Neutral endopeptidase (human)	2	10	-12	>10
CA ²⁺ /calmodulin dependent protein kinase II	2	100	53	~100
Acetyl CoA synthetase	2	100	-4	>100
Acyl CoA-cholesterol acyl transferase	2	100	6	>100
HMG-CoA reductase	2	30	16	>30
Protein kinase (non-selective)	2	300	39	>300
Cyclooxygenase-I	2	300	44	>300

* Approximate estimation of IC₅₀

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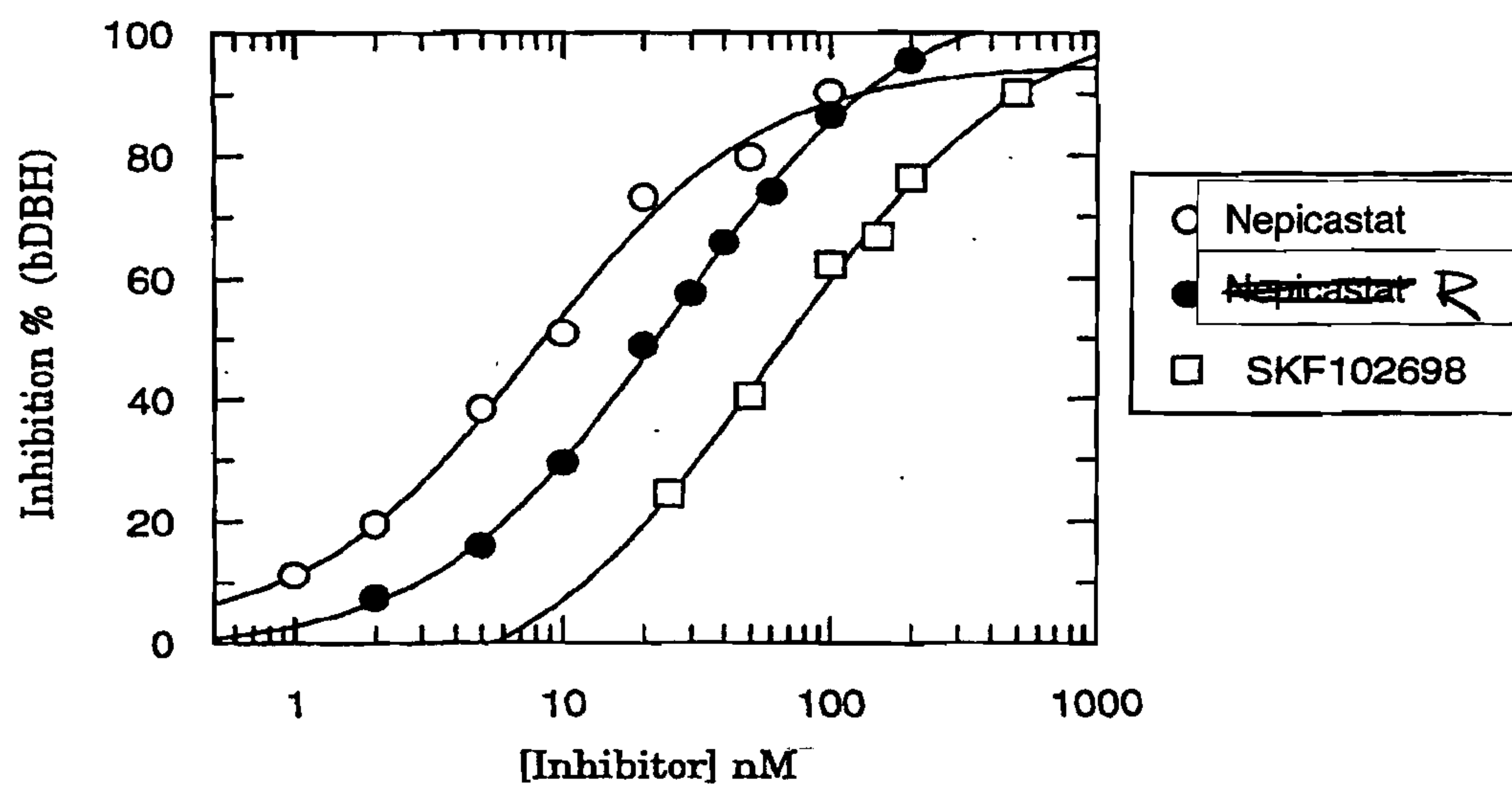
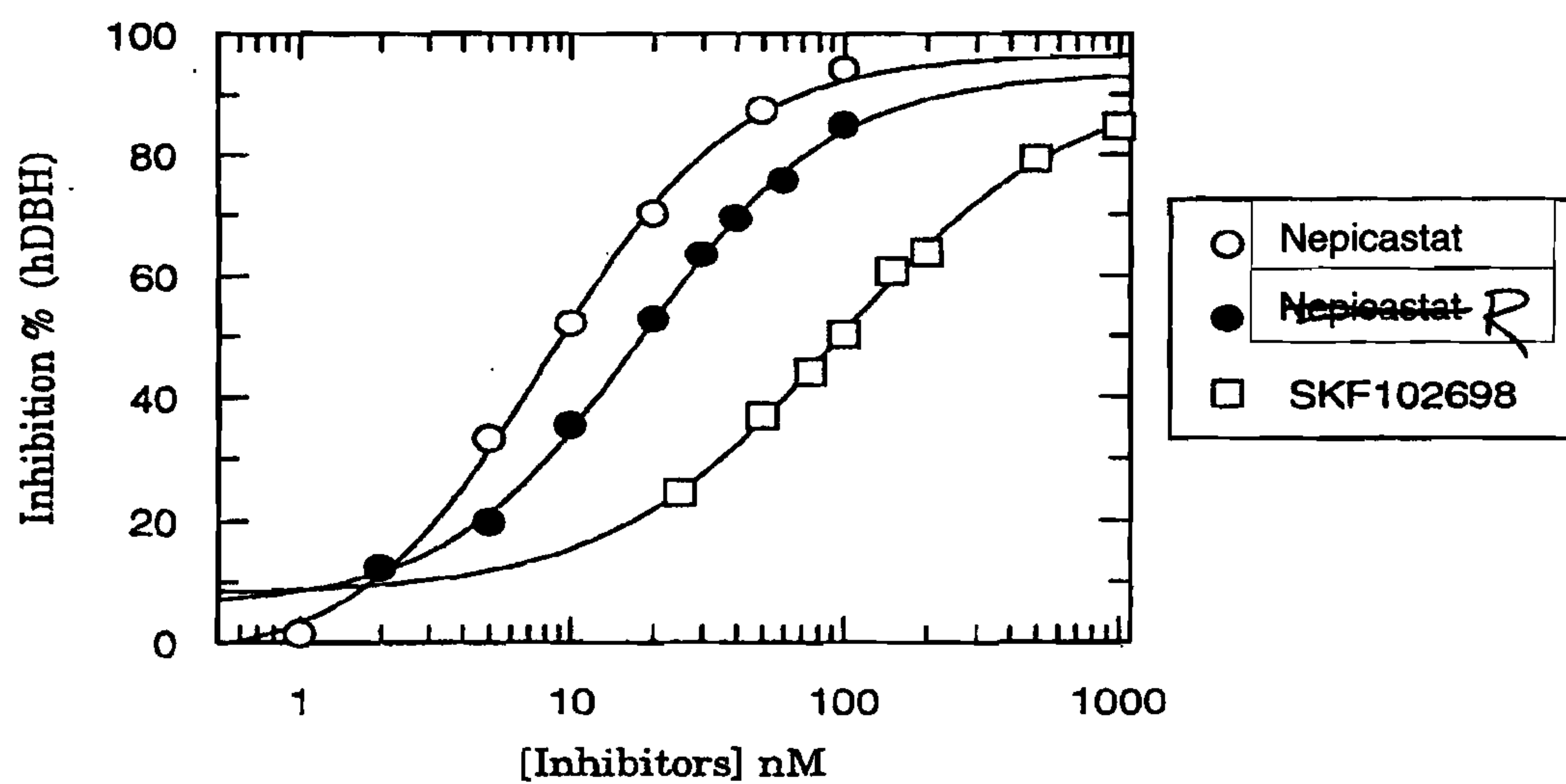
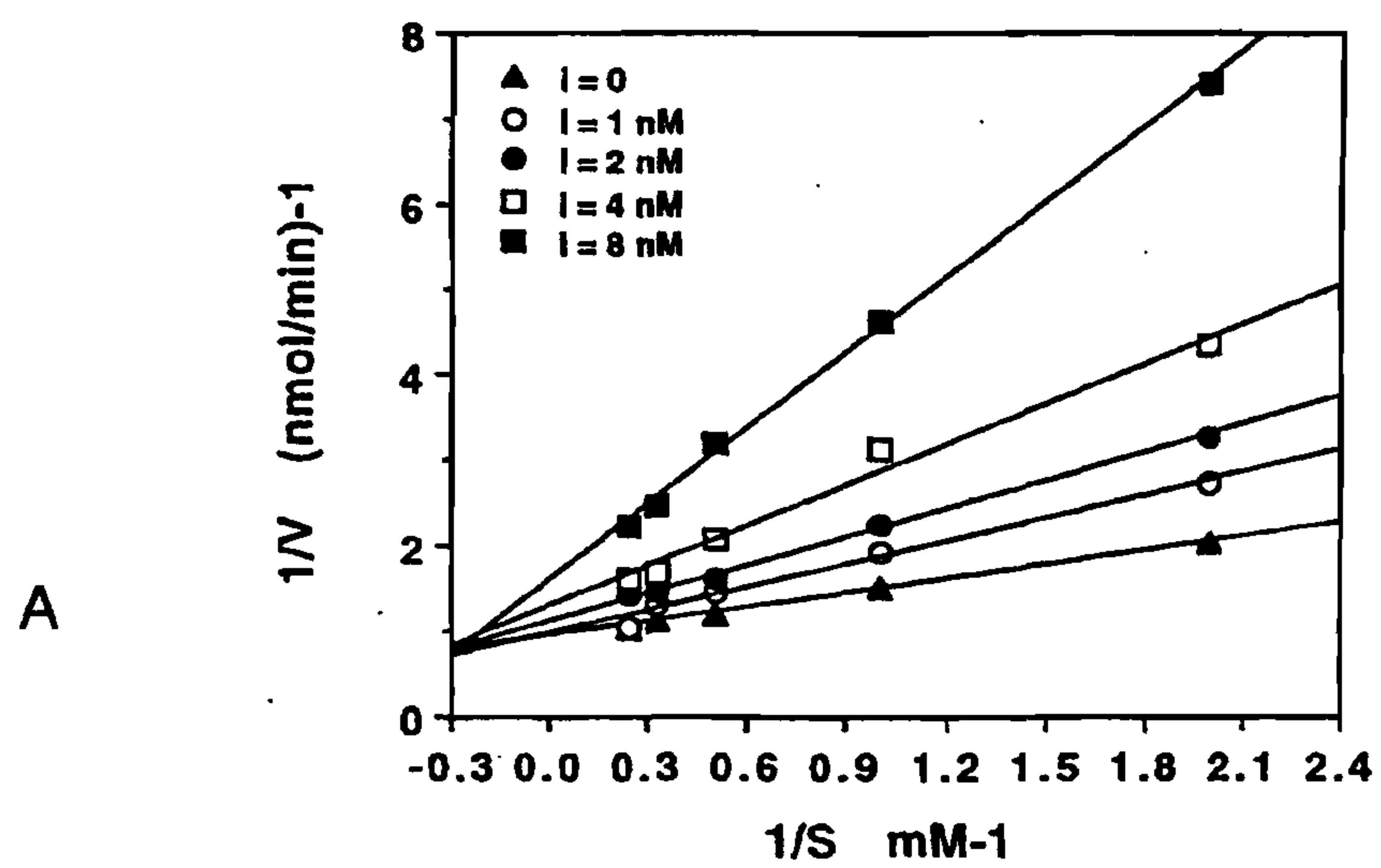
Figure 93**Figure 94**

Figure 95

	SKF 102698 nM	S nM	R nM
Enzyme			
Bovine (Sigma)	67.0 ± 4.2	8.5 ± 0.8	25.1 ± 0.6
Human (cell line)	85.0 ± 3.7	9.0 ± 0.8	18.3 ± 0.6

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Figure 96



B

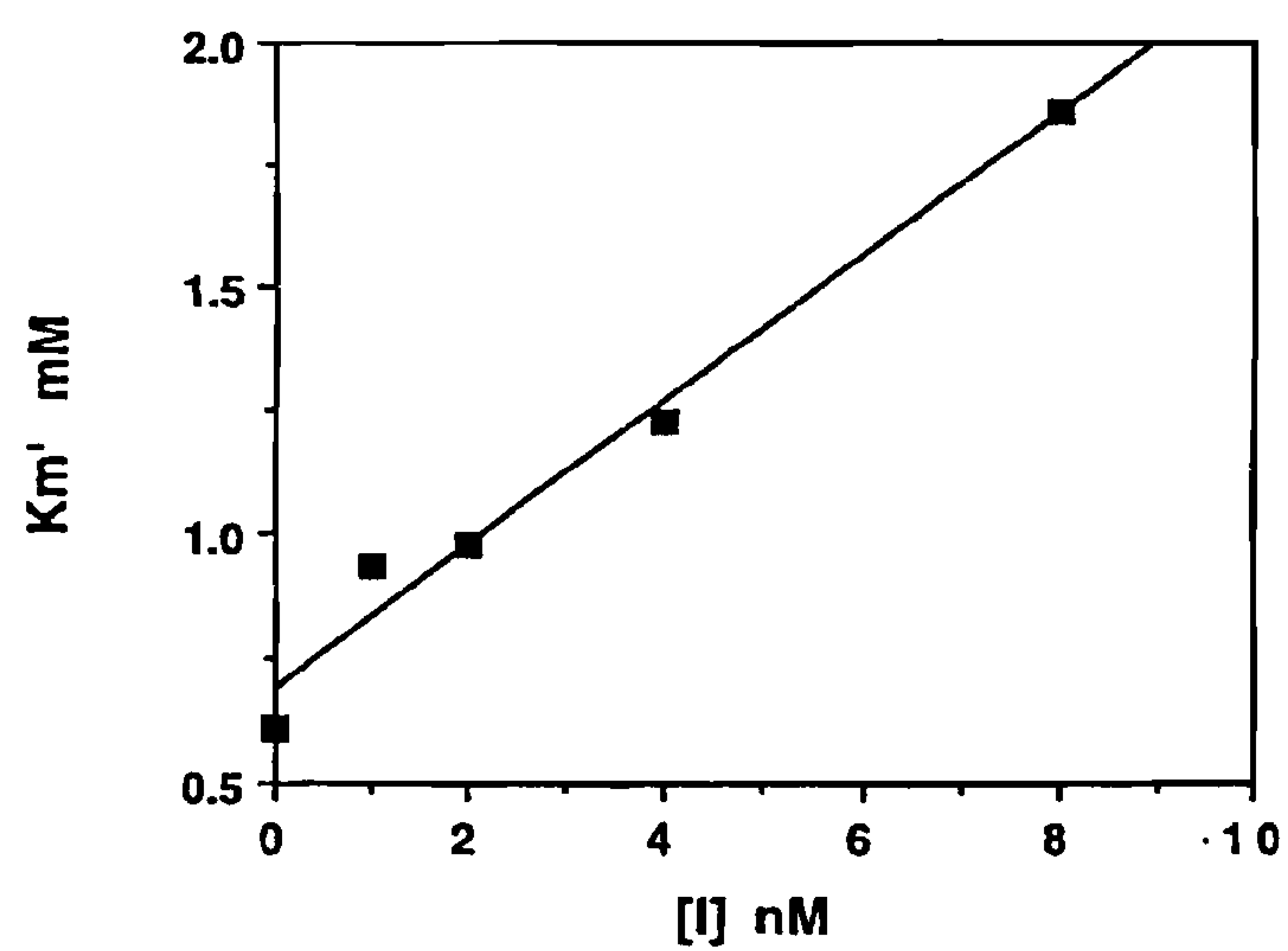


Figure 97**TABLE 1**

Receptor	Radioligand	Tissue	Drug defining NSB
alpha1a (cl)	[3H]prazosin	alpha1a expressed in rat fibroblast	phentolamine
alpha1b (cl)	[3H]prazosin	alpha1b expressed in rat fibroblast	phentolamine
alpha1c (cl)	[3H]prazosin	alpha1c expressed in rat fibroblast	phentolamine
alpha2a	[3H]rauwolscine	rabbit spleen	phentolamine
alpha2b	[3H]rauwolscine	rat kidney	phentolamine
beta1	[3H]CGP-12177	rabbit lung	isoproterenol
beta2	[3H]CGP 12177	rat lung	Isoproterenol
M1	[3H]pirenzepine	rat cortex	atropine
D1	[3H]SCH23990	rat striatum	butaclamol
D2	[3H]spiperone	rat striatum	butaclamol
mu	[3H]DAGO	guinea-pig brain	naloxone
5-HT1a	[3H]8-OH-DPAT	rat brain	5-HT
5-HT1c/2c	[3H]mesulergine	pig choroid plexus	methylsergide
5-HT2a	[3H]ketanserin	rat cortex	methylsergide

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Figure 98

TABLE 2
RECEPTOR PROFILE OF **Nepicastat**

	N	P _{ki} (SEM)	nH (SEM)	I.O.P. Protocol	Notebook (Page)
alpha1a (cloned)	3	6.94 (0.12)	1.31 (0.13)	20-93-0563-s	18027 (58-71)
alpha1b (cloned)	3	6.86 (0.03)	0.99 (0.04)	20-93-0561-3	18027 (58-71)
alpha1c (cloned)	3	6.69 (0.12)	1.04 (0.17)	20-93-0562-s	18027 (58-71)
alpha2a	3	6.19 (0.15)	1.20 (0.31)	20-89-0153-s	18027 (58-71)
alpha2b	3	5.99 (0.37)	0.91 (0.33)	20-89-0154-s	18027 (58-71)
beta1	3	5.38 (0.17)	0.87 (0.08)	20-89-0155-s	18027 (58-71)
beta2	3	6.02 (0.26)	0.77 (0.14)	20-89-0156-s	18027 (58-71)
M1	3	4.3 (0.07)	0.96 (0.04)	20-89-0163-s	18191 (123)
D1	3	< 5		20-89-0160-s	18498 (130)
D2	3	< 5		20-89-0161-s	18498 (130)
mu	3	4.46 (0.13)	1.15 (0.08)	20-89-0315-s	18498 (130)
5-HT1a	3	3.9 (0.15)	0.82 (0.03)	20-89-0168-s	18191 (123)
5-HT2c	3	4.3 (0.17)	0.80 (0.07)	20-89-0169-s	18028 (49)
5-HT2	3	4.16 (0.12)	0.86 (0.17)	20-89-0170-s	18028 (49)

Values are the mean (sem) of at least N determinations.

Figure 99

VARIABLE	GROUP	N	MEAN	STD DEV	P-LEVEL*
TEMPERATURE	Vehicle	6	36.7	0.20	0.001
	30 mg/kg	5	36.1*	0.56	
	100 mg/kg	5	35.5*	2.10*	
	300 mg/kg	6	35.8#	0.27	

Nepicastat
Nepicastat
Nepicastat

Figure 100

SYNTEX Report 513-Nepicastat

GROUP 1 - VEHICLE		TREATMENT DAY	
CLINICAL DESCRIPTION / LOCATION		PT1	1
ANIMALS ALIVE AT END OF INTERVAL		6	0
ANIMALS EXAMINED DURING INTERVAL		6	6
NORMAL		6	6
ANALGESIA TEST NORMAL		-	6
ATAXIA TEST NORMAL		-	6
CORE BODY TEMPERATURE		-	6
CORNEA REFLEX NORMAL		-	6
GRIP STRENGTH NORMAL		-	6
INDUCED ACTIVITY NORMAL		-	6
ORIENTATION NORMAL		-	6
PASSIVITY TEST NORMAL		-	6
PINNA REFLEX NORMAL		-	6
RIGHTING REFLEX NORMAL		-	6
KILLED BY DESIGN		-	6
STUDY PHASE: PT-PRETREATMENT			

Figure 101

GROUP 2 - 30 MG/KG Nepicastat		TREATMENT DAY	
CLINICAL DESCRIPTOR / LOCATION		PT1	5
ANIMALS ALIVE AT END OF INTERVAL		6	0
ANIMALS EXAMINED DURING INTERVAL		6	5
NORMAL		6	5
ANALGESIA TEST NORMAL		-	5
ATAxia TEST NORMAL		-	5
CORE BODY TEMPERATURE		-	5
CORNEA REFLEX NORMAL		-	5
GRIP STRENGTH NORMAL		-	5
INDUCED ACTIVITY NORMAL		-	5
ORIENTATION NORMAL		-	5
PASSIVITY TEST NORMAL		-	5
PINNA REFLEX NORMAL		-	5
RIGHTING REFLEX NORMAL		-	5
FOUND DEAD		-	1
KILLED BY DESIGN		-	5
STUDY PHASE: PT=PRETREATMENT			

SYNTEX Report 513-M-94-Nepicastat

Figure 102

GROUP 3 - 100 mg/kg Nepicastat	CLINICAL DESCRIPTION / LOCATION	TREATMENT DAY	
		PT1	1
	ANIMALS ALIVE AT END OF INTERVAL	6	0
	ANIMALS EXAMINED DURING INTERVAL	6	5
	NORMAL	6	1
	ABNORMAL		
	GAIT	-	1
	POSTURE	-	1
	ANALGESIA TEST NORMAL	-	5
	ATAxia TEST NORMAL	-	5
	CORE BODY TEMPERATURE	-	5
	CORNEA REFLEX NORMAL	-	5
	GRIP STRENGTH NORMAL	-	5
	INACTIVE	-	1
	INDUCED ACTIVITY ABNORMAL	-	1
	INDUCED ACTIVITY NORMAL	-	4
	ORIENTATION NORMAL	-	5
	OTHER REACTION	-	4
	PASSIVITY TEST ABNORMAL	-	1
	PASSIVITY TEST NORMAL	-	4
	PINNA REFLEX NORMAL	-	5
	RIGHTING REFLEX NORMAL	-	5
	FOUND DEAD	-	1
	KILLED BY DESIGN	-	5
STUDY PHASE: PT=PRETREATMENT			

Figure 103

SYNTEX Report 513-M-94-Nepicastat

GROUP 4 • 300 MG/KG Nepicastat		TREATMENT DAY	
CLINICAL DESCRIPTOR / LOCATION		PT1	1
ANIMALS ALIVE AT END OF INTERVAL		8	0
ANIMALS EXAMINED DURING INTERVAL		8	6
NORMAL		6	6
ANALGESIA TEST NORMAL		-	6
ATAxia TEST NORMAL		-	6
CORE BODY TEMPERATURE		-	6
CORNER REFLEX NORMAL		-	6
GRIP STRENGTH NORMAL		-	6
INDUCED ACTIVITY NORMAL		-	6
ORIENTATION NORMAL		-	6
PASSIVITY TEST NORMAL		-	6
PINNA REFLEX NORMAL		-	6
RIGHTING REFLEX NORMAL		-	6
KILLED BY DESIGN		-	6
STUDY PHASE: PT=PRETREATMENT			

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Figure 104**Nepicastat MOTOR ACTIVITY EXPERIMENT: HORIZONTAL
ACTIVITY****Hour = 0.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	4095.4	2866.2	1013.4
30 mg/kg	8	4023.0	2495.6	882.3
10 mg/kg	8	4449.8	4084.0	1443.9
3 mg/kg	8	3284.9	2506.6	886.2
Clon	8	3355.4	2630.0	929.9
H ₂ O	8	5767.1	4082.7	1443.5
PEG	8	4495.3	2294.7	811.3
SKF	8	4366.8	3502.0	1238.1

Hour = 1

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	2192.8	1433.2	506.7
30 mg/kg	8	2678.6	1514.3	535.4
10 mg/kg	8	3538.4	2955.7	1045.0
3 mg/kg	8	2040.0	1302.5	460.5
Clon	8	1573.7	1035.4	366.1
H ₂ O	8	3347.9	2121.4	750.0
PEG	8	1466.5	1096.3	387.6
SKF	8	1754.0	1252.2	442.7

p < 0.05 versus H₂O control

* p < 0.05 versus peg control

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Figure 105

Nepicastat

**MOTOR ACTIVITY EXPERIMENT: HORIZONTAL
ACTIVITY****HOURL = 1.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1376.2	979.8	346.4
30 mg/kg	8	2004.5	1380.7	488.2
10 mg/kg	8	2446.8	1719.4	607.9
3 mg/kg	8	1693.5	1793.4	634.1
Clon	8	1456.9	849.5	300.4
H ₂ O	8	2142.9	1489.4	526.6
PEG	8	1389.9	1040.9	368
SKF	8	1410.7	1163.1	411.2

HOURL = 2

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	742.5	811.4	286.9
30 mg/kg	8	1209	1241.3	438.9
10 mg/kg	8	2331.4	1656.5	585.7
3 mg/kg	8	1380.1	1464.1	517.6
Clon	8	1352.6 [#]	411.4	145.5
H ₂ O	8	584.4	881.8	311.8
PEG	8	889.4	753.8	266.5
SKF	8	888.2	478.9	169.3

p < 0.05 versus H₂O control

* p < 0.05 versus peg control

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Figure 106**Nepicastat****MOTOR ACTIVITY EXPERIMENT: HORIZONTAL
ACTIVITY****HOURL = 2.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	823.4	813.3	287.5
30 mg/kg	8	782.5	859.2	303.8
10 mg/kg	8	1193.2	1535.9	543.0
3 mg/kg	8	439.4	549.4	194.2
Clon	8	1382.9 [#]	679.2	240.1
H ₂ O	8	839.0	1272.4	449.9
PEG	8	1594.6	1001.9	354.2
SKF	8	695.5 [*]	607.4	214.7

HOURL = 3

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1167.6	1809.8	639.9
30 mg/kg	8	298.8	236.6	83.7
10 mg/kg	8	318.5	291.6	103.1
3 mg/kg	8	262.3	249.7	88.3
Clon	8	1394.0	974.6	344.6
H ₂ O	8	557.7	975.5	344.9
PEG	8	1397.4	1453.0	513.7
SKF	8	430.9	425.7	150.5

[#] p < 0.05 versus h2o control^{*} p < 0.05 versus peg control

Figure 107**Nepicastat MOTOR ACTIVITY EXPERIMENT: HORIZONTAL ACTIVITY****HOURL = 3.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	632.5	521.6	184.4
30 mg/kg	8	773.9	1235.4	436.8
10 mg/kg	8	172.9	155.1	54.8
3 mg/kg	8	610.5	821.8	290.6
Clon	8	971.1	640.6	226.5
H ₂ O	8	993.1	1268.4	448.4
PEG	8	696.7	718.6	254.1
SKF	8	377.3	291.3	103.0

HOURL = 4

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	740.5	902.3	319.0
30 mg/kg	8	894.7	818.7	289.5
10 mg/kg	7	324.1	568.9	215.0
3 mg/kg	8	490.6	555.0	196.2
Clon	8	791.4	725.7	256.6
H ₂ O	8	546.6	754.9	266.9
PEG	8	309.9	252.4	89.2
SKF	8	932.1	1054.3	372.8

p < 0.05 versus H₂O control

* p < 0.05 versus peg control

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Figure 108**Nepicastat****MOTOR ACTIVITY EXPERIMENT: NO. OF
MOVEMENTS****HOURL = 0.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	195.3	92.2	32.6
30 mg/kg	8	171.8	63.1	22.3
10 mg/kg	8	186.1	124.9	44.1
3 mg/kg	8	148.8	87.9	31.1
Clon	8	150.0	73.1	25.8
H ₂ O	8	238.1	99.9	35.3
PEG	8	172.6	69.3	24.5
SKF	8	182.1	89.0	31.5

HOURL = 1

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	111.4	60.2	21.3
30 mg/kg	8	123.9	39.3	13.9
10 mg/kg	8	98.4	57.1	20.2
3 mg/kg	8	92.5	45.9	16.2
Clon	8	93.1	45.9	16.2
H ₂ O	8	144.8	55.2	19.5
PEG	8	57.0	45.6	16.1
SKF	8	82.1	47.6	16.8

p < 0.05 versus H₂O control

* p < 0.05 versus peg control

Figure 109**Nepicastat****MOTOR ACTIVITY EXPERIMENT: NO. OF
MOVEMENTS****HOURL = 1.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	66.6	45.0	15.9
30 mg/kg	8	84.0	57.5	20.3
10 mg/kg	8	78.4	61.2	21.6
3 mg/kg	8	67.0	55.3	19.6
Clon	8	68.4	24.3	8.6
H ₂ O	8	92.7	60.0	21.2
PEG	8	45.7	40.2	14.2
SKF	8	71.9*	40.4	14.3

HOURL = 2

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	35.2	32.2	11.4
30 mg/kg	8	52.7	50.9	18.0
10 mg/kg	8	91.1	72.5	25.6
3 mg/kg	8	57.0	53.4	18.9
Clon	8	64.9 [#]	16.8	5.9
H ₂ O	8	23.3	37.2	13.1
PEG	8	32.0	28.3	10.0
SKF	8	49.4	25.3	8.9

[#] p < 0.05 versus H₂O control* p < 0.05 versus peg control

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Figure 110**Nepicastat****MOTOR ACTIVITY EXPERIMENT: NO. OF
MOVEMENTS****HOURL = 2.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	46.1	58.9	20.8
30 mg/kg	8	31.0	31.3	11.1
10 mg/kg	8	37.6	33.6	11.9
3 mg/kg	8	16.9	23.1	8.2
Clon	8	71.4	26.9	9.5
H ₂ O	8	45.6	59.2	20.9
PEG	8	72.1	42.1	14.9
SKF	8	29.5*	28.1	10.0

HOURL = 3

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	45.7	72.8	25.8
30 mg/kg	8	18.0	37.8	13.4
10 mg/kg	8	29.3	48.1	17.0
3 mg/kg	8	7.7	8.3	2.9
Clon	8	67.1	40.7	14.4
H ₂ O	8	26.1	45.3	16.0
PEG	8	63.1	62.9	22.2
SKF	8	22.3	17.9	6.3

p < 0.05 versus H₂O control

* p < 0.05 versus peg control

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Figure 111**Nepicastat****MOTOR ACTIVITY EXPERIMENT: NO. OF
MOVEMENTS****hour = 3.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	26.8	20.9	7.4
30 mg/kg	8	30.9	52.2	18.5
10 mg/kg	8	5.4	5.7	2.0
3 mg/kg	8	22.8	33.3	11.8
Clon	8	42.2	23.0	8.1
H ₂ O	8	34.6	50.3	17.8
PEG	8	27.9	27.7	9.8
SKF	8	11.8	11.8	4.2

hour = 4

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	31.8	41.9	14.8
30 mg/kg	8	31.8	32.9	11.6
10 mg/kg	7	14.7	25.6	9.7
3 mg/kg	8	16.9	26.1	9.2
Clon	8	35.6	30.5	10.8
H ₂ O	8	19.4	28.6	10.1
PEG	8	7.4	9.5	3.4
SKF	8	42.1*	52.4	18.5

* p < 0.05 versus H₂O control

* p < 0.05 versus peg control

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Figure 112**Nepicastat****MOTOR ACTIVITY EXPERIMENT: REST
TIME (SECONDS)****HOURL = 0.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1603.6	116.3	41.1
30 mg/kg	8	1609.5	93.0	32.9
10 mg/kg	8	1587.4	194.2	68.7
3 mg/kg	8	1654.6	106.6	37.7
Clon	8	1641.4	109.1	38.6
H ₂ O	8	1511.4	173.3	61.3
PEG	8	1585.6	105.9	37.4
SKF	8	1581.5	146.0	51.6

HOURL = 1

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1719.5	40.5	14.3
30 mg/kg	8	1695.4	31.2	11.0
10 mg/kg	8	1707.1	62.5	22.1
3 mg/kg	8	1720.1	45.6	16.1
Clon	8	1733.2 [#]	39.1	13.8
H ₂ O	8	1665.1	70.9	25.1
PEG	8	1755.7	46.8	16.6
SKF	8	1741.5	43.0	15.2

[#] p < 0.05 versus H₂O control^{*} p < 0.05 versus peg control

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Figure 113**Nepicastat****MOTOR ACTIVITY EXPERIMENT: REST
TIME (SECONDS)****HOUR = 1.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1753.2	32.8	11.6
30 mg/kg	8	1732.6	54.2	19.2
10 mg/kg	8	1731.1	62.3	22.0
3 mg/kg	8	1740.2	78.6	27.8
Clon	8	1747.7	25.2	8.9
H ₂ O	8	1722.5	55.5	19.6
PEG	8	1759.9	43.8	15.5
SKF	8	1750.4	40.6	14.4

HOUR = 2

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1770.9	27.0	9.5
30 mg/kg	8	1761.1	39.8	14.1
10 mg/kg	8	1726.4	67.7	23.9
3 mg/kg	8	1753.1	59.1	20.9
Clon	8	1750.2 [#]	18.4	6.5
H ₂ O	8	1784.5	19.1	6.8
PEG	8	1779.2	22.4	7.9
SKF	8	1772.6	16.9	6.0

[#] p < 0.05 versus H₂O control^{*} p < 0.05 versus peg control

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Figure 114**Nepicastat****MOTOR ACTIVITY EXPERIMENT: REST
TIME (SECONDS)****HOURL = 2.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1764.6	51.0	18.0
30 mg/kg	8	1778.5	23.5	8.3
10 mg/kg	8	1770.6	39.7	14.0
3 mg/kg	8	1790.6	16.1	5.7
Clon	8	1746.7	26.8	9.5
H ₂ O	8	1756.6	54.8	19.4
PEG	8	1742.5	36.5	12.9
SKF	8	1782.4	24.0	8.5

HOURL = 3

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1757.4	76.8	27.2
30 mg/kg	8	1789.2	26.4	9.3
10 mg/kg	8	1777.0	38.4	13.6
3 mg/kg	8	1798.1	1.6	0.6
Clon	8	1754.2	31.2	11.0
H ₂ O	8	1782.0	35.0	12.4
PEG	8	1745.0	53.5	18.9
SKF	8	1787.5	13.8	4.9

p < 0.05 versus H₂O control

* p < 0.05 versus peg control

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Figure 115**Nepicastat****MOTOR ACTIVITY EXPERIMENT: REST
TIME (SECONDS)****Hour = 3.5**

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1781.0	17.6	6.2
30 mg/kg	8	1782.0	32.7	11.6
10 mg/kg	8	1797.2	4.6	1.6
3 mg/kg	8	1781.1	27.3	9.6
Clon	8	1764.4	26.3	9.3
H ₂ O	8	1777.4	34.0	12.0
PEG	8	1785.0	17.7	6.3
SKF	8	1793.1	8.0	2.8

Hour = 4

TRT	N Obs	Mean	Std Dev	Std Error
100 mg/kg	8	1778.4	29.8	10.5
30 mg/kg	8	1778.6	22.3	7.9
10 mg/kg	7	1790.6	18.7	7.1
3 mg/kg	8	1788.9	19.6	6.9
Clon	8	1773.5	27.2	9.6
H ₂ O	8	1785.9	20.5	7.2
PEG	8	1796.2	5.5	2.0
SKF	8	1770.9	38.0	13.4

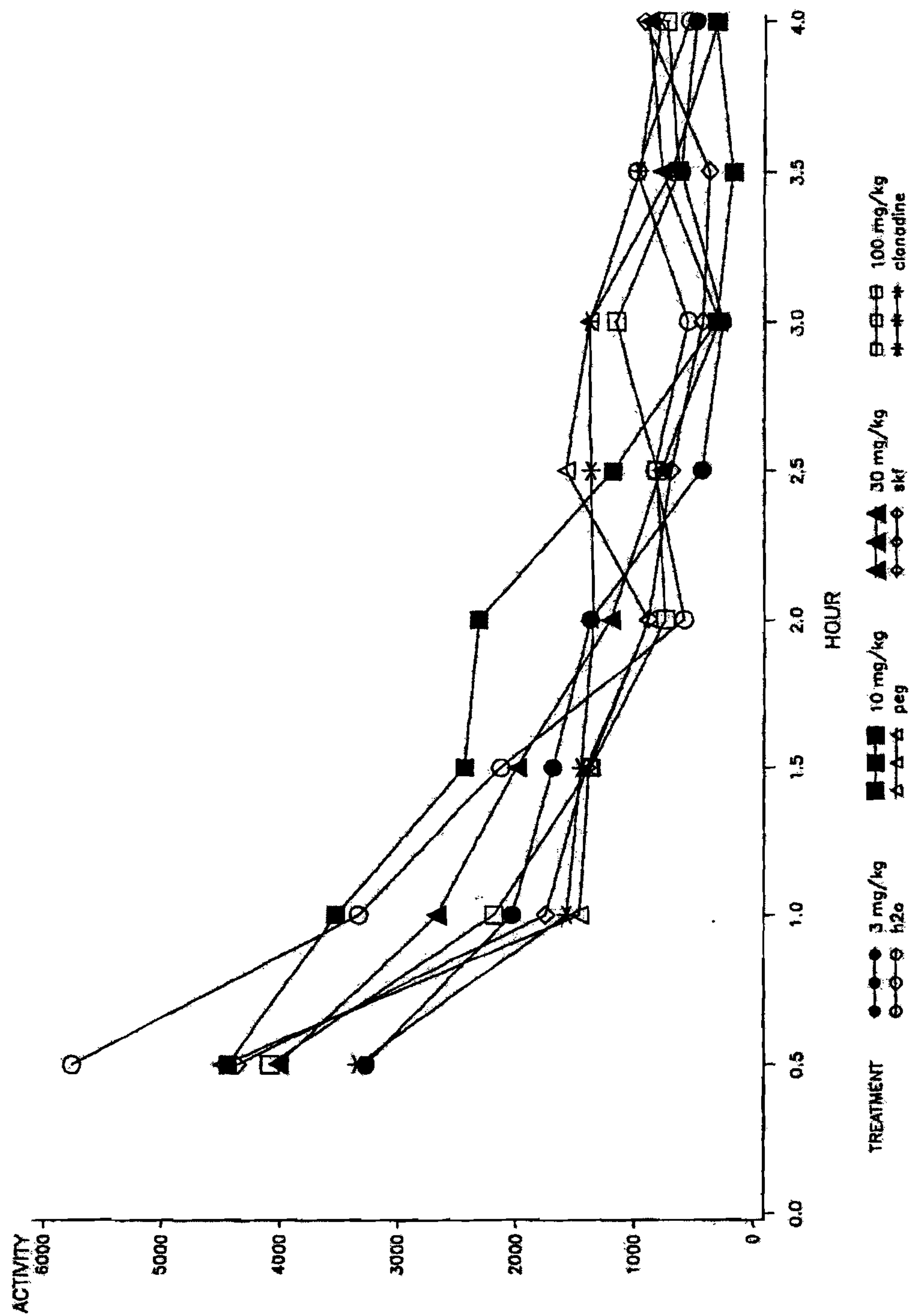
p < 0.05 versus H₂O control

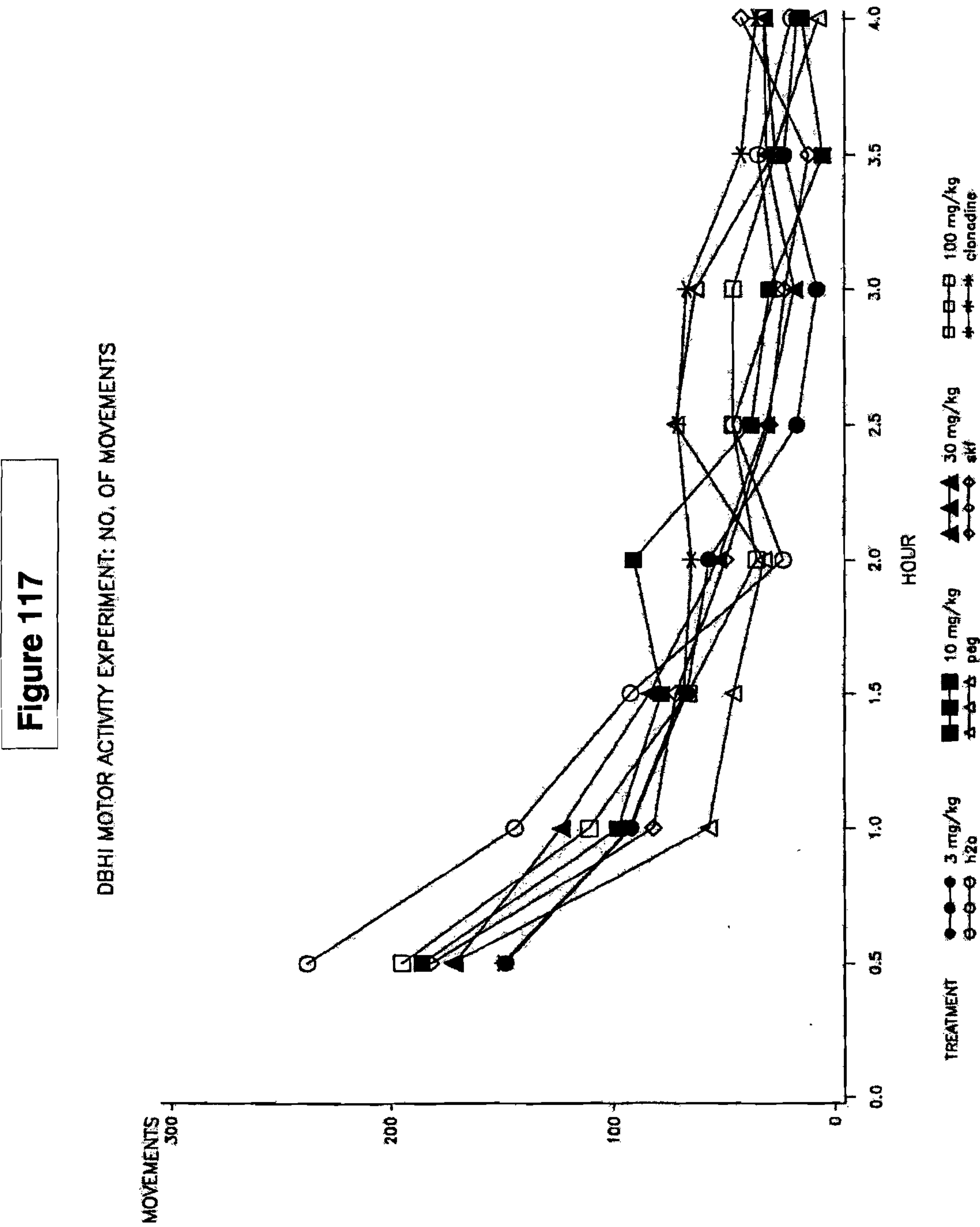
* p < 0.05 versus peg control

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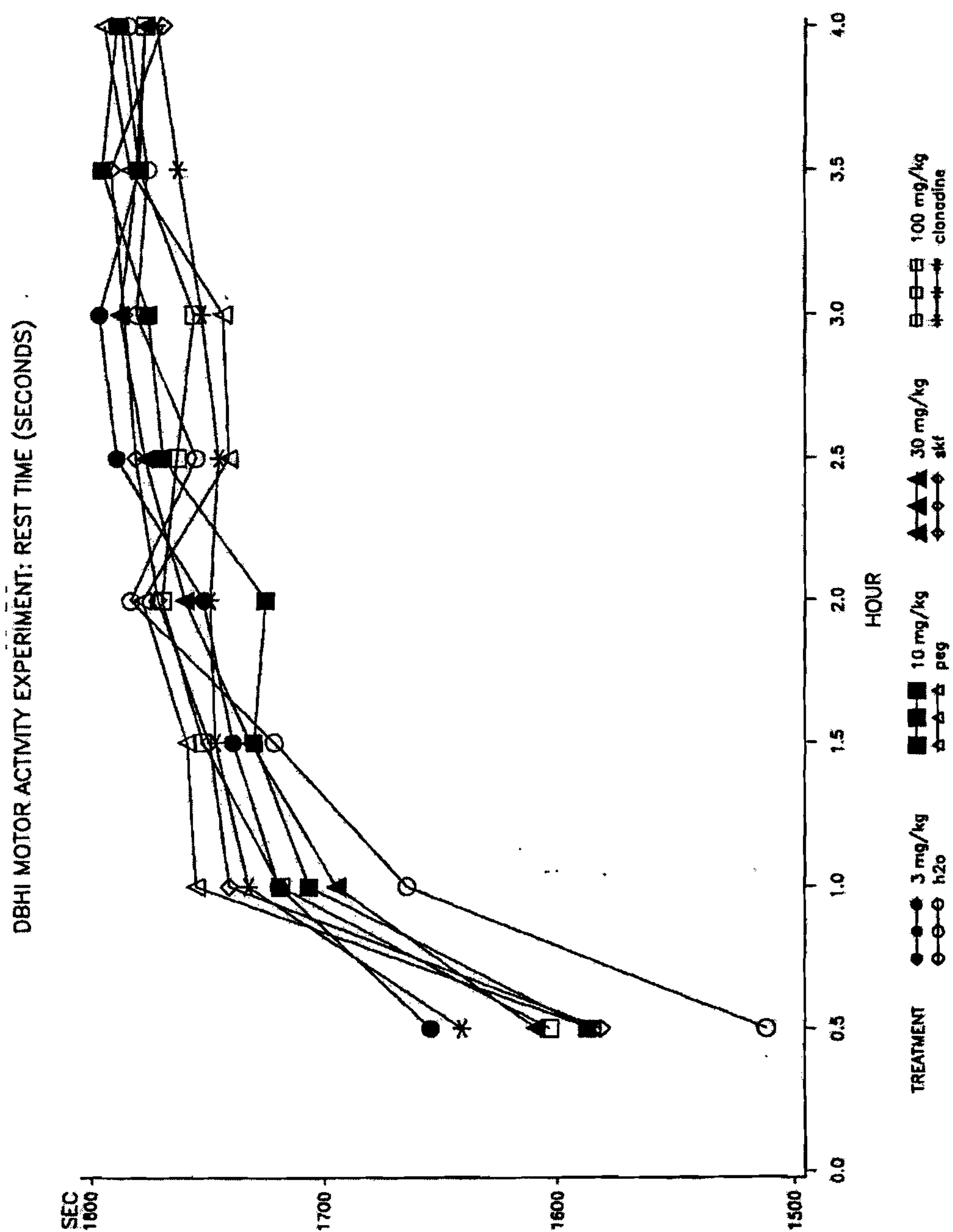
Figure 116

DBHI MOTOR ACTIVITY EXPERIMENT: HORIZONTAL ACTIVITY





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Figure 118

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Figure 119

**SUMMARY STATISTICS AND SIGNIFICANCE ASSESSMENTS
FOR MAXIMUM STARTLE RESP**

TIME	TREAT	N Obs	Mean	Std. Dev.	
1	CLON	480	737.54	524.31	p = 0.0077
	H2O	480	1352.50	933.46	
	PEG	480	1463.49	1076.67	
	Nepicastat 10	480	1564.15	1078.59	
	Nepicastat 100	480	1376.38	1151.97	
	Nepicastat 3	480	1597.92	972.93	
	Nepicastat 30	480	1545.02	1087.56	
	SKF	480	1094.69	1017.18	
2	CLON	480	656.62	435.4	p = 0.0888
	H2O	480	985.22	794.85	
	PEG	480	996.36	912.69	
	Nepicastat 10	480	1093.86	960.92	
	Nepicastat 100	480	886.69	862.58	
	Nepicastat 3	480	1127.73	800.09	
	Nepicastat 30	480	1137.35	915.18	
	SKF	480	746.93	764.51	
3	CLON	480	706.10	458.95	
	H2O	480	881.24	696.91	
	PEG	480	803.49	796.92	
	Nepicastat 10	480	961.71	814.65	
	Nepicastat 100	480	819.82	839.06	
	Nepicastat 3	480	1017.07	689.53	
	Nepicastat 30	480	975.97	731.30	
	SKF	480	613.17	586.79	

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Figure 120**SUMMARY STATISTICS AND SIGNIFICANCE ASSESSMENTS
FOR MAXIMUM STARTLE RESP**

TIME	TREAT	N Obs	Mean	Std. Dev.
4	CLON	480	752.08	519.93
	H2O	480	764.93	583.7
	PEG	480	769.18	602.22
	Nepicastat 10	480	854.33	629.26
	Nepicastat 100	480	749.82	682.81
	Nepicastat 3	480	1018.84	744.02
	Nepicastat 30	480	892.59	671.77
	SKF	480	616.16	605.69

SKF WAS NOT SIGNIFICANTLY DIFFERENT FROM PEG AT ANY TIME

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Figure 121**SUMMARY STATISTICS AND SIGNIFICANCE ASSESSMENTS
FOR MAXIMUM STARTLE RESP**

TIME	TREAT	N Obs	Mean	Std. Dev.	
1	CLON	480	84.18	81.47	p = 0.0036
	H2O	480	172.21	144.37	
	PEG	480	215.29	254.75	
	Nepicastat 10	480	219.75	239.35	
	Nepicastat 100	480	198.02	257.26	
	Nepicastat 3	480	188.68	150.76	
	Nepicastat 30	480	214.96	218.82	
	SKF	480	165.15	229.24	
2	CLON	480	65.24	47.77	p = 0.0352
	H2O	480	110.52	95.33	
	PEG	480	133.05	164.81	
	Nepicastat 10	480	142.7	195.15	
	Nepicastat 100	480	108.81	146.15	
	Nepicastat 3	480	125.09	95.06	
	Nepicastat 30	480	156.85	206.82	
	SKF	480	100.46	134.74	
3	CLON	480	74.6	57.08	p = 0.0921
	H2O	480	101.61	111.38	
	PEG	480	102.6	149.94	
	Nepicastat 10	480	114.54	149.4	
	Nepicastat 100	480	97.54	137.62	
	Nepicastat 3	480	107.16	78.16	
	Nepicastat 30	480	111.73	106.91	
	SKF	480	79.78	41.05	

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Figure 122**SUMMARY STATISTICS AND SIGNIFICANCE ASSESSMENTS
FOR MAXIMUM STARTLE RESP**

TIME	TREAT	N Obs	Mean	Std. Dev.
4	CLON	480	75.04	57.32
	H2O	480	79	69.63
	PEG	480	87.61	86.03
	Nepicastat 10	480	91.21	74.78
	Nepicastat 100	480	76.98	72.07
	Nepicastat 3	480	105.2	85.41
	Nepicastat 30	480	98.81	106.02
	SKF	480	75.29	108.16

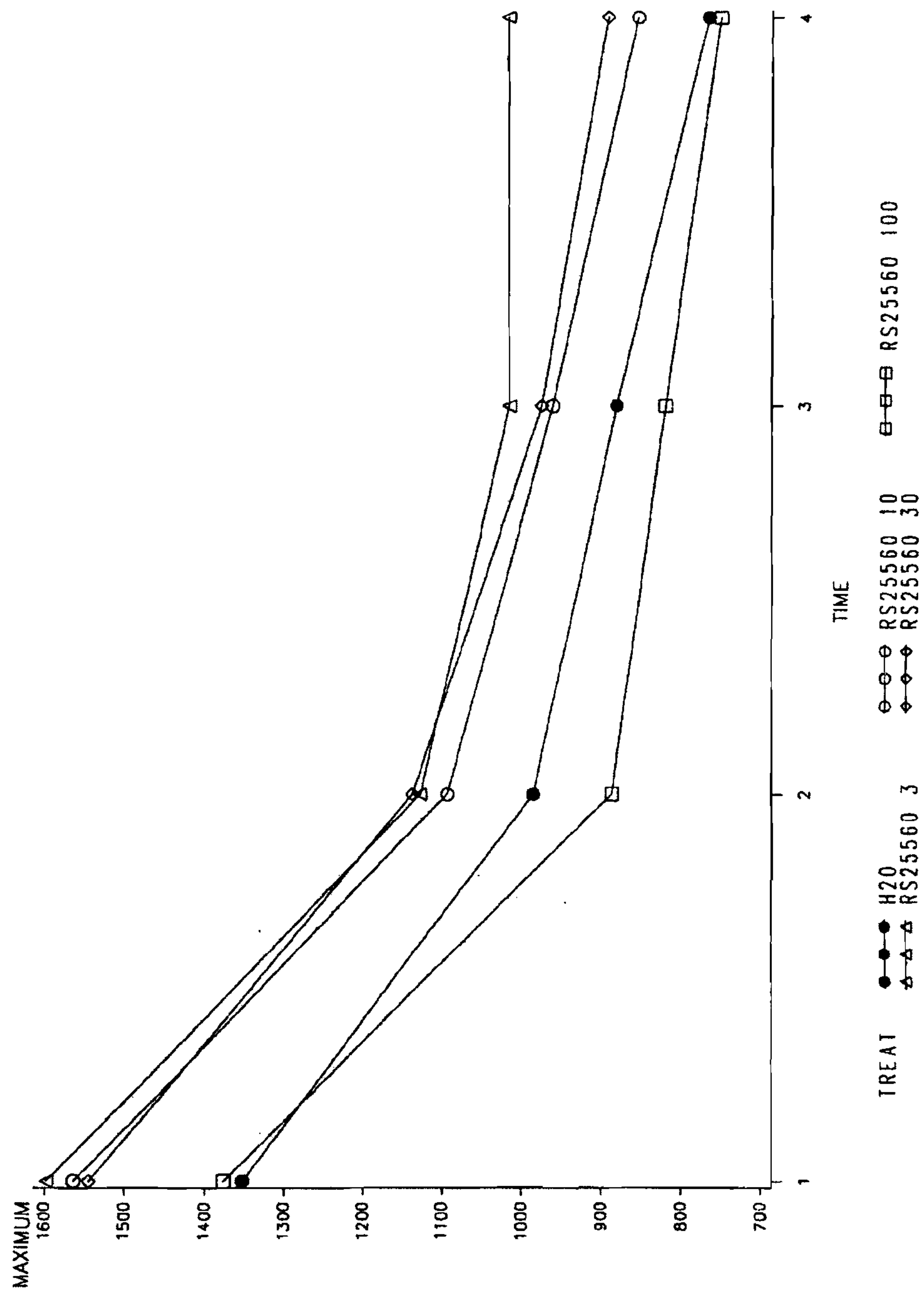
SKF WAS NOT SIGNIFICANTLY DIFFERENT FROM PEG AT ANY TIME

+ OVERALL TREATMENT EFFECT WAS marginally SIGNIFICANT ($p = 0.703$)

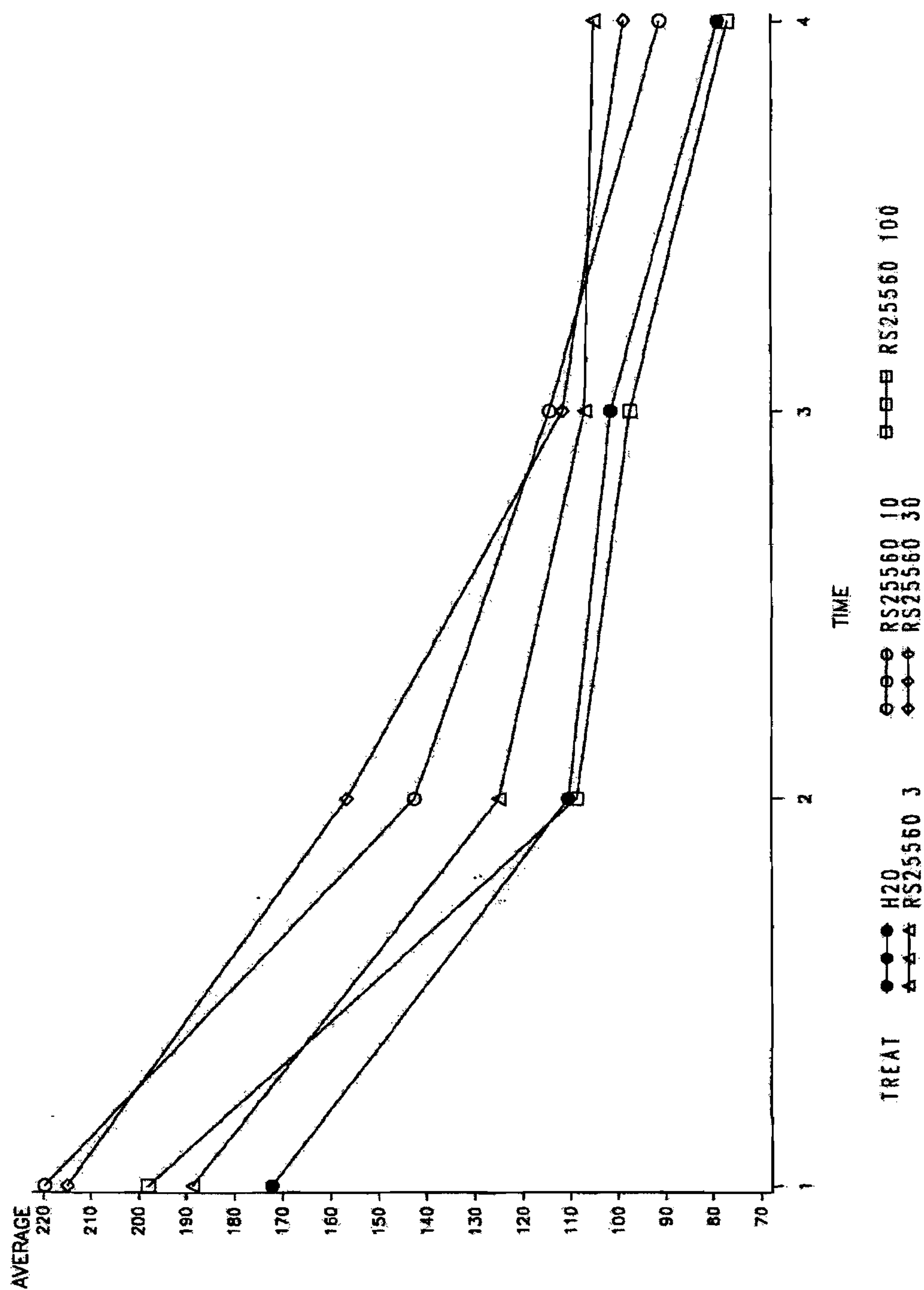
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Figure 123

NEPICASTAT AND H2O VERSUS TIME WITH RESPECT TO ST_MAX



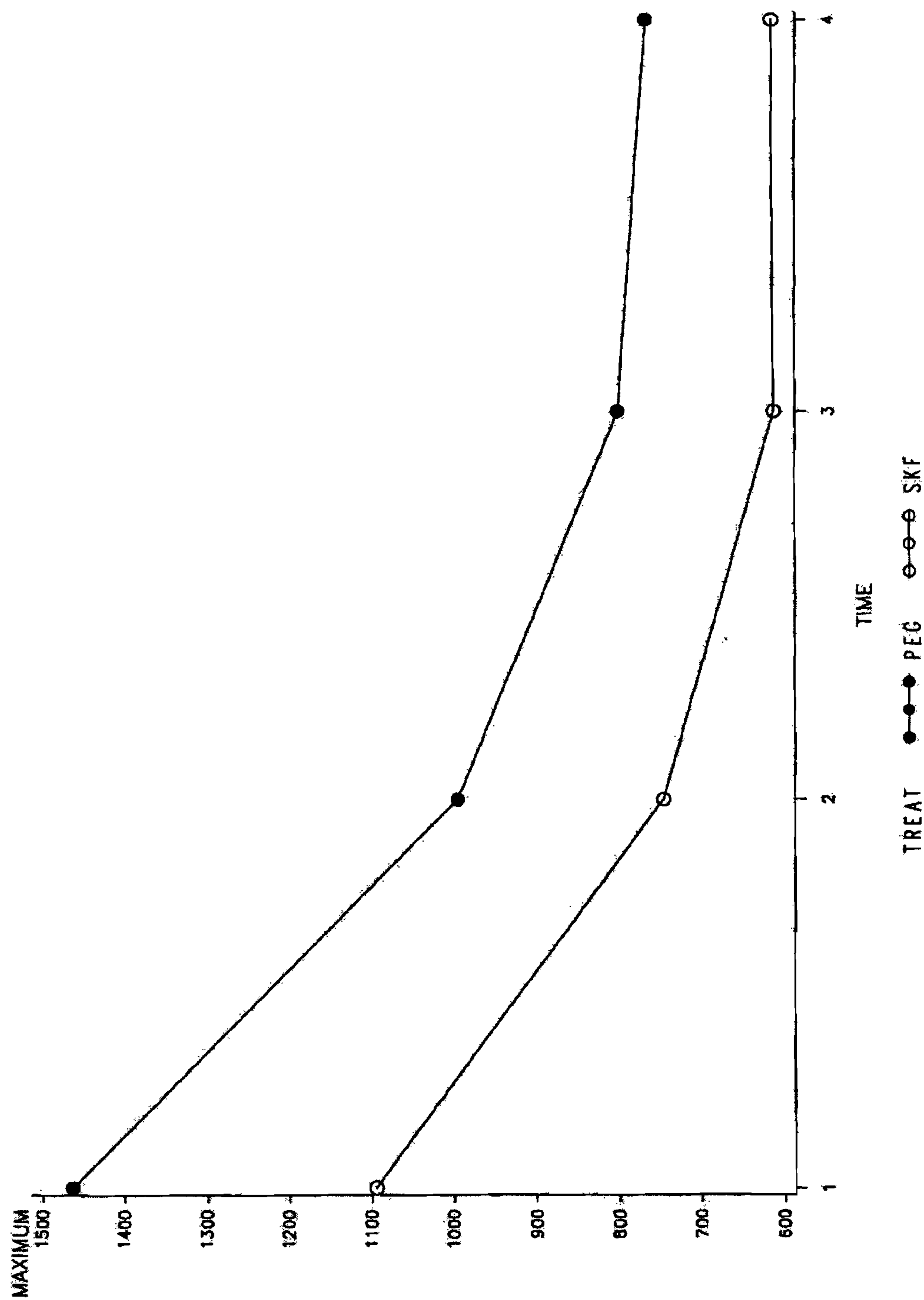
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Figure 124RS25560 AND H₂O VERSUS TIME WITH RESPECT TO ST_AVG

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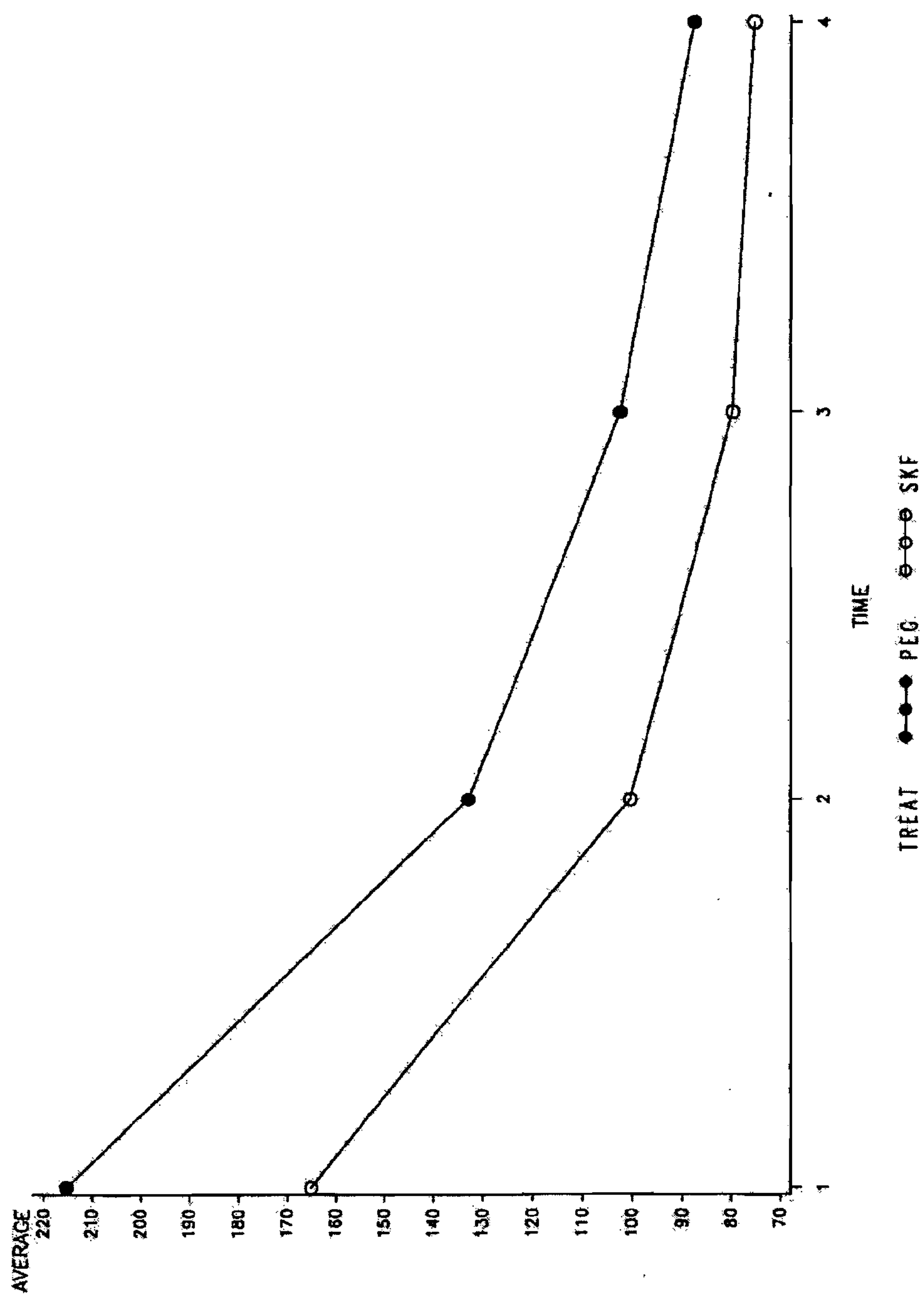
Figure 125

PEG AND SKF VERSUS TIME WITH RESPECT TO ST_MAX



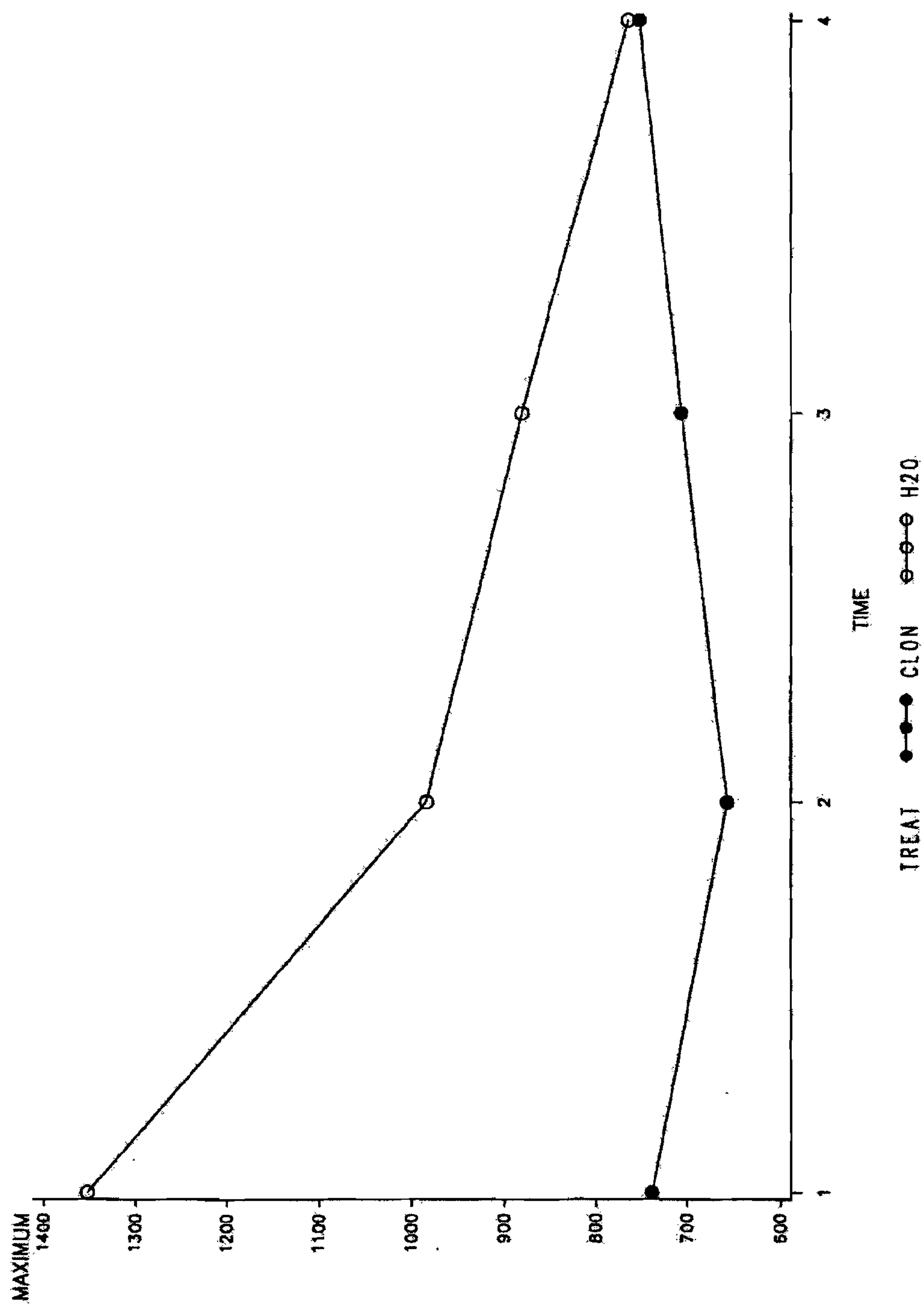
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Figure 126



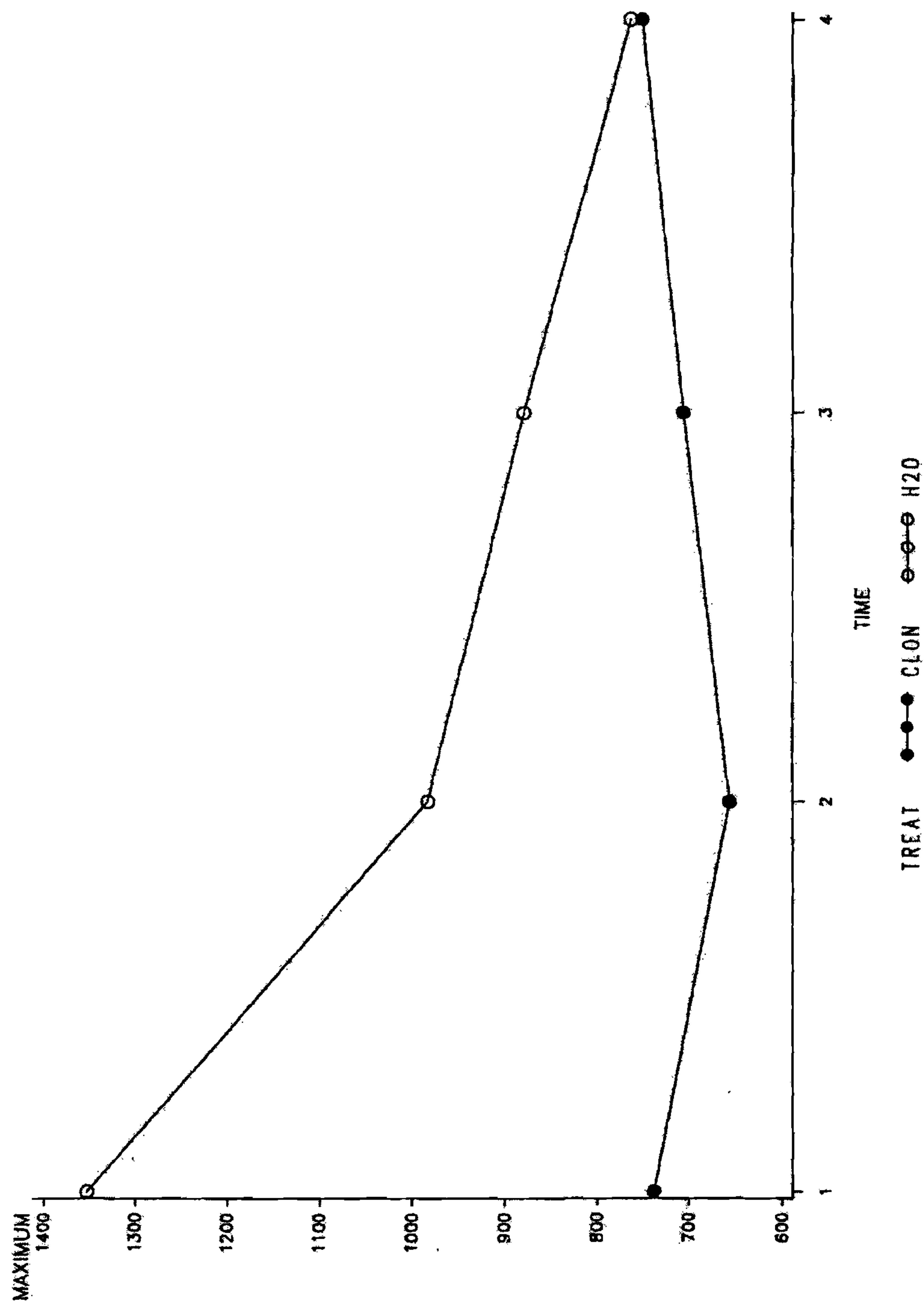
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Figure 127



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Figure 128



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Figure 129

PRE-TREATMENT ACOUSTIC STARTLE REACTIVITY AND STARTING DATE FOR EACH RAT

Day Started	Animal ID	Treatment	base startle	box #	Day Started	Animal ID	Treatment	base startle	box #
10/22/94	104	dH ₂ O	120.11	8	10/22/94	69	Cyclodextrin	81.79	5
10/23/94	78	dH ₂ O	420.63	6	10/23/94	11	Cyclodextrin	113.68	3
10/24/94	98	dH ₂ O	149.63	2	10/24/94	30	Cyclodextrin	60.95	6
10/25/94	23	dH ₂ O	95.16	7	10/25/94	9	Cyclodextrin	94.84	1
10/26/94	49	dH ₂ O	85.00	1	10/26/94	95	Cyclodextrin	389.42	7
10/27/94	24	dH ₂ O	402.79	8	10/27/94	33	Cyclodextrin	210.42	1
10/28/94	67	dH ₂ O	208.21	3	10/28/94	100	Cyclodextrin	200.68	4
10/29/94	89	dH ₂ O	136.47	1	10/29/94	40	Cyclodextrin	446.53	8
10/30/94	5	dH ₂ O	293.26	5	10/30/94	26	Cyclodextrin	156.16	2
10/31/94	77	dH ₂ O	228.68	5	10/31/94	43	Cyclodextrin	130.47	3
11/1/94	71	dH ₂ O	175.00	7	11/1/94	76	Cyclodextrin	293.37	4
11/2/94	59	dH ₂ O	36.47	3	11/2/94	73	Cyclodextrin	236.47	1
		Mean:	196.12				Mean:	201.23	
10/22/94	14	clonidine	550.89	6	10/22/94	52	SKF-102698	88.95	4
10/23/94	66	clonidine	82.05	2	10/23/94	103	SKF-102698	268.00	7
10/24/94	81	clonidine	86.05	1	10/24/94	83	SKF-102698	222.00	3
			6						
10/25/94	68	clonidine	120.79	4	10/25/94	70	SKF-102698	119.37	6
10/26/94	35	clonidine	146.63	3	10/26/94	10	SKF-102698	82.32	2
10/27/94	4	clonidine	333.42	4	10/27/94	94	SKF-102698	333.11	6
10/28/94	15	clonidine	238.16	7	10/28/94	34	SKF-102698	240.16	2
10/29/94	19	clonidine	216.95	3	10/29/94	44	SKF-102698	576.74	4
10/30/94	72	clonidine	64.58	8	10/30/94	22	SKF-102698	135.95	6
10/31/94	41	clonidine	140.00	1	10/31/94	38	SKF-102698	173.84	6
11/1/94	88	clonidine	273.26	8	11/1/94	82	SKF-102698	156.26	2
11/2/94	85	clonidine	172.05	5	11/2/94	80	SKF-102698	65.11	8
		Mean:	201.54				Mean:	205.15	

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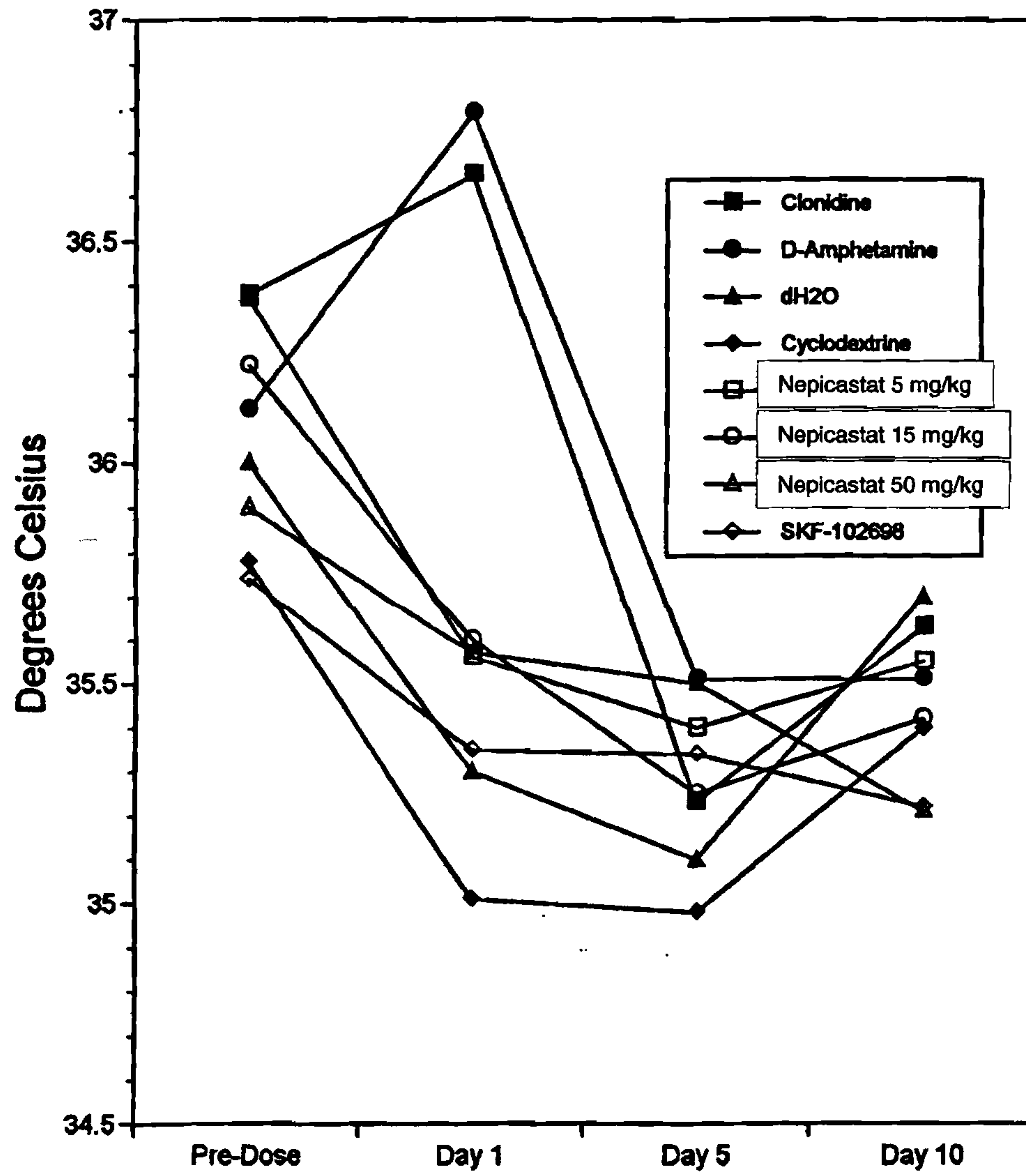
Figure 130

PRE-TREATMENT ACOUSTIC STARTLE REACTIVITY AND STARTING DATE FOR EACH RAT

Day Started	Animal ID	Treatment	base startle	box #	Day Started	Animal ID	Treatment	base startle	box #
10/22/94	31	d-Amphetamine	122.84	7	10/22/94	57	5 mg/kg b.i.d.	165.68	1
10/23/94	97	d-Amphetamine	335.58	1	10/23/94	8	5 mg/kg b.i.d.	267.74	8
10/24/94	64	d-Amphetamine	209.16	8	10/24/94	36	5 mg/kg b.i.d.	83.00	4
10/25/94	50	d-Amphetamine	82.05	2	10/25/94	101	5 mg/kg b.i.d.	124.42	5
10/26/94	16	d-Amphetamine	271.32	8	10/26/94	29	5 mg/kg b.i.d.	349.37	5
10/27/94	93	d-Amphetamine	192.47	3	10/27/94	75	5 mg/kg b.i.d.	242.26	3
10/28/94	46	d-Amphetamine	56.37	6	10/28/94	56	5 mg/kg b.i.d.	99.89	8
10/29/94	58	d-Amphetamine	127.95	2	10/29/94	87	5 mg/kg b.i.d.	65.68	7
10/30/94	39	d-Amphetamine	238.79	7	10/30/94	17	5 mg/kg b.i.d.	212.74	1
10/31/94	2	d-Amphetamine	162.95	2	10/31/94	48	5 mg/kg b.i.d.	194.84	8
11/1/94	51	d-Amphetamine	111.00	3	11/1/94	54	5 mg/kg b.i.d.	139.05	6
11/2/94	12	d-Amphetamine	335.00	4	11/2/94	18	5 mg/kg b.i.d.	561.11	2
		Mean:	205.46				Mean:	208.82	
10/22/94	99	15 mg/kg b.i.d.	60.26	3	10/22/94	74	50 mg/kg b.i.d.	132.32	2
10/23/94	13	15 mg/kg b.i.d.	122.63	5	10/23/94	60	50 mg/kg b.i.d.	81.58	4
10/24/94	63	15 mg/kg b.i.d.	112.26	7	10/24/94	53	50 mg/kg b.i.d.	124.58	5
10/25/94	32	15 mg/kg b.i.d.	254.63	8	10/25/94	91	50 mg/kg b.i.d.	398.74	3
10/26/94	6	15 mg/kg b.i.d.	560.42	6	10/26/94	20	50 mg/kg b.i.d.	207.84	4
10/27/94	42	15 mg/kg b.i.d.	311.65	2	10/27/94	55	50 mg/kg b.i.d.	205.47	7
10/28/94	65	15 mg/kg b.i.d.	72.47	1	10/28/94	61	50 mg/kg b.i.d.	264.74	5
10/29/94	45	15 mg/kg b.i.d.	265.47	5	10/29/94	102	50 mg/kg b.i.d.	258.42	6
10/30/94	27	15 mg/kg b.i.d.	145.87	3	10/30/94	28	50 mg/kg b.i.d.	113.42	4
10/31/94	84	15 mg/kg b.i.d.	228.63	4	10/31/94	47	50 mg/kg b.i.d.	62.74	7
11/1/94	25	15 mg/kg b.i.d.	168.74	1	11/1/94	37	50 mg/kg b.i.d.	438.05	1
11/2/94	79	15 mg/kg b.i.d.	192.32	7	11/2/94	86	50 mg/kg b.i.d.	168.16	6
		Mean:	207.97				Mean:	205.59	

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Figure 131



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Figure 132**MEAN BODY CORE TEMPERATURES (°celcius)**

-----Pre-Dose Baseline-----				
TRT	N	MEAN	Change from Baseline	St. Dev.
Clonidine 20 mg/kg, b.i.d.	8	36.39	---	---
d-Amphetamine 2 mg/kg, b.i.d.	11	36.11	---	---
dH ₂ O	10	36.04	---	---
Cyclodextrine	10	35.78	---	---
Nepicastat 15 mg/kg, b.i.d.	11	36.23	---	---
Nepicastat 5 mg/kg, b.i.d.	9	36.26	---	---
Nepicastat 50 mg/kg, b.i.d.	8	35.90	---	---
SKF 102698 50 mg/kg, b.i.d.	8	35.75	---	---

-----Day 1-----				
TRT	N	MEAN	Change from Baseline	St. Dev.
Clonidine 20 mg/kg, b.i.d.	8	36.65	0.26*	0.96
d-Amphetamine 2 mg/kg, b.i.d.	11	36.79	0.68**	0.82
dH ₂ O	10	35.33	-0.71	0.94
Cyclodextrine	10	35.02	-0.76	0.67
Nepicastat 15 mg/kg, b.i.d.	11	35.59	-0.64	0.67
Nepicastat 5 mg/kg, b.i.d.	9	35.53	-0.72	0.81
Nepicastat 50 mg/kg, b.i.d.	8	35.51	-0.39	0.93
SKF 102698 50 mg/kg, b.i.d.	8	35.35	-0.40	0.79

**p<0.01 versus dH₂O*p<0.05 versus dH₂O

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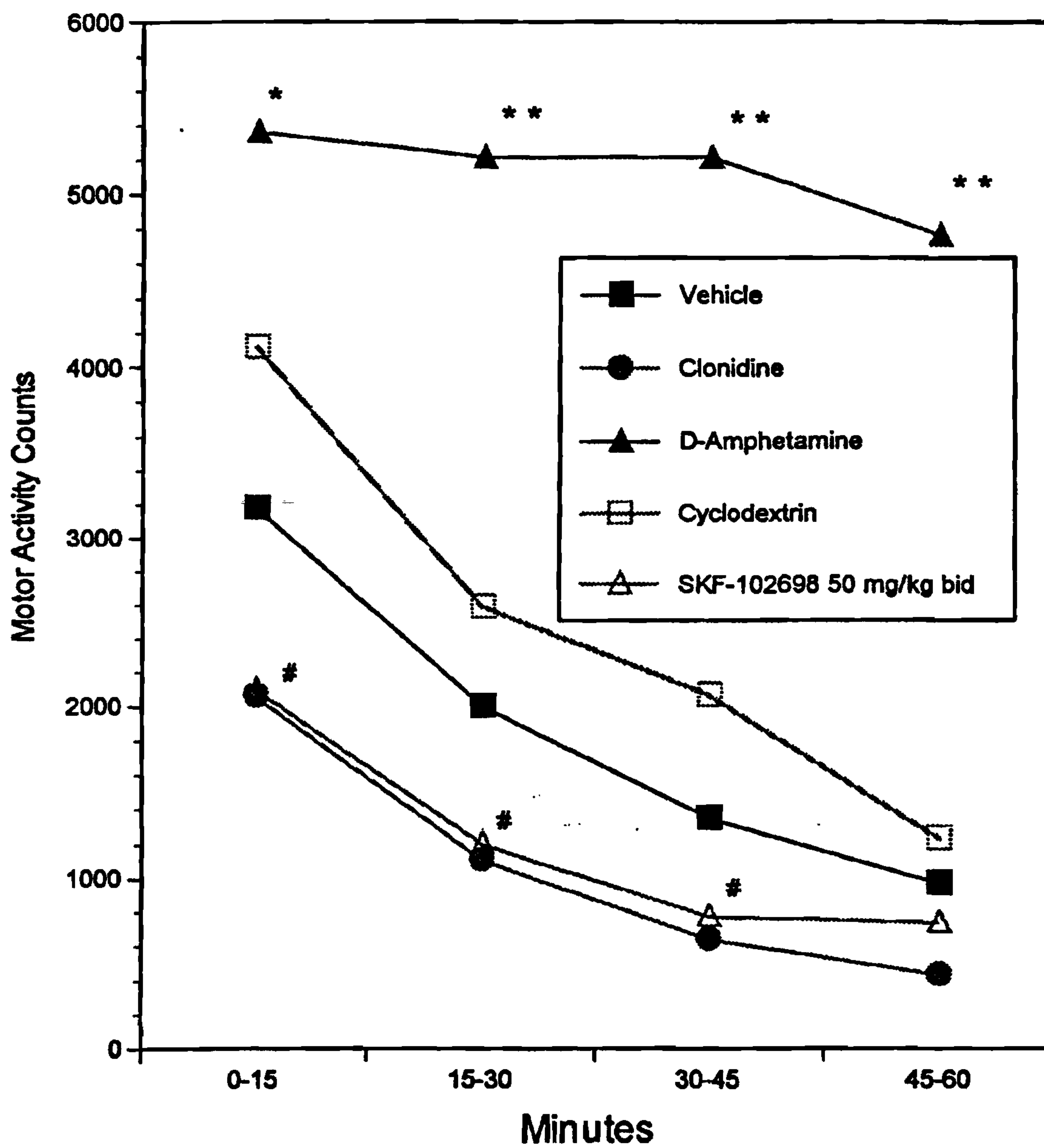
Figure 133

MEAN BODY CORE TEMPERATURES (°celcius)

-----Day 5-----				
TRT	N	MEAN	Change from Baseline	St. Dev.
Clonidine 20 mg/kg, b.i.d.	8	35.23	-1.16	1.43
d-Amphetamine 2 mg/kg, b.i.d.	11	35.51	-0.60	1.36
dH ₂ O	10	35.14	-0.90	0.78
Cyclodextrine	10	34.98	-0.80	1.22
Nepicastat 15 mg/kg, b.i.d.	11	35.25	-0.97	1.57
Nepicastat 5 mg/kg, b.i.d.	9	35.46	-0.80	1.23
Nepicastat 50 mg/kg, b.i.d.	8	35.46	-0.44	1.37
SKF 102698 50 mg/kg, b.i.d.	8	35.51	-0.41	1.08

-----Day 10-----				
TRT	N	MEAN	Change from Baseline	St. Dev.
Clonidine 20 mg/kg, b.i.d.	8	35.65	-0.74	0.73
d-Amphetamine 2 mg/kg, b.i.d.	11	35.51	-0.60	1.13
dH ₂ O	10	35.69	-0.35	1.17
Cyclodextrine	10	35.39	-0.39	1.64
Nepicastat 15 mg/kg, b.i.d.	11	35.43	-0.80	1.58
Nepicastat 5 mg/kg, b.i.d.	9	35.80	-0.46	1.06
Nepicastat 50 mg/kg, b.i.d.	8	35.45	-0.45	1.48
SKF 102698 50 mg/kg, b.i.d.	8	35.23	-0.52	1.44

Figure 134



* $p < 0.05$ versus vehicle
** $p < 0.01$ versus vehicle
$p < 0.05$ versus cyclodextrine

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Figure 135**SPONTANEOUS MOTOR ACTIVITY**

-----0-15 Minutes-----				
TRT	N	MEDIAN	MEAN	STD
Clonidine 20 mg/kg, b.i.d.	8	1708.0	2058.13	1319.50
d-Amphetamine 2 mg/kg, b.i.d.	11	6280.0*	5360.55	3007.37
dH ₂ O	10	3103.0	3172.00	2293.21
Cyclodextrine	10	4517.0	4117.90	2100.53
Nepicastat 15 mg/kg, b.i.d.	11	4183.0	3724.64	1744.07
Nepicastat 5 mg/kg, b.i.d.	10	3309.5	2623.90	1838.93
Nepicastat 50 mg/kg, b.i.d.	8	3234.5	3319.88	2335.99
SKF 102698 50 mg/kg, b.i.d.	9	2009.0#	2097.89	1243.61

-----15-30 Minutes-----				
TRT	N	MEDIAN	MEAN	STD
Clonidine 20 mg/kg, b.i.d.	8	914.5	1105.75	899.41
d-Amphetamine 2 mg/kg, b.i.d.	11	6542.0**	5214.55	3073.80
dH ₂ O	10	1327.0	1999.40	1822.47
Cyclodextrine	10	3061.5	2584.00	1597.48
Nepicastat 15 mg/kg, b.i.d.	11	2499.0	2353.64	1467.16
Nepicastat 5 mg/kg, b.i.d.	10	878.5	1230.80	1057.36
Nepicastat 50 mg/kg, b.i.d.	8	2017.5	1927.13	1526.12
SKF 102698 50 mg/kg, b.i.d.	9	1290.0#	1197.89	851.56

**p<0.01 versus dH₂O*p<0.05 versus dH₂O

#p<0.05 versus Cyclodextrine

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Figure 136

SPONTANEOUS MOTOR ACTIVITY

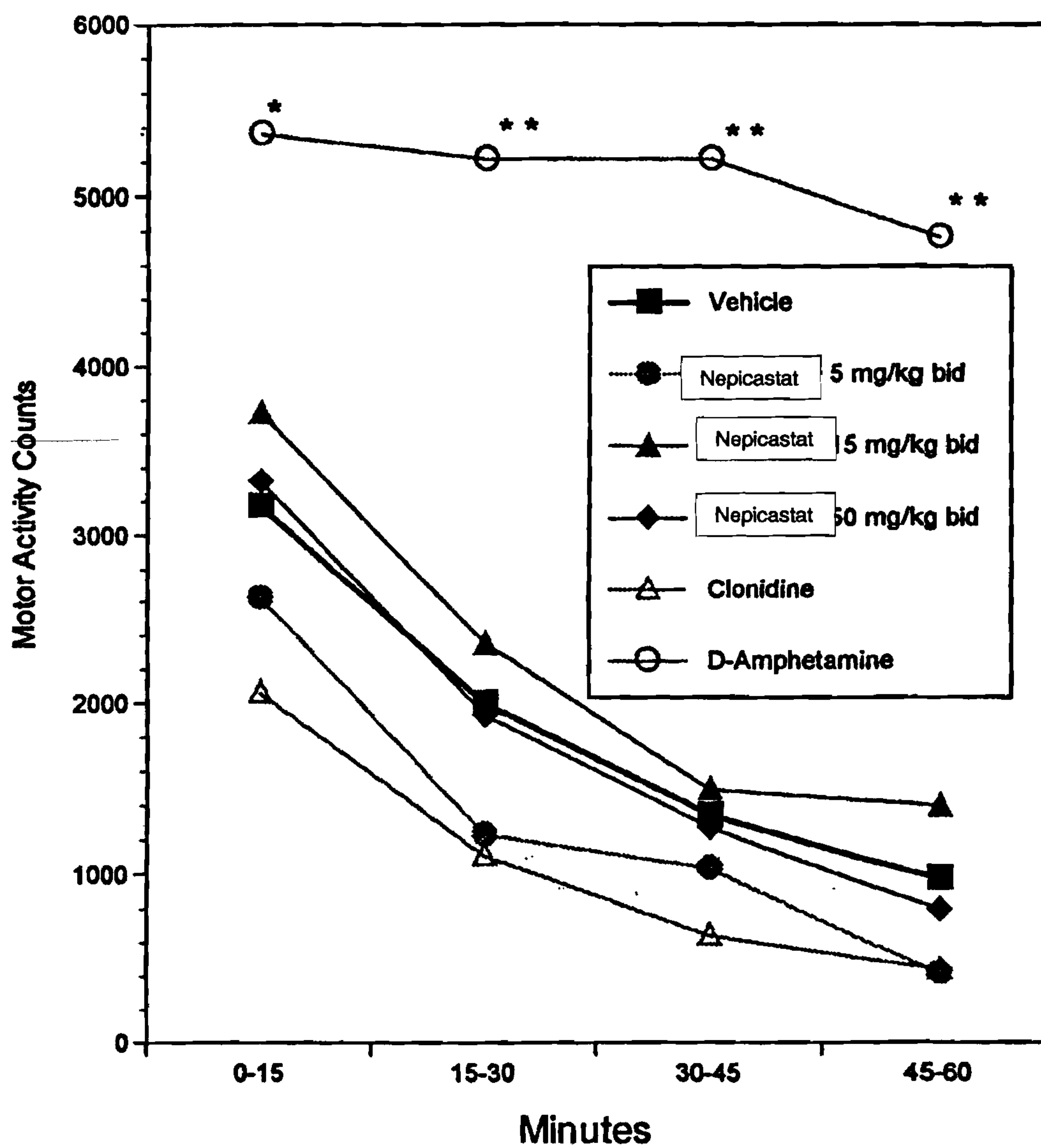
-----30-45 Minutes-----				
TRT	N	MEDIAN	MEAN	STD
Clonidine 20 mg/kg, b.i.d.	8	430.0	636.50	446.35
d-Amphetamine 2 mg/kg, b.i.d.	11	5693.0**	5217.09	3108.40
dH ₂ O	10	778.5	1343.30	1313.10
Cyclodextrine	10	2526.5	2061.50	1630.56
Nepicastat 15 mg/kg, b.i.d.	11	940.0	1490.18	1368.84
Nepicastat 5 mg/kg, b.i.d.	10	788.5	1034.80	868.39
Nepicastat 50 mg/kg, b.i.d.	8	1113.5	1269.63	1161.22
SKF 102698 50 mg/kg, b.i.d.	9	384.0#	772.67	652.68

-----45-60 Minutes-----				
TRT	N	MEDIAN	MEAN	STD
Clonidine 20 mg/kg, b.i.d.	8	153.0	431.37	460.26
d-Amphetamine 2 mg/kg, b.i.d.	11	4842.0**	4763.27	2610.13
dH ₂ O	10	359.0	970.40	1129.12
Cyclodextrine	10	951.0	1228.00	1145.70
Nepicastat 15 mg/kg, b.i.d.	11	838.0	1395.82	1463.45
Nepicastat 5 mg/kg, b.i.d.	10	289.5	412.00	460.84
Nepicastat 50 mg/kg, b.i.d.	8	306.0	792.12	1098.57
SKF 102698 50 mg/kg, b.i.d.	9	558.0	734.33	775.86

**p<0.01 versus dH₂O*p<0.05 versus dH₂O

#p<0.05 versus Cyclodextrine

Figure 137



* p<0.05 versus vehicle

** p<0.01 versus vehicle

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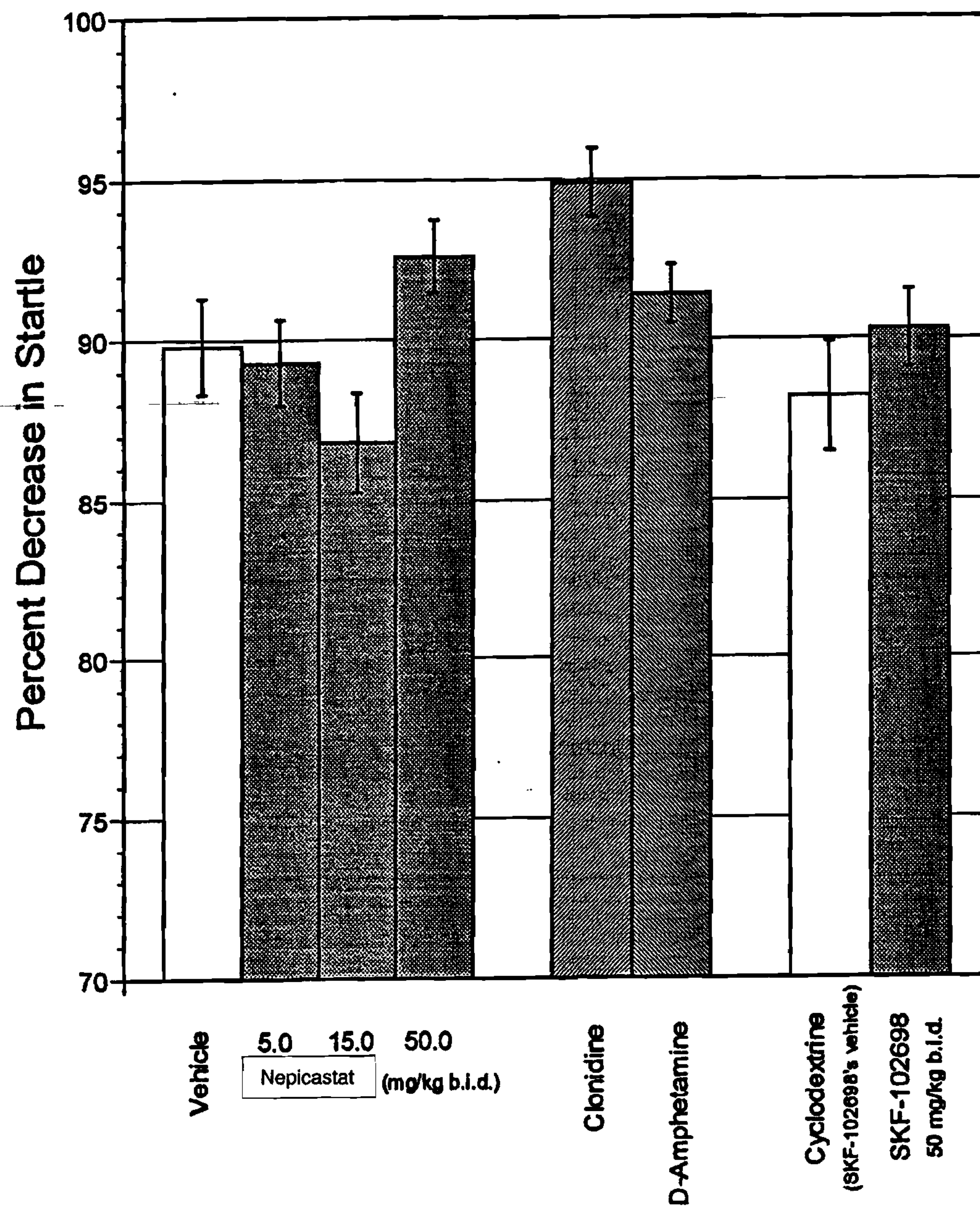
Figure 138

Figure 139

SUMMARY STATISTICS AND P-VALUES FOR OVERALL PAIRWISE TREATMENT
COMPARISONS FOR PERCENT PREPULSE INHIBITION IN RATS

TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	36	94.9%	6.4%	0.0782		
d-Amphetamine 2 mg/kg, b.i.d.	48	91.4%	6.4%	NS		
Nepicastat 15 mg/kg, b.i.d.	44	86.8%	10.3%	NS		
Nepicastat 5 mg/kg, b.i.d.	44	89.3%	8.8%	NS		
Nepicastat 50 mg/kg, b.i.d.	36	92.6%	6.8%	NS		NS
SKF 102698 50 mg/kg, b.i.d.	36	90.3%	7.3%		NS	
Cyclodextrine	44	88.2%	11.2%	NS		
dH ₂ O	44	89.8%	9.9%			

Figure 140

SUMMARY STATISTICS AND P-VALUES FOR PAIRWISE TREATMENT COMPARISONS WITHIN
TIME FOR PERCENT PREPULSE INHIBITION IN RATS
(for startles 1-15)

TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	9	95.3%	4.8%	0.0450		
d-Amphetamine 2 mg/kg, b.i.d.	12	92.0%	6.7%	NS		
Nepicastat 15 mg/kg, b.i.d.	11	87.4%	8.6%	NS		
Nepicastat 5 mg/kg, b.i.d.	11	86.6%	8.7%	NS		
Nepicastat 50 mg/kg, b.i.d.	9	90.7%	11.0%	NS		NS
SKF 102698 50 mg/kg, b.i.d.	9	83.6%	8.8%		NS	
Cyclodextrine	11	84.1%	11.3%	NS		
dH ₂ O	11	90.4%	5.3%			

(for startles 16-30)

TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	9	96.1%	4.1%	0.0326		
d-Amphetamine 2 mg/kg, b.i.d.	12	91.8%	4.7%	NS		
Nepicastat 15 mg/kg, b.i.d.	11	82.1%	11.9%	NS		
Nepicastat 5 mg/kg, b.i.d.	11	87.6%	9.8%	NS		
Nepicastat 50 mg/kg, b.i.d.	9	92.5%	4.6%	NS		NS
SKF 102698 50 mg/kg, b.i.d.	9	90.3%	6.7%		NS	
Cyclodextrine	11	83.3%	15.1%	NS		
dH ₂ O	11	89.1%	8.2%			

Figure 141

SUMMARY STATISTICS AND P-VALUES FOR PAIRWISE TREATMENT COMPARISONS WITHIN
TIME FOR PERCENT PREPULSE INHIBITION IN RATS
(for startles 31-45)

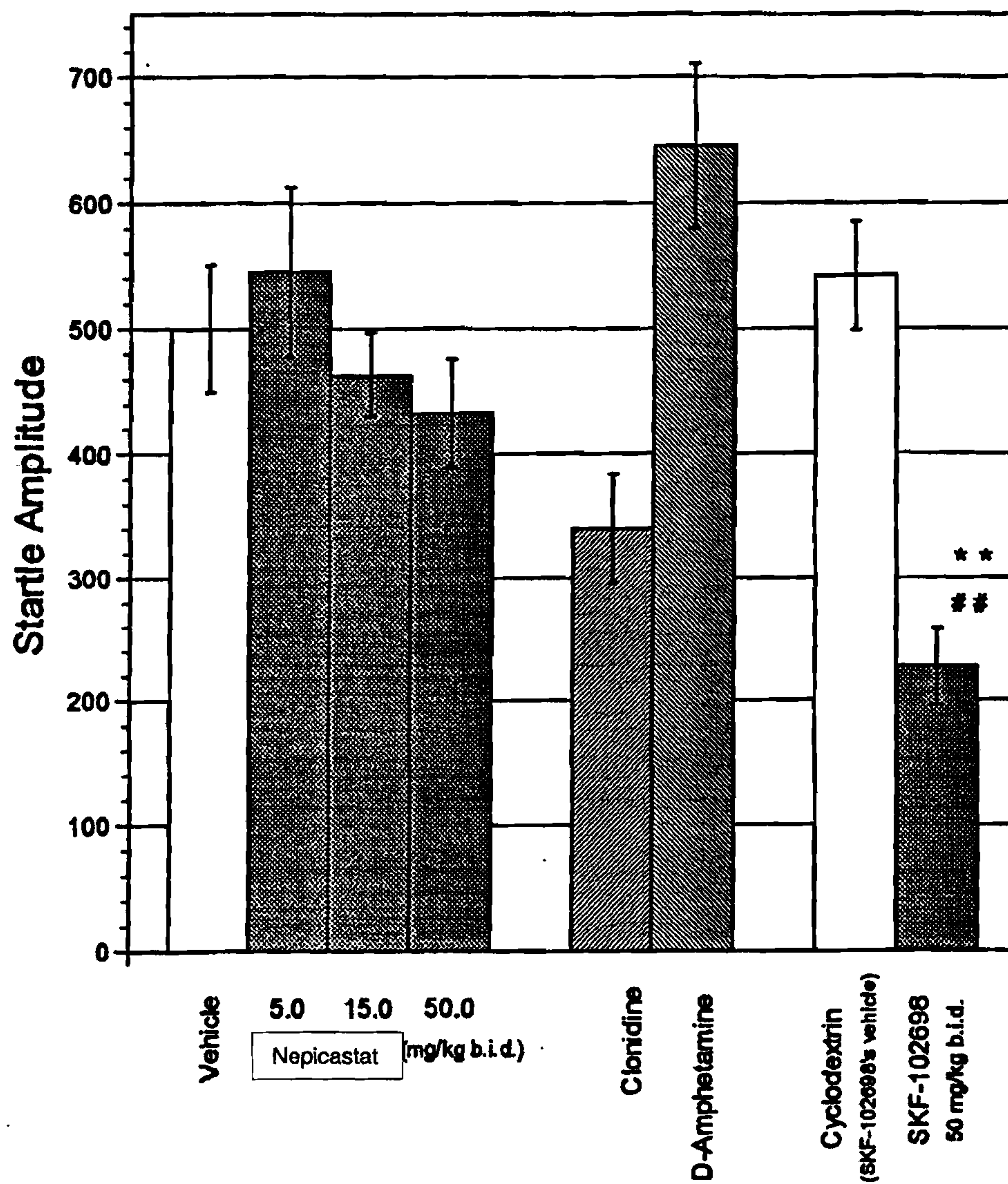
TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	9	95.8%	5.1%	NS		
d-Amphetamine 2 mg/kg, b.i.d.	12	89.5%	9.0%	NS		
Nepicastat 15 mg/kg, b.i.d.	11	88.5%	10.1%	NS		
Nepicastat 5 mg/kg, b.i.d.	11	91.0%	7.3%	NS		
Nepicastat 50 mg/kg, b.i.d.	9	92.8%	5.7%	NS		NS
SKF 102698 50 mg/kg, b.i.d.	9	94.7%	4.5%		NS	
Cyclodextrine	11	92.8%	4.7%	NS		
dH ₂ O	11	92.7%	7.8%			

(for startles 46-60)

TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	9	92.3%	10.2%	NS		
d-Amphetamine 2 mg/kg, b.i.d.	12	92.5%	4.8%	NS		
Nepicastat 15 mg/kg, b.i.d.	11	89.3%	10.1%	NS		
Nepicastat 5 mg/kg, b.i.d.	11	91.8%	9.4%	NS		
Nepicastat 50 mg/kg, b.i.d.	9	94.2%	4.2%	NS		NS
SKF 102698 50 mg/kg, b.i.d.	9	92.5%	3.5%		NS	
Cyclodextrine	11	92.4%	8.2%	NS		
dH ₂ O	11	87.0%	15.8%			

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Figure 142



**p<0.005 versus cyclodextrine

###p<0.005 versus Nepicastat 50 mg/kg, b.i.d.

Figure 143

SUMMARY STATISTICS AND P-VALUES FOR OVERALL PAIRWISE TREATMENT
COMPARISONS FOR ACOUSTIC STARTLE REACTIVITY IN RATS

TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	36	340.54	262.01	NS		
d-Amphetamine 2 mg/kg, b.i.d.	48	645.38	451.70	NS		
Nepicastat 15 mg/kg, b.i.d.	44	463.47	221.49	NS		
Nepicastat 5 mg/kg, b.i.d.	44	545.44	447.12	NS		
Nepicastat 50 mg/kg, b.i.d.	36	432.91	261.64	NS		0.0047
SKF 102698 50 mg/kg, b.i.d.	36	227.13	186.56		0.0007	
Cyclodextrine	44	542.11	287.10	NS		
dH ₂ O	44	500.12	335.27			

Figure 144

SUMMARY STATISTICS AND P-VALUES FOR PAIRWISE TREATMENT COMPARISONS WITHIN
TIME FOR ACOUSTIC STARTLE REACTIVITY IN RATS
(for startles 1-15)

TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	9	459.80	354.44	NS		
d-Amphetamine 2 mg/kg, b.i.d.	12	726.45	515.64	NS		
Nepicastat 5 mg/kg, b.i.d.	11	590.91	263.52	NS		
Nepicastat mg/kg, b.i.d.	11	780.82	453.96	NS		
Nepicastat 0 mg/kg, b.i.d.	9	669.04	272.06	NS		0.0169
SKF 102698 50 mg/kg, b.i.d.	9	340.89	249.38		0.0085	
Cyclodextrine	11	725.93	319.13	NS		
dH ₂ O	11	692.89	273.10			

(for startles 16-30)

TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	9	260.29	126.42	NS		
d-Amphetamine 2 mg/kg, b.i.d.	12	735.85	478.72	NS		
Nepicastat 5 mg/kg, b.i.d.	11	499.46	236.03	NS		
Nepicastat mg/kg, b.i.d.	11	551.09	441.75	NS		
Nepicastat 0 mg/kg, b.i.d.	9	390.47	228.21	NS		NS
SKF 102698 50 mg/kg, b.i.d.	9	246.78	194.28		0.0116	
Cyclodextrine	11	485.58	184.76	NS		
dH ₂ O	11	477.66	265.91			

Figure 145

**SUMMARY STATISTICS AND P-VALUES FOR PAIRWISE TREATMENT COMPARISONS WITHIN
TIME FOR ACOUSTIC STARTLE REACTIVITY IN RATS
(for startles 31-45)**

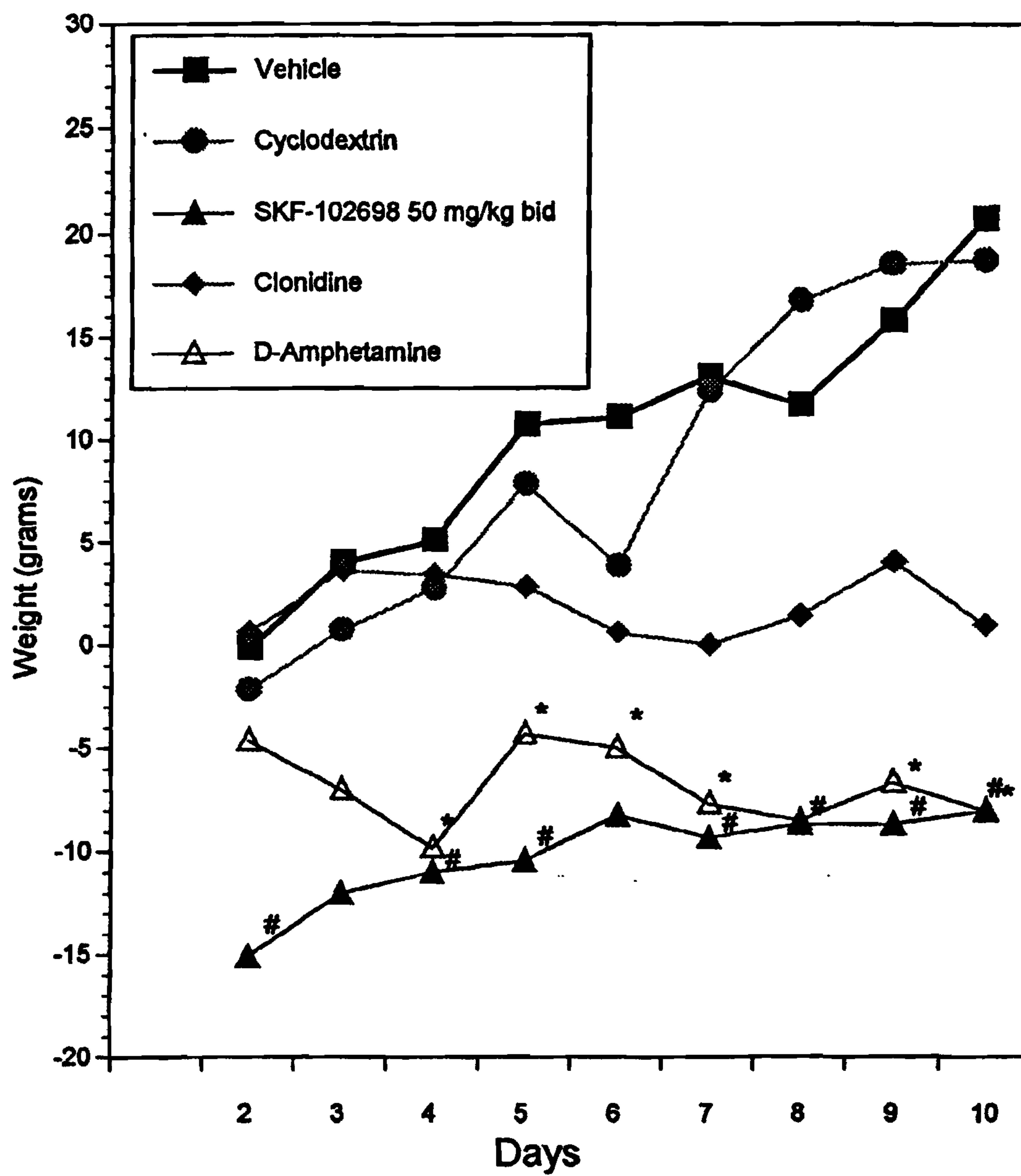
TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	9	349.42	276.69	NS		
d-Amphetamine 2 mg/kg, b.i.d.	12	586.60	387.76	NS		
Nepicastat 15 mg/kg, b.i.d.	11	403.51	152.02	NS		
Nenicastat 5 mg/kg, b.i.d.	11	374.42	379.49	NS		
Nepicastat 50 mg/kg, b.i.d.	9	408.16	254.32	NS		0.0198
SKF 102698 50 mg/kg, b.i.d.	9	164.89	121.59		0.0061	
Cyclodextrine	11	550.58	354.10	NS		
dH ₂ O	11	329.49	277.82			

(for startles 46-60)

TREAT	N Obs	Mean	Std Dev	p vs. dH ₂ O	p vs. Cyclodextrine	p vs. SKF 102698
Clonidine 20 mg/kg, b.i.d.	9	292.67	237.18	NS		
d-Amphetamine 2 mg/kg, b.i.d.	12	532.60	438.87	NS		
Nepicastat 15 mg/kg, b.i.d.	11	360.00	166.31	NS		
Nepicastat 5 mg/kg, b.i.d.	11	475.44	464.56	NS		
Nepicastat 50 mg/kg, b.i.d.	9	263.98	105.67	NS		NS
SKF 102698 50 mg/kg, b.i.d.	9	155.96	111.81		0.0019	
Cyclodextrine	11	406.35	178.98	NS		
dH ₂ O	11	500.40	432.91			

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Figure 146

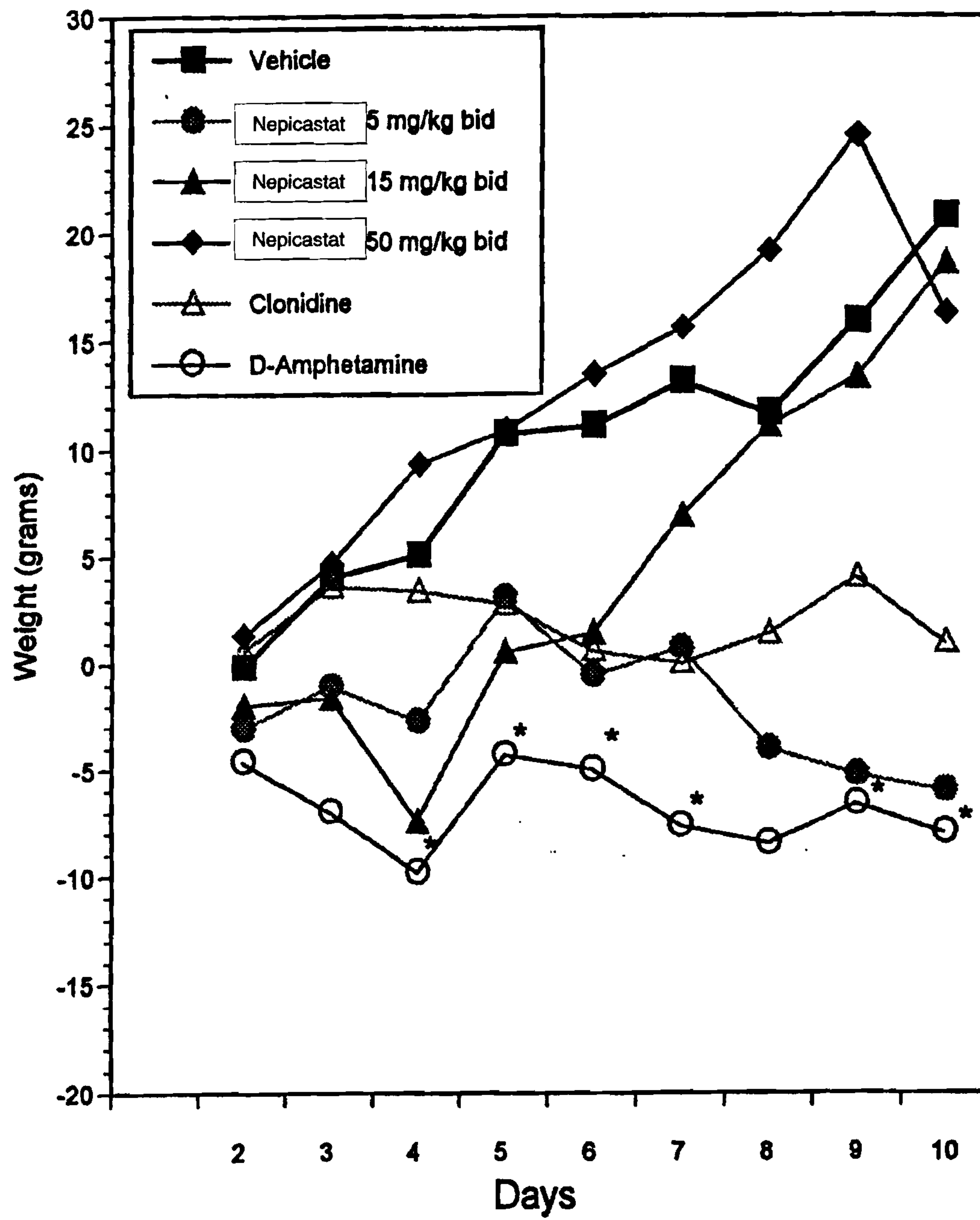


*p<0.005 versus vehicle

#p<0.005 versus cyclodextrin

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Figure 147



*p<0.005 versus vehicle

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Figure 148

Roche Safety and Tolerability Study			Nepicastat		
4/28/97 to 5/16/97					
MPTP Lesioned Animals: L-374, L-353, L-358					
Drug Preparation: Drug prepared in Mango juice @ concentration of 5 mg/ml.					
Animal #	Weight/sex	Route of Admin	Drug Acceptance	Adverse Effects	Comments
Week 1: 5 mg/kg BID					
374	0.712 kg/F	Oral (syringe)	Good	None	None
353	0.739 kg/M	Oral (syringe)	Good	None	None
358	0.991 kg/F	Oral (syringe)	Good	None	None
Week 2: 5 mg/kg BID					
Animal #	Weight	Route of Admin	Drug Acceptance	Adverse Effects	Comments
374	0.674 kg	Oral (syringe)	Good	None	None
353	0.714 kg	Oral (syringe)	Good	None	None
358	0.934 kg	Oral (syringe)	Good	None	None
Week 3: 5 mg/kg BID					
Animal #	Weight	Route of Admin	Drug Acceptance	Adverse Effects	Comments
374	0.697 kg	Oral (syringe) 5/12/97-5/13/97 am Oral Gavage 5/13/97 pm-5/16/97 pm	Poor Spilling out 1/4 med Good	None	None
353	0.703 kg	Oral (syringe) 5/12/97-5/13/97 am Oral Gavage 5/13/97 pm-5/16/97 pm	Poor Spilling out 1/4 med Good	None	None
358	0.937 kg	Oral (syringe) 5/12/97-5/13/97 am Oral Gavage 5/13/97 pm-5/16/97 pm	Poor Spilling out 1/4 med Good	None	None

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Figure 149

Roche Safety and Tolerability Study				Nepicastat	
1/7/97 to 1/27/97					
Drug Preparation: Drug prepared in Mango Juice @ concentration of 5 mg/ml. Drug administered by oral syringe					
NON- Lesioned Animals: L-314, L-316, L-317					
Animals				Week 1 0.5 mg/kg BID (5 days)	
	Weight/Sex	Route of Admin	Drug Acceptance	Adverse Effects	Comments
314	0.957 kg/M	Oral (syringe)	Good	None	None
316	0.809 kg/M	Oral (syringe)	Good	None	None
317	0.839 kg/M	Oral (syringe)	Good, but easily distracted with interruption in receiving drug	None	animal easily distracted noted on since start of study
Animals				Week 2 2 mg/kg BID (5 days)	
	Weight	Route of Admin	Drug Acceptance	Adverse Effects	Comments
314	0.957 kg	Oral (syringe)	Good	None	None
316	0.809 kg	Oral (syringe)	Good	None	None
317	0.839 kg	Oral (syringe)	Good, but easily distracted with interruption in receiving drug	None	Animal remained easily distracted
Animals				Week 3 5 mg/kg BID (5 days)	
	Weight	Route of Admin	Drug Acceptance	Adverse Effects	Comments
314	1.01 kg	Oral (syringe)	Good on day 1, dribbling out day 2, restraint required days 3,4,5	None	Refusing med by day 2 Required restraint days 2,3,4,5
316	0.808 kg	Oral (syringe)	Good on days 1 & 2, required restraint on days 3,4,5	None	Refusing med by day 3 Required restraint days 3,4,5
317	0.87 kg	Oral (syringe)	good	4-5 Light colored loose stool on days 4 & 5, gone by day 6	Required restraint throughout week #2
Conclusion: Animals less willing to voluntarily accept drug at concentration of 5 mg/kg, and all requiring restraint by day 3 at highest dose. Drug delivery to be adjusted for next Safety and Tolerability study (MPTP-lesioned monkeys) by diluting drug in larger volume of Mango juice. One (L-317) out of 3 animals with loose stool observed on days 4 and 5 at highest dose (5 mg/kg bid), normal stools by day 6. Animal L-317 with easily distracted behavior since beginning of study. Final assessment is that drug appears to be tolerated with minimal side effects.					

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Figure 150

Parkinsonian Dream Scale VIII

<u>Parkinsonian Features:</u>	<u>Score R/L</u>
1. Tremor (right/left)	____/____
0- absent	
1- occasional, or barely detectable (normal for aged), occurring while active	
2- frequent, or easily detectable, occurring while active or at rest	
3- continuous, or intense, occurring while active and rest	
2. Freezing	_____
0- absent	
1- occasional episodes of short duration (< 5 seconds)	
2- occasional episodes of moderate duration (6-10 seconds)	
3- frequent episodes or episodes of long duration (>10 seconds)	
3. Locomotion	_____
0- uses limbs smoothly and symmetrically	
1- walks slowly (normal for aged)	
2- walks very slowly and with effort, may drag limb or refuse to bear weight	
3- no ambulation, even with stimulation	
4. Manual Dexterity/ Fine motor skills (right/left)	____/____
(≤1/2 inch food presented on flat surface or spoon)	
0- normal function- able to grasp/retrieve small objects, aims accurately, independent use.	
1- reduced grasping and slow, some loss of maneuverability of food	
2- gross handling of food item only/ may drop food or multiple attempts to retrieve food, marked slowness	
3- unable to grasp/retrieve small food item	
5. Bradykinesia(right arm/ left arm)	____/____
0- quick, precise movements	
1- mild slowing of movements (normal for aged)	
2- slow deliberate movements with marked impairment initiating movements	
3- no movements	
6. Hypokinesia	_____
0- moves freely, alert, responsive	
1- reduced activity (normal for aged), moves less frequently (without provocation)	
2- minimal activity, moves with provocation, may have reduced facial expression	
3- akinetic (essentially no movements) even with provocation	
7. Balance	_____
0- requires no assistance for maintaining posture	
1- requires assistance for standing	
2- requires assistance for walking, or falls	
3- face down, or unable to maintain posture	
8. Posture	_____
0- normal posture (cat squat)	
1- reduced posture (normal for aged, head down)	
2- stooped posture- haunched	
3- unable to maintain posture, recumbent	

TOTAL SCORE _____

Figure 151

A : Lesioning Schedule Group A				
Treatment	Animal #	Sex/Weight	MPTP Lesioning Dates	
Placebo	346	F/755 grams	2/10/97	5/7/97
	351	M/858	"	5/7/97
	353	F/993	"	
	355	F/822	"	
	357	F/861	"	
	415	M/657	5/7/97	7/7/97

Lesioning Schedule Group B					
Treatment	Animal #	Sex/Weight	MPTP Lesioning Dates		
1 mg/kg/day Nepicastat	159	F/788 grams	6/23/97		
	255	F/755	"		
	256	F/870	"	7/21/97	
	265	M/823	"		
	411	F/640	2/10/97	5/7/97	7/7/97
	373	F/	6/23/97 (died)		

Table 1C: Lesioning Schedule Group C

Treatment	Animal #	Sex/Weight	MPTP Lesioning Dates		
4 mg/kg/day Nepicastat	371	F/571 grams	5/7/97	7/7/97	
	372	F/500	5/7/97	6/16/97	7/7/97
	375	F/666	5/7/97		
	414	F/662	5/7/97	7/7/97	
	416	F/619	5/7/97	7/7/97	
	430	M/1000	5/7/97		

Table 1D: Lesioning Schedule Group D

Treatment	Animal #	Sex/Weight	MPTP Lesioning Dates	
Nepicastat 10 mg/kg/day	350	M/840 grams	2/3/97	5/7/97
	352	M/977	2/10/97	
	356	M/980	"	
	358	F/705	"	
	374	F/627	"	
	425	M/913	"	5/7/97

Figure 152**Table 2A: Treatment Schedule Group A**

Treatment	Animal #	Post MPTP Baseline	L-Dopa 2.5 mg/kg	L-Dopa 5 mg/kg	L-Dopa 7.5 mg/kg	Water
Placebo	346	6/9/97	6/16/97	6/24/97	6/30/97	7/22/97
	351	"	"	"	"	"
	353	"	"	"	"	"
	355	3/31/97	4/29/97/	5/6/97	5/13/97	6/27/97
	357	"	"	"	"	"
	415	8/4/97	8/11/97	8/18/97	8/25/97	9/16/97

Table 2B: Treatment Schedule Group B

Treatment	Animal #	Post MPTP Baseline	L-Dopa 2.5 mg/kg	L-Dopa 5 mg/kg	L-Dopa 7.5 mg/kg	RS-25560-197
1 mg/kg/day	159	8/18/97	9/2/97	9/9/97	9/15/97	10/7/97
Nepicastat						
	255	9/22/97	9/29/97	10/6/97	10/13/97	11/3/97
	256	8/18/97	9/2/97	9/9/97	9/15/97	10/7/97
	265	8/18/97	9/2/97	9/9/97	9/15/97	10/7/97
	411	9/9/97	9/29/97	10/6/97	10/13/97	11/3/97
	373	Died				

Table 2C: Treatment Schedule Group C

Treatment	Animal #	Post MPTP Baseline	L-Dopa 2.5 mg/kg	L-Dopa 5 mg/kg	L-Dopa 7.5 mg/kg	RS-25560-197
Nepicastat	371	8/4/97	8/11/97	8/18/97	8/25/97	9/16/97
4 mg/kg/day						
	372	8/6/97	8/11/97	8/18/97	8/25/97	9/16/97
	375	8/6/97	8/11/97	8/18/97	8/25/97	9/16/97
	414	8/6/97	8/11/97	8/18/97	8/25/97	9/16/97
	416	8/4/97	8/11/97	8/18/97	8/25/97	9/16/97
	430	8/4/97	8/11/97	8/18/97	8/25/97	9/16/97

Table 2D: Treatment Schedule Group D

Treatment	Animal #	Post MPTP Baseline	L-Dopa 2.5 mg/kg	L-Dopa 5 mg/kg	L-Dopa 7.5 mg/kg	RS-25560-197
	350	6/9/97	6/16/97	6/24/97	6/30/97	7/22/97
Nepicastat						
10 mg/kg/day						
	352	3/31/97	4/29/97/	5/6/97	5/13/97	6/27/97
	356	"	"	"	"	"
	358	6/3/97	6/10/97	6/17/97	6/24/97	7/14/97
	374	6/3/97	6/10/97	6/17/97	6/24/97	7/14/97
	425	6/9/97	6/16/97	6/24/97	6/30/97	7/22/97

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Figure 153A

Animal #	Baseline Activity Monitoring	Post-MPTP Activity Monitoring
346	342.9 movements/10 mins	193.6 movements/10 mins
351	161.2	93.8
353	721.3	301.0
355	391.5	105.9
357	512.9	516.0
415	134.8	57.8
Average	377.43	211.35
Stan. Dev.	90.11	173.02

Figure 153B

Animal #	Post-MPTP CRS
346	8.7
351	13.1
353	15.4
355	5.8
357	4.8
415	5.5
Average	8.9
Stan. Dev.	4.43

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Figure 154A

Animal #	Baseline Activity Monitoring	Post-MPTP Activity Monitoring
159	419 movements/10 mins	65.8 movements/10 mins
255	237	28
256	687	85.9
265	381	583
411	44	59.3
Average	353.6	164.4
Stan. Dev.	237.5	234.9

Figure 154B

Animal #	Post-MPTP CRS
159	12.5
255	16.1
256	12.9
265	4.3
411	5.8
Average	10.32
Stan. Dev.	5.04

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Figure 155A

Animal #	Baseline Activity Monitoring	Post-MPTP Activity Monitoring
371	120 movements/10 mins	34.4 movements/10 mins
372	63	56.9
375	244	150
414	231	56.1
416	147.8	81.4
430	136.1	176.6
Average	156.98	92.57
Stan. Dev.	69	57.39

Figure 155B

Animal #	Post-MPTP CRS
371	17.3
372	7.5
375	7.5
414	6.5
416	7.5
430	7.5
Average	8.97
Stan. Dev.	4.10

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Figure 156A

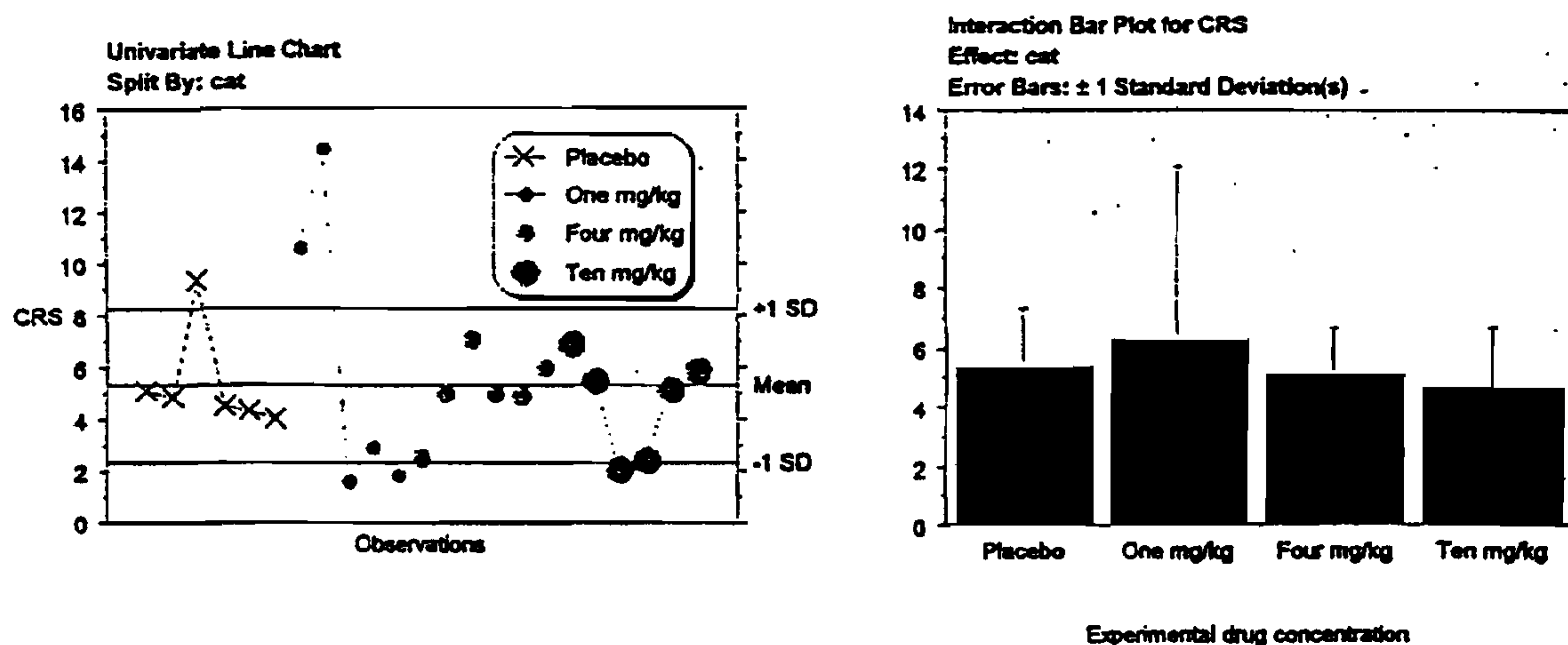
Animal #	Baseline Activity Monitoring	Post-MPTP Activity Monitoring
350	555 movements/10 mins	113 movements/10 mins
352	505	33.4
356	274	16
358	749	267
374	741	443
425	417	243
Average	540	185.9
Stan. Dev.	185	163

Figure 156A

Animal #	Post-MPTP CRS
350	10.2
352	7.6
356	5.3
358	4.0
374	5.4
425	15.6
Average	8.02
Stan. Dev.	4.31

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Figure 157



Descriptive Statistics
Split By: cat

	CRS, Total	CRS, Placebo	CRS, One mg/kg	CRS, Four mg/kg	CRS, Ten mg/kg
Mean	5.3	5.3	6.3	5.1	4.7
Std. Dev.	3.0	2.0	5.9	1.5	2.0
Std. Error	.8	.8	2.6	.6	.8
Count	23	6	5	6	6
Minimum	1.6	4.0	1.6	2.5	2.1
Maximum	14.4	9.3	14.4	7.1	6.9

Kruskal-Wallis Test for CRS
Grouping Variable: cat

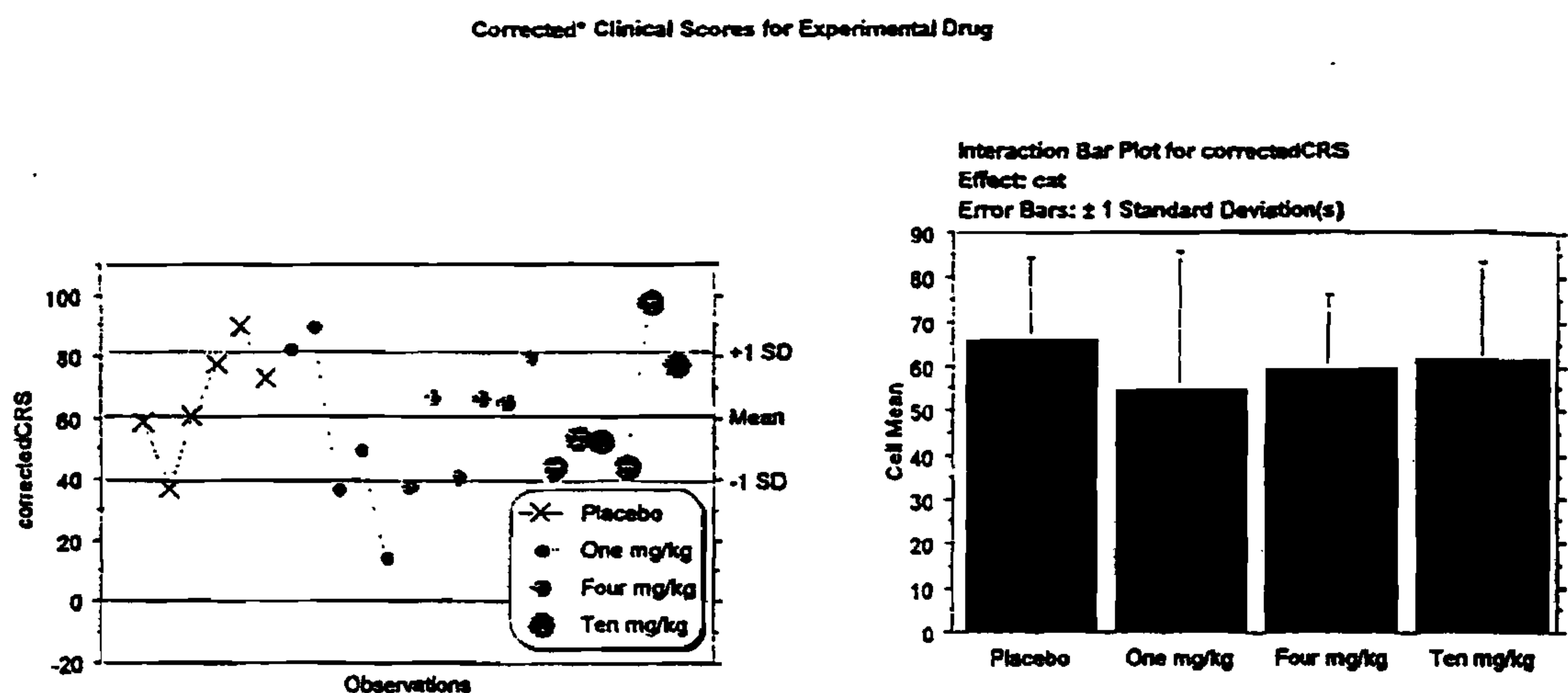
DF	3
# Groups	4
# Ties	1
H	.381
P-Value	.9441
H corrected for ties	.381
Tied P-Value	.9441

Kruskal-Wallis Rank Info for CRS
Grouping Variable: cat

	Count	Sum Ranks	Mean Rank
Placebo	6	69.000	11.500
One mg/kg	5	54.000	10.800
Four mg/kg	6	79.000	13.167

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Figure 158



Descriptive Statistics
Split By: cat

	Experimental drug concentration				
	correctedCRS, Total	correctedCRS, Placebo	correctedCRS, One mg/kg	correctedCRS, Four mg/kg	correctedCRS, Ten mg/kg
Mean	60.8	65.9	54.8	59.7	61.8
Std. Dev.	20.9	18.3	31.3	16.4	21.6
Std. Error	4.4	7.5	14.0	6.7	8.8
Count	23	6	5	6	6
Minimum	14.4	36.6	14.4	38.5	44.2
Maximum	98.1	89.6	89.4	80.0	98.1

*Corrected clinical scores are clinical scores of experimental drug at each concentration as a ratio of Post MPTP clinical scores

Kruskal-Wallis Test for correctedCRS
Grouping Variable: cat

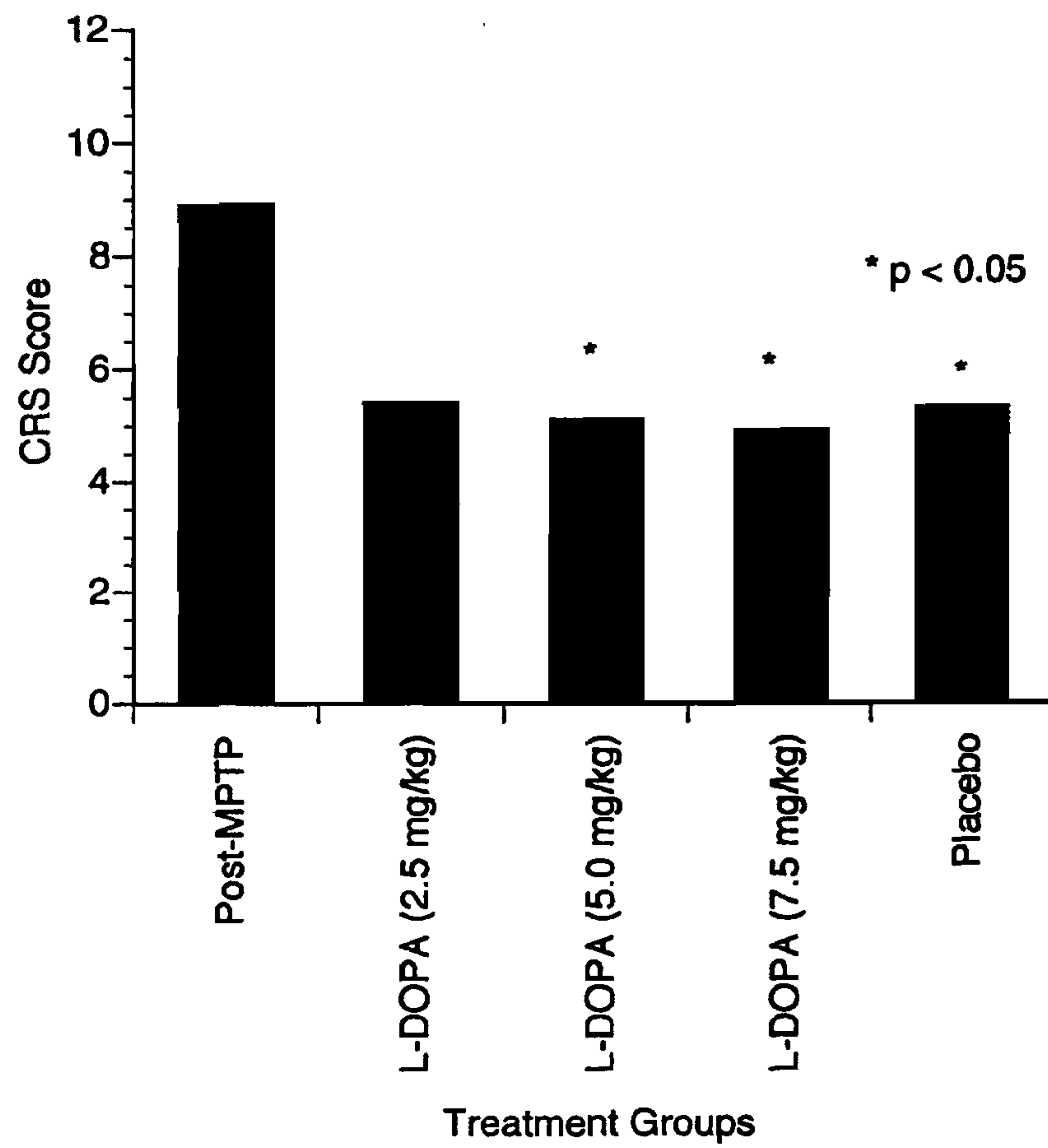
DF	3
# Groups	4
# Ties	1
H	.463
P-Value	.9269
H corrected for ties	.463
Tied P-Value	.9269

Kruskal-Wallis Rank Info for correctedCRS
Grouping Variable: cat

	Count	Sum Ranks	Mean Rank
Placebo	6	80.000	13.333
One mg/kg	5	53.000	10.600
Four mg/kg	6	70.000	11.667

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Figure 159



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Figure 160**Comparing Clinical Scores* of Placebo to Post MPTP and L-Dopa drug concentrations****Friedman Test for 5 Variables**

DF	4
# Groups	5
# Ties	3
Chi Square	9.833
P-Value	.0433
Chi Square corrected for ties	10.085
Tied P-Value	.0390

Friedman Rank Info for 5 Variables

	Count	Sum Ranks	Mean Rank
Post MPTP	6	28.500	4.750
L-Dopa2.5mg	6	18.000	3.000
L-Dopa5mg	6	14.500	2.417
L-Dopa7.5mg	6	14.000	2.333
Placebo	6	15.000	2.500

Descriptive Statistics

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Post MPTP	8.9	4.4	1.8	6	4.8	15.4	0
L-Dopa2.5mg	5.4	3.2	1.3	6	.3	9.6	0
L-Dopa5mg	5.1	1.5	.6	6	3.0	7.7	0
L-Dopa7.5mg	4.9	2.5	1.0	6	2.5	9.8	0
Placebo	5.3	2.0	.8	6	4.0	9.3	0

*Note: Each primate was clinically scored Post MPTP and the same primate was subsequently clinically scored after dosage with 2.5 mg, 5 mg and 7.5 mg of L-Dopa then clinically scored with no experimental drug.

Figure 161

Groups	Sum Rank		Λ	# of Treatments(a)	# of Subjects per group(b)	SE	q	p	q' 0.05(2), Infinity,p	Conclude
	Post	Test gp								
Post to Ldopa7.5mg	28.5	14	14.5	5	6	5.48	2.65	5	2.44	Reject H0
Post to Ldopa5mg	28.5	14.5	14	5	6	5.48	2.55	5	2.44	Reject H0
Post to Ldopa2.5mg	28.5	18	10.5	5	6	5.48	1.92	5	2.44	Accept H0
Post to Placebo ^a	28.5	15	13.5	5	6	5.48	2.46	5	2.44	Reject H0

^aTable B.7 Critical Values of q' for two-tailed Dunnnett's test p.539 Biostatistical Analysis JH Zar 2nd ed Prentice-Hall, Inc NJ84

Summary

The CRS measurement is significantly lower for the ^aplacebo and L-dopa dosage at 5 mg/kg and 7.5 mg/kg compared to the post MPTP CRS measurement.

^aNote: Placebo is borderline significant.

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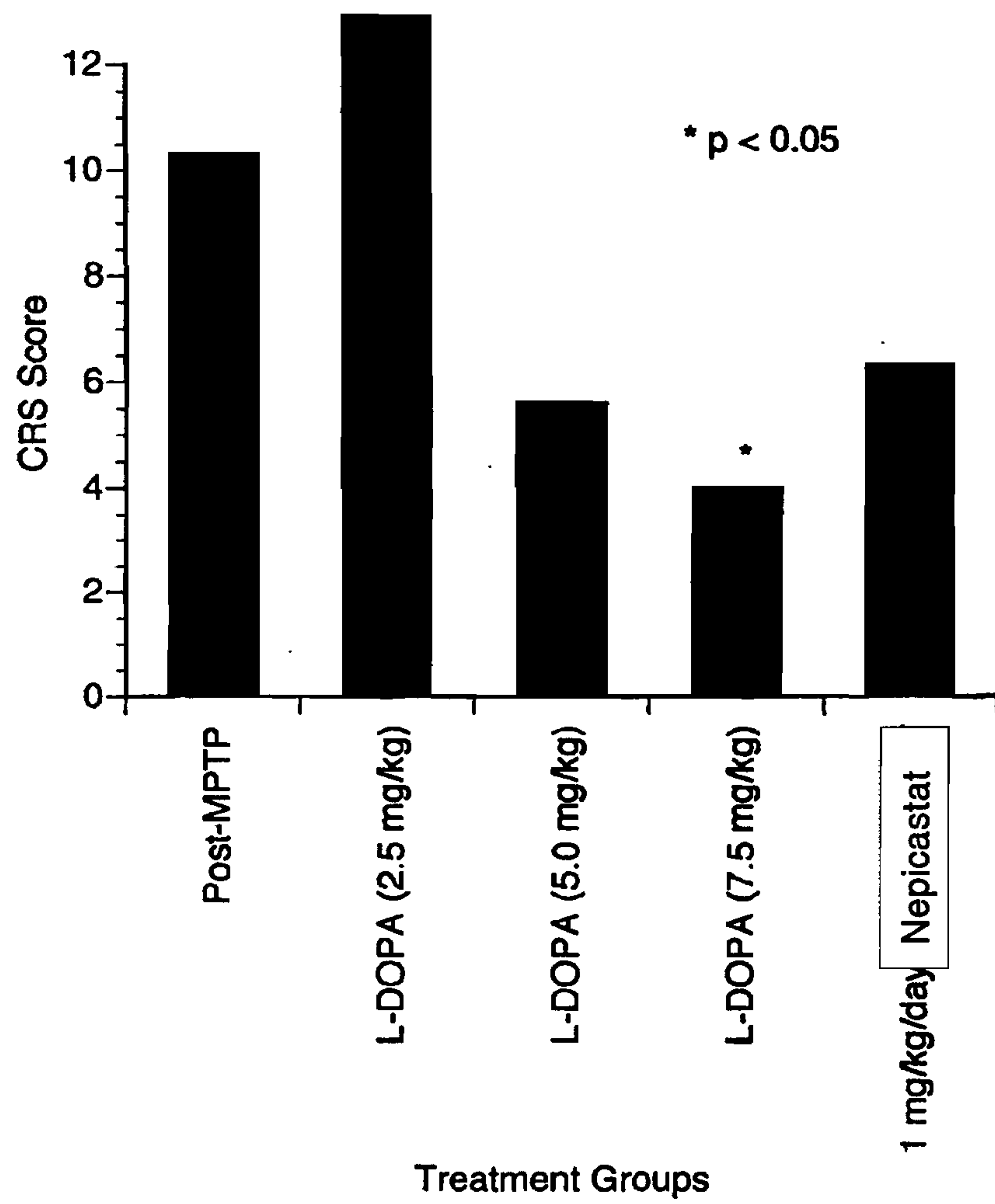
Figure 162

Figure 163

Comparing Clinical Scores* of 1 mg/kg Experimental Drug to Post MPTP and L-Dopa drug concentrations

Friedman Test for 5 Variables

DF	4
# Groups	5
# Ties	0
Chi Square	13.440
P-Value	.0093
Chi Square corrected for ties	13.440
Tied P-Value	.0093

Friedman Rank Info for 5 Variables

	Count	Sum Ranks	Mean Rank
Post MPTP	5	22.000	4.400
L-Dopa2.5mg	5	22.000	4.400
L-Dopa5mg	5	10.000	2.000
L-Dopa7.5mg	5	9.000	1.800
Onemg/kg	5	12.000	2.400

Descriptive Statistics

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Post MPTP	10.3	5.0	2.3	5	4.3	16.1	0
L-Dopa2.5mg	12.9	3.9	1.7	5	6.9	16.8	0
L-Dopa5mg	5.6	4.5	2.0	5	.9	10.9	0
L-Dopa7.5mg	4.0	2.3	1.0	5	.9	7.1	0
Onemg/kg	6.3	5.9	2.6	5	1.6	14.4	0

*Note: Each primate was clinically scored Post MPTP and the same primate was subsequently clinically scored after dosage with 2.5 mg, 5 mg and 7.5 mg of L-Dopa then clinically scored with 1 mg/kg of experimental drug.

Figure 164

Groups	Sum Rank		Δ	# of Treatments	# of Subjects per group	q	p	q' 0.05(2)	Conclusion
	Post	Test gp							
Post to Ldopa7.5mg	22	9	13	5	5	5.00	5.00	2.44	Reject H0
Post to Ldopa5mg	22	10	12	5	5	5.00	5.00	2.44	AcceptH0*
Post to Ldopa2.5mg	22	22	0	5	5	5.00	5.00	2.44	x
Post to ED1mg	22	12	10	5	5	5.00	5.00	2.44	Accept H0

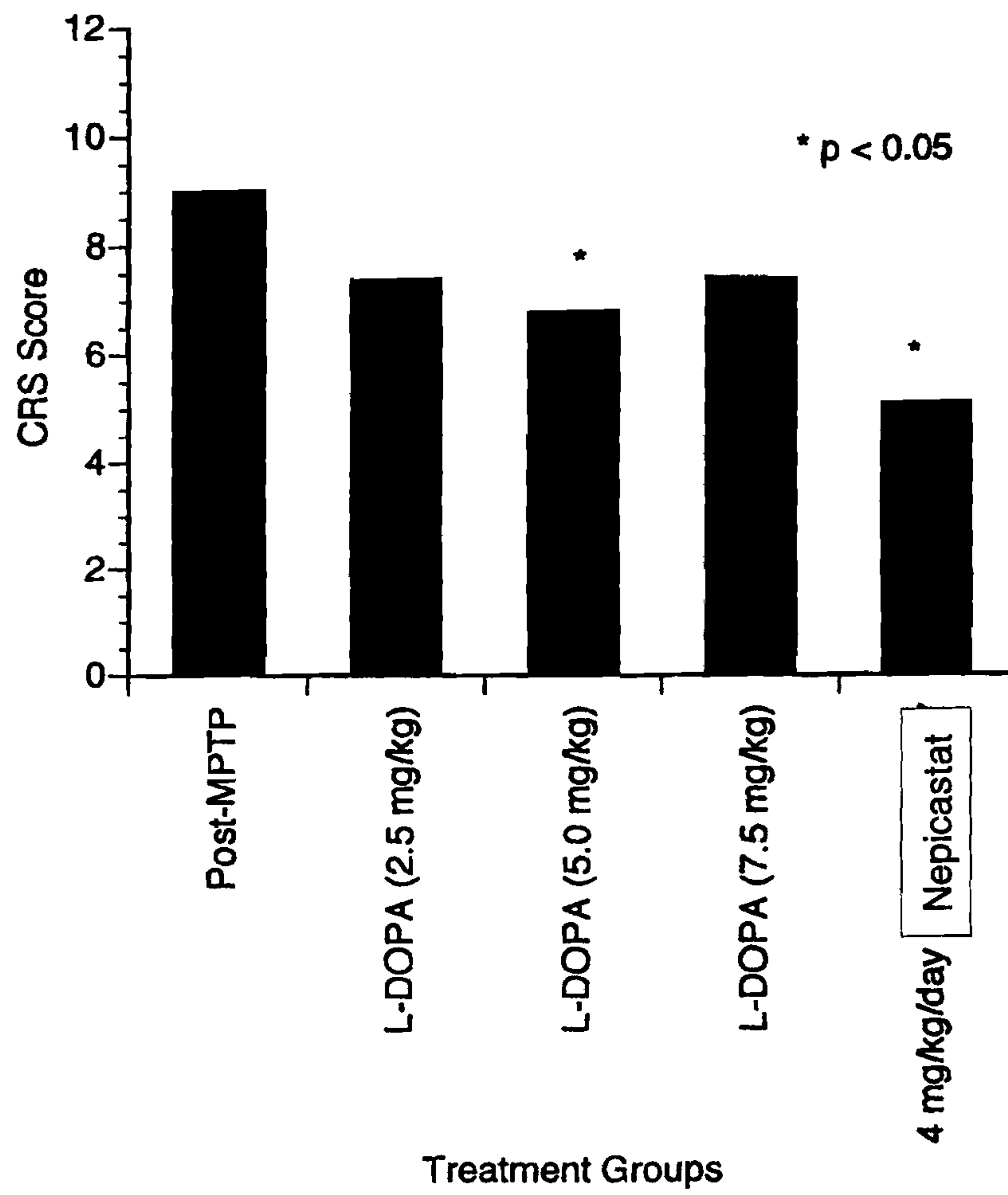
Summary

The CRS measurement is significantly lower for the L-dopa dosage at 7.5 mg/kg compared to the post MPTP CRS measurement.

*borderline

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Figure 165



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Figure 166**Comparing Clinical Scores* of 4 mg/kg Experimental Drug to Post MPTP and L-Dopa drug concentrations****Friedman Test for 5 Variables**

DF	4
# Groups	5
# Ties	0
Chi Square	20.000
P-Value	.0005
Chi Square corrected for ties	20.000
Tied P-Value	.0005

Friedman Rank Info for 5 Variables

	Count	Sum Ranks	Mean Rank
Post MPTP	6	30.000	5.000
L-Dopa2.5mg	6	21.000	3.500
L-Dopa5mg	6	13.000	2.167
L-Dopa7.5mg	6	19.000	3.167
fourmg/kg	6	7.000	1.167

Descriptive Statistics

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Post MPTP	9.0	4.1	1.7	6	6.5	17.3	0
L-Dopa2.5mg	7.4	1.4	.6	6	5.3	9.7	0
L-Dopa5mg	6.8	1.9	.8	6	5.1	10.5	0
L-Dopa7.5mg	7.4	1.4	.6	6	6.3	10.1	0
fourmg/kg	5.1	1.5	.6	6	2.5	7.1	0

*Note: Each primate was clinically scored Post MPTP and the same primate was subsequently clinically scored after dosage with 2.5 mg, 5 mg and 7.5 mg of L-Dopa then clinically scored with 1 mg/kg of experimental drug.

Figure 167

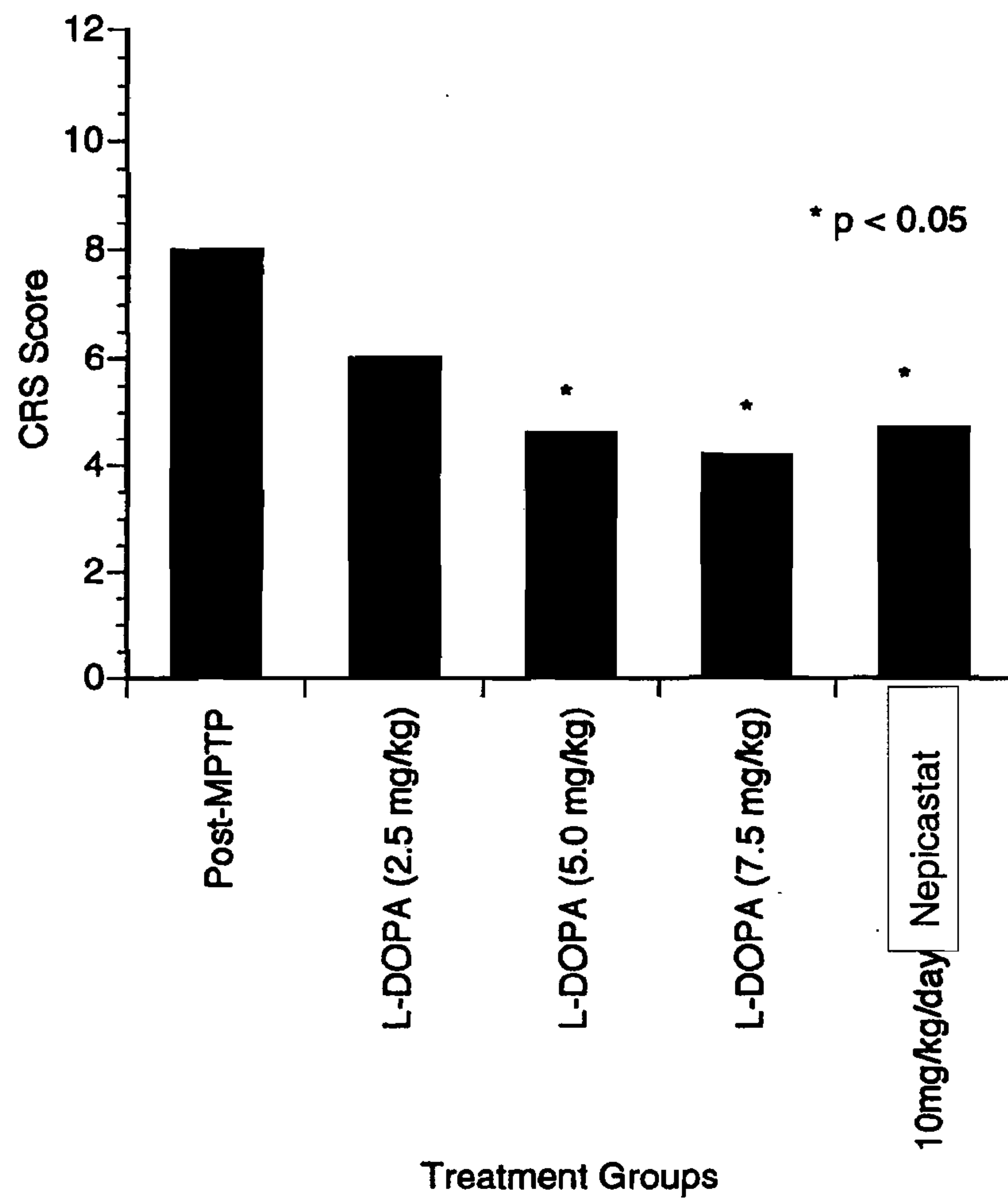
Groups	Sum Rank		Δ	# of Treatments(a)	# of Subjects per group(b)	SE	q	p	q' 0.05(2) infinity,p	Conclude
	Post	Test gp								
Post to Ldopa7.5mg	30	19	11	5	6	5.48	2.01	5	2.44	Accept H0
Post to Ldopa5mg	30	13	17	5	6	5.48	3.10	5	2.44	Reject H0
Post to Ldopa2.5mg	30	21	9	5	6	5.48	1.64	5	2.44	Accept H0
Post to ED4mg	30	7	23	5	6	5.48	4.20	5	2.44	Reject H0

*Table B.7 Critical Values of q for two-tailed Dunnett's test p.539 Biostatistical Analysis JH Zar 2nd ed Prentice-Hall, Inc NJ84

Summary

The CRS measurement is significantly lower for the experimental drug at 4 mg/kg and L-dopa dosage at 5 mg/kg compared to the post MPTP CRS measurement. Note from some reason the the 7.5 mg/kg L-dopa dose is not significantly lower.

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Figure 168

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Figure 169**Comparing Clinical Scores* of 10 mg/kg Experimental Drug to Post MPTP and L-Dopa drug concentrations****Friedman Test for 5 Variables**

DF	4
# Groups	5
# Ties	0
Chi Square	12.800
P-Value	.0123
Chi Square corrected for ties	12.800
Tied P-Value	.0123

Friedman Rank Info for 5 Variables

	Count	Sum Ranks	Mean Rank
Post MPTP	6	29.000	4.833
L-Dopa2.5mg	6	20.000	3.333
L-Dopa5mg	6	15.000	2.500
L-Dopa7.5mg	6	11.000	1.833
Tenmg/kg	6	15.000	2.500

Descriptive Statistics

	Mean	Std. Dev.	Std. Error	Count	Minimum	Maximum	# Missing
Post MPTP	8.0	4.3	1.8	6	4.0	15.6	0
L-Dopa2.5mg	6.0	2.6	1.1	6	2.9	10.3	0
L-Dopa5mg	4.6	1.9	.8	6	2.6	7.9	0
L-Dopa7.5mg	4.2	1.2	.5	6	2.6	5.6	0
Tenmg/kg	4.7	2.0	.8	6	2.1	6.9	0

*Note: Each primate was clinically scored Post MPTP and the same primate was subsequently clinically scored after dosage with 2.5 mg, 5 mg and 7.5 mg of L-Dopa then clinically scored with 10 mg/kg of experimental drug.

Figure 170

Groups	Sum Rank		Δ	# of		# of Subjects	SE	q	p	q' 0.05(2) / p'	Conclude
	Post	Test gp		Treatments	per group						
Post to Ldopa7.5mg		29	11	18	5	6	5.48	3.29	5	2.44	Reject H0
Post to Ldopa5mg		29	15	14	5	6	5.48	2.56	5	2.44	Reject H0
Post to Ldopa2.5mg		29	20	9	5	6	5.48	1.64	5	2.44	Accept Ho
Post to ED10mg		29	15	14	5	6	5.48	2.56	5	2.44	Reject Ho

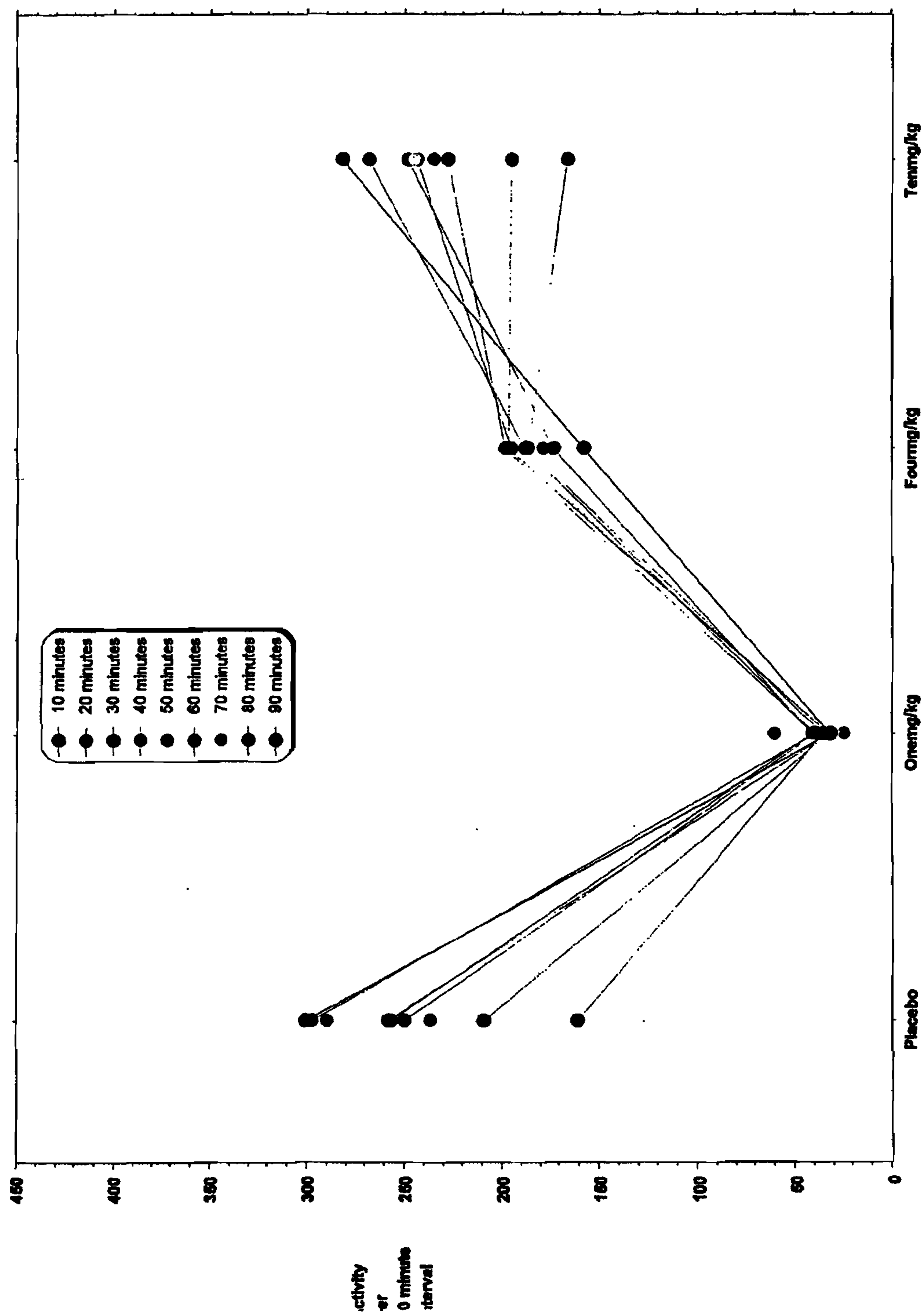
*Table B.7 Critical Values of q' for two-tailed Dunnett's test p.539 Biostatistical Analysis JH Zar 2nd ed Prentice-Hall, Inc NJ'84

Summary

The CRS measurement is significantly lower for the experimental drug at 10 mg/kg experimental drug and L-dopa dosage at 5, 7.5 mg/kg compared to the post MPTP CRS measurement.
is not significantly lower.

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Figure 171
Activity Counts Measure over 10 minute Intervals for Placebo and 1,4,10 mg/kg of
Experimental Drug
After 10 to 90 minutes Post Dosing



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Figure 172

Descriptive Statistics**Split By: Minutes****Row exclusion: ActivityDataEXdrug.svd**

	Mean	Std. Dev.	Count	Minimum	Maximum
Placebo, Total	251.3	209.6	54	18.2	767.8
Placebo, 10 minutes	161.3	73.2	6	100.8	274.4
Placebo, 20 minutes	209.6	146.1	6	69.0	457.8
Placebo, 30 minutes	258.6	189.5	6	71.0	562.2
Placebo, 40 minutes	249.8	222.0	6	65.8	611.6
Placebo, 50 minutes	236.6	237.2	6	20.2	641.2
Placebo, 60 minutes	257.4	263.7	6	18.2	712.0
Placebo, 70 minutes	289.8	267.7	6	65.0	767.8
Placebo, 80 minutes	301.1	267.9	6	45.0	765.0
Placebo, 90 minutes	297.5	254.9	6	54.4	733.8
Onemg/kg, Total	37.1	33.2	45	0.0	122.2
Onemg/kg, 10 minutes	34.8	15.0	5	16.2	57.6
Onemg/kg, 20 minutes	32.3	26.1	5	.8	61.6
Onemg/kg, 30 minutes	32.4	32.5	5	0.0	85.2
Onemg/kg, 40 minutes	39.3	26.9	5	0.0	75.2
Onemg/kg, 50 minutes	60.5	46.6	5	0.0	122.2
Onemg/kg, 60 minutes	40.3	30.8	5	.2	79.4
Onemg/kg, 70 minutes	24.5	39.6	5	0.0	94.0
Onemg/kg, 80 minutes	31.3	40.4	5	0.0	87.4
Onemg/kg, 90 minutes	38.8	45.6	5	0.0	108.6
Fourmg/kg, Total	181.4	106.0	54	34.2	448.4
Fourmg/kg, 10 minutes	186.7	83.2	6	93.0	314.2
Fourmg/kg, 20 minutes	196.5	81.8	6	92.4	314.2
Fourmg/kg, 30 minutes	195.3	123.0	6	48.0	394.8
Fourmg/kg, 40 minutes	198.5	144.6	6	34.2	448.4
Fourmg/kg, 50 minutes	178.8	131.7	6	69.6	440.8
Fourmg/kg, 60 minutes	173.2	136.0	6	81.6	442.8
Fourmg/kg, 70 minutes	157.6	117.2	6	57.2	378.6
Fourmg/kg, 80 minutes	158.2	96.5	6	79.4	337.0
Fourmg/kg, 90 minutes	188.1	90.3	6	98.6	363.6
Tenmg/kg, Total	234.8	176.7	54	4.0	679.4
Tenmg/kg, 10 minutes	166.6	153.7	6	23.0	375.6
Tenmg/kg, 20 minutes	195.1	154.5	6	29.6	420.6
Tenmg/kg, 30 minutes	243.6	166.8	6	47.2	482.4
Tenmg/kg, 40 minutes	228.3	185.7	6	5.4	513.8
Tenmg/kg, 50 minutes	235.5	183.8	6	4.0	480.0
Tenmg/kg, 60 minutes	248.8	204.6	6	9.6	543.6
Tenmg/kg, 70 minutes	245.1	192.7	6	14.0	528.4
Tenmg/kg, 80 minutes	281.7	200.2	6	37.2	603.0
Tenmg/kg, 90 minutes	268.7	237.2	6	23.4	679.4

Results for totals may not agree with results for individual cells because of missing values for split variables.

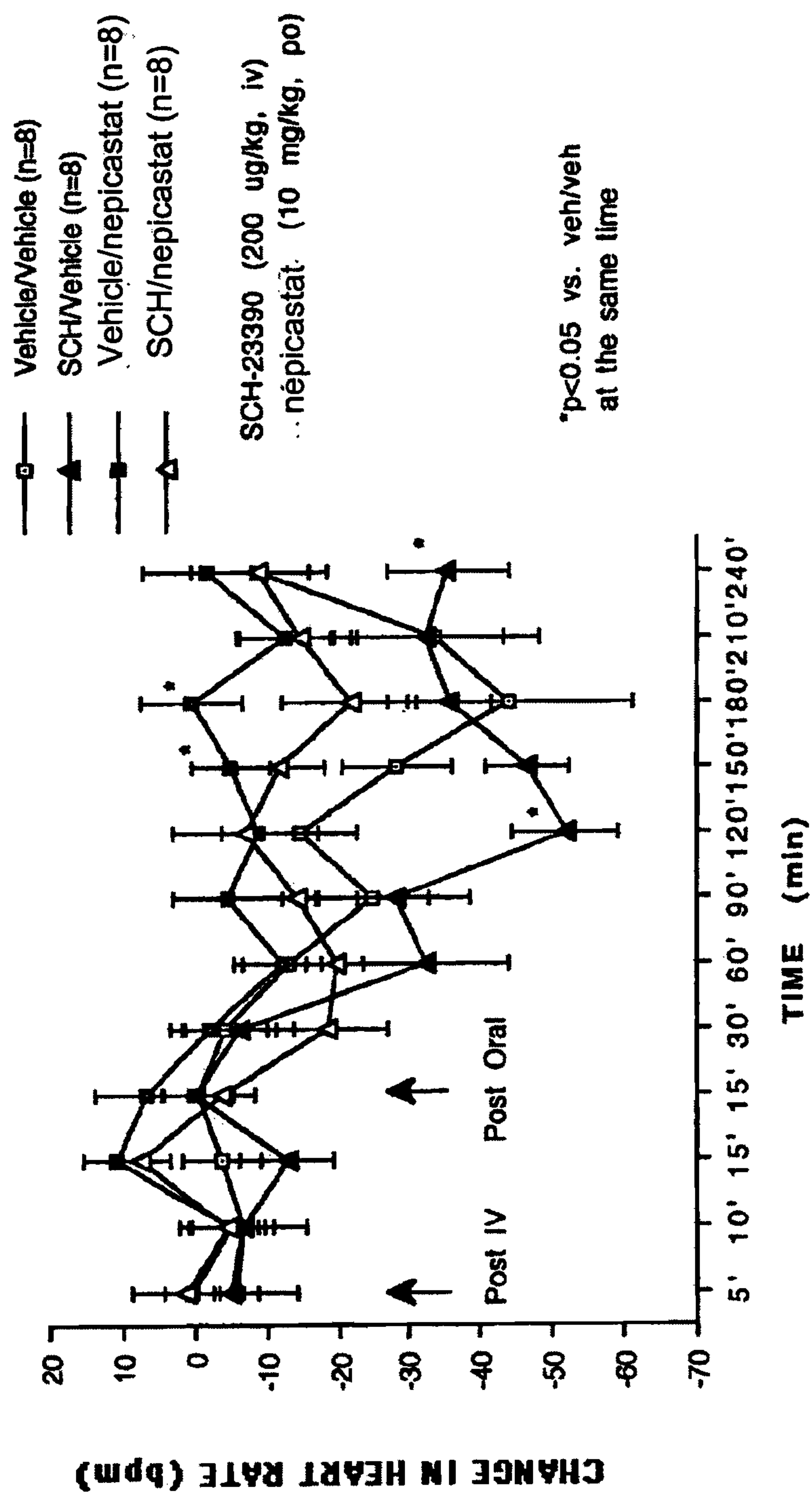
Figure 173

BASELINE HEART RATE AND MEAN ARTERIAL PRESSURE

Parameter	Treatment (iv/po)	N	Mean	Standard Error
Heart Rate (bpm)	Veh/Veh	8	471.0	7.4
	Veh/nepicastat	8	457.0	6.5
	SCH/Veh	8	454.0	7.6
	SCH/nepicastat	8	448.1	10.6
Mean Arterial Pressure	Veh/Veh	8	160.6	3.2
	Veh/nepicastat	8	160.9	2.7
	SCH/Veh	8	157.0	2.6
	SCH/nepicastat	8	159.5	2.7

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Figure 174



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Figure 175

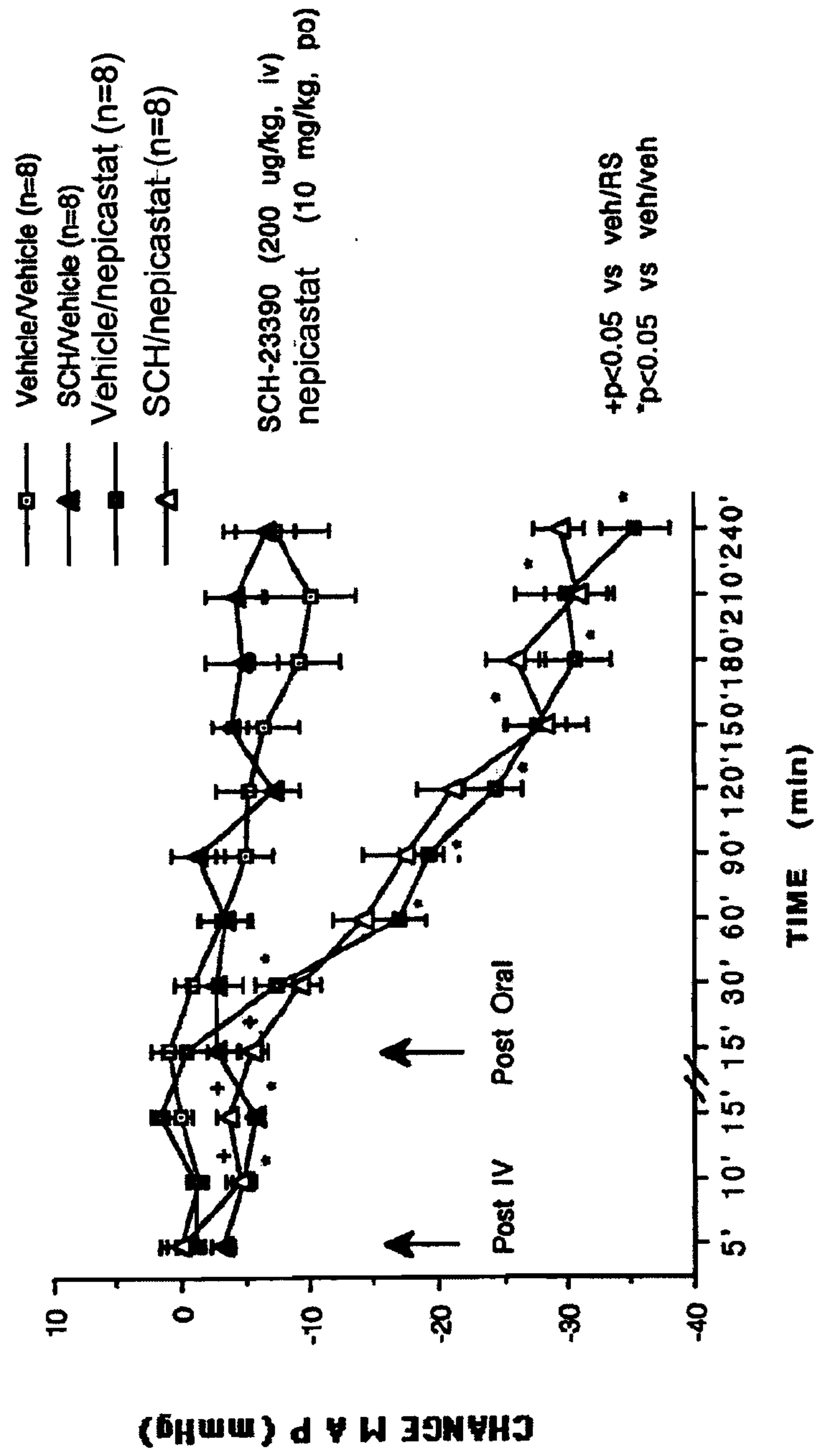
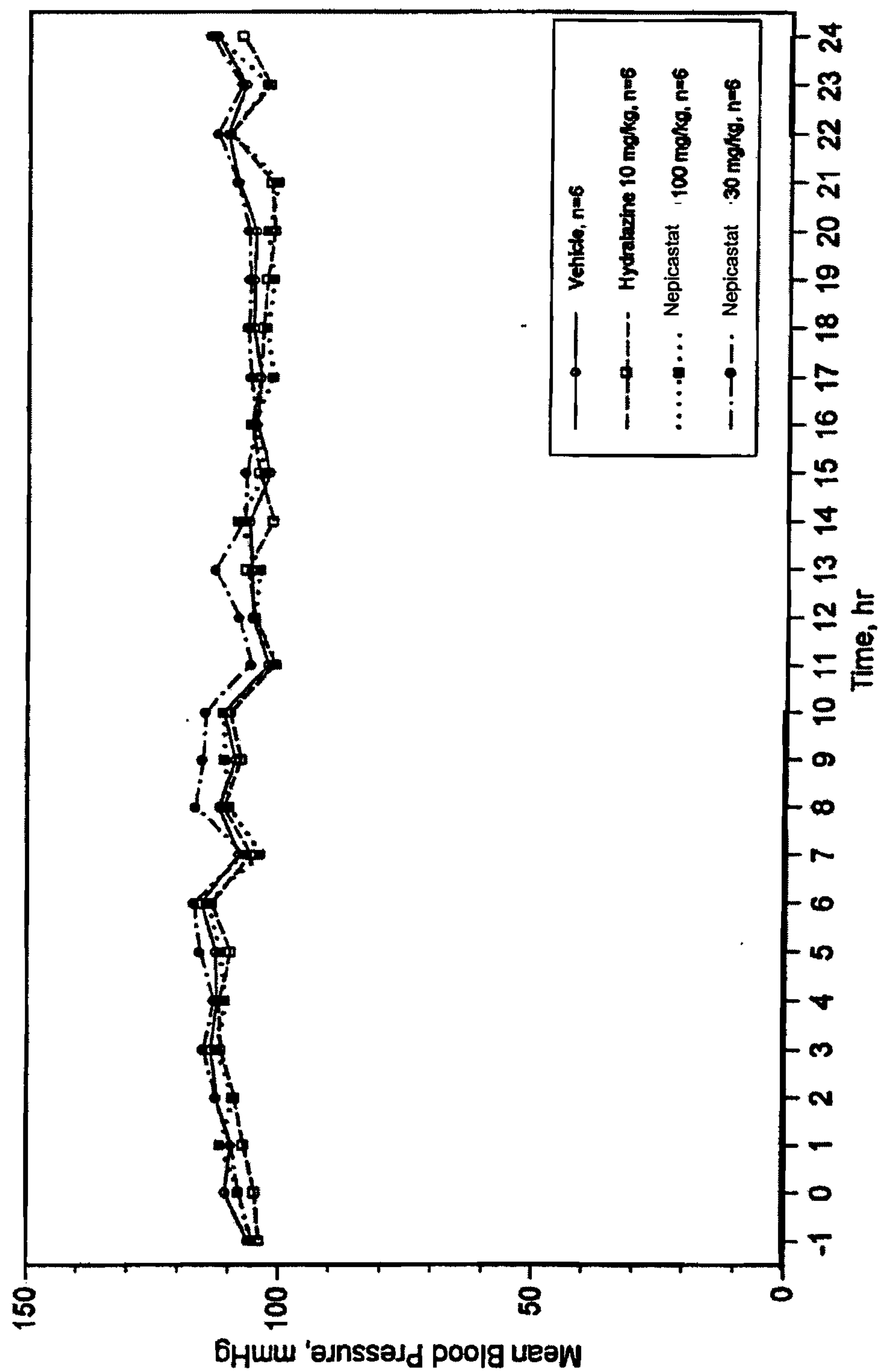


Figure 2

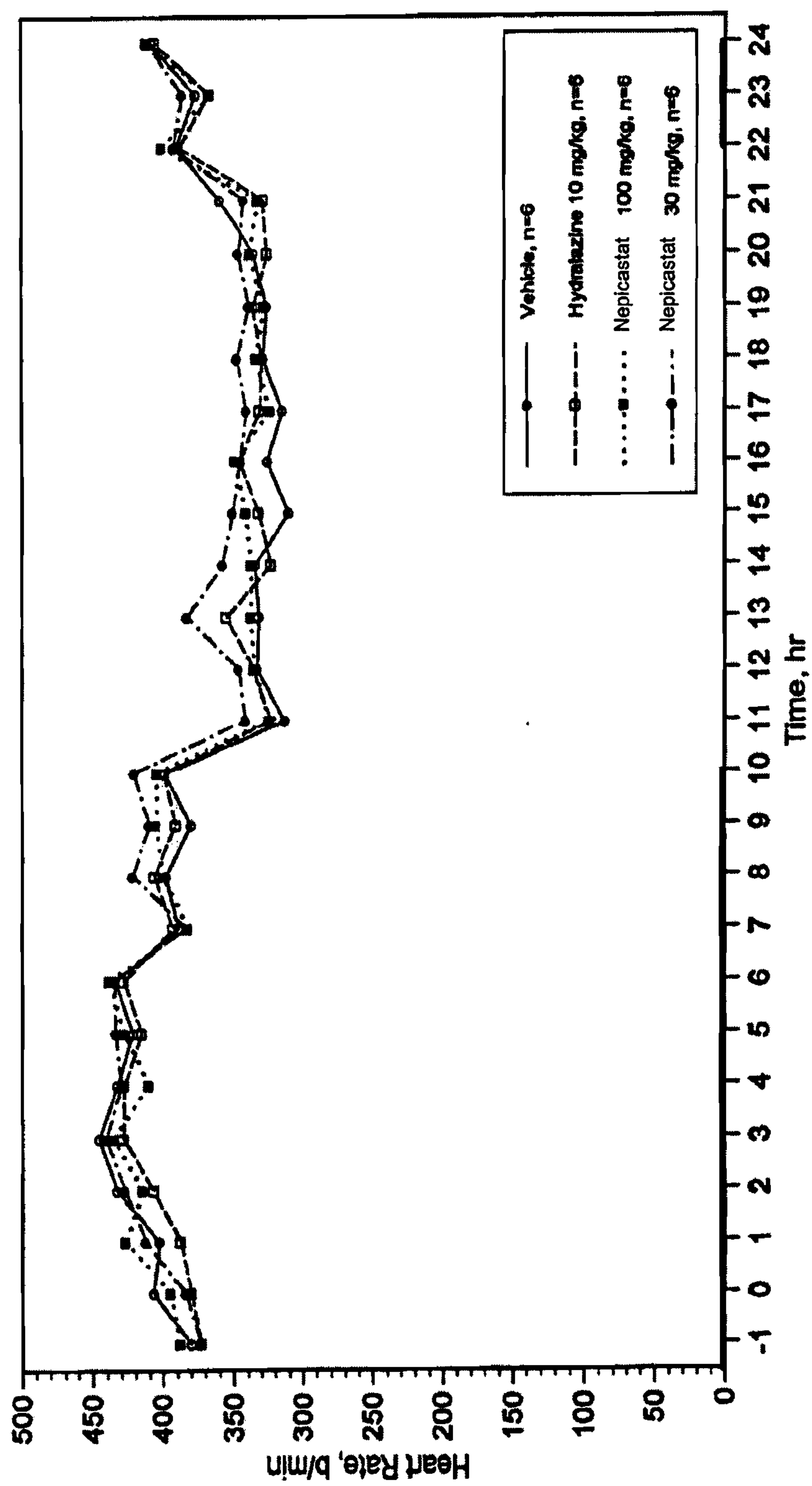
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Figure 176



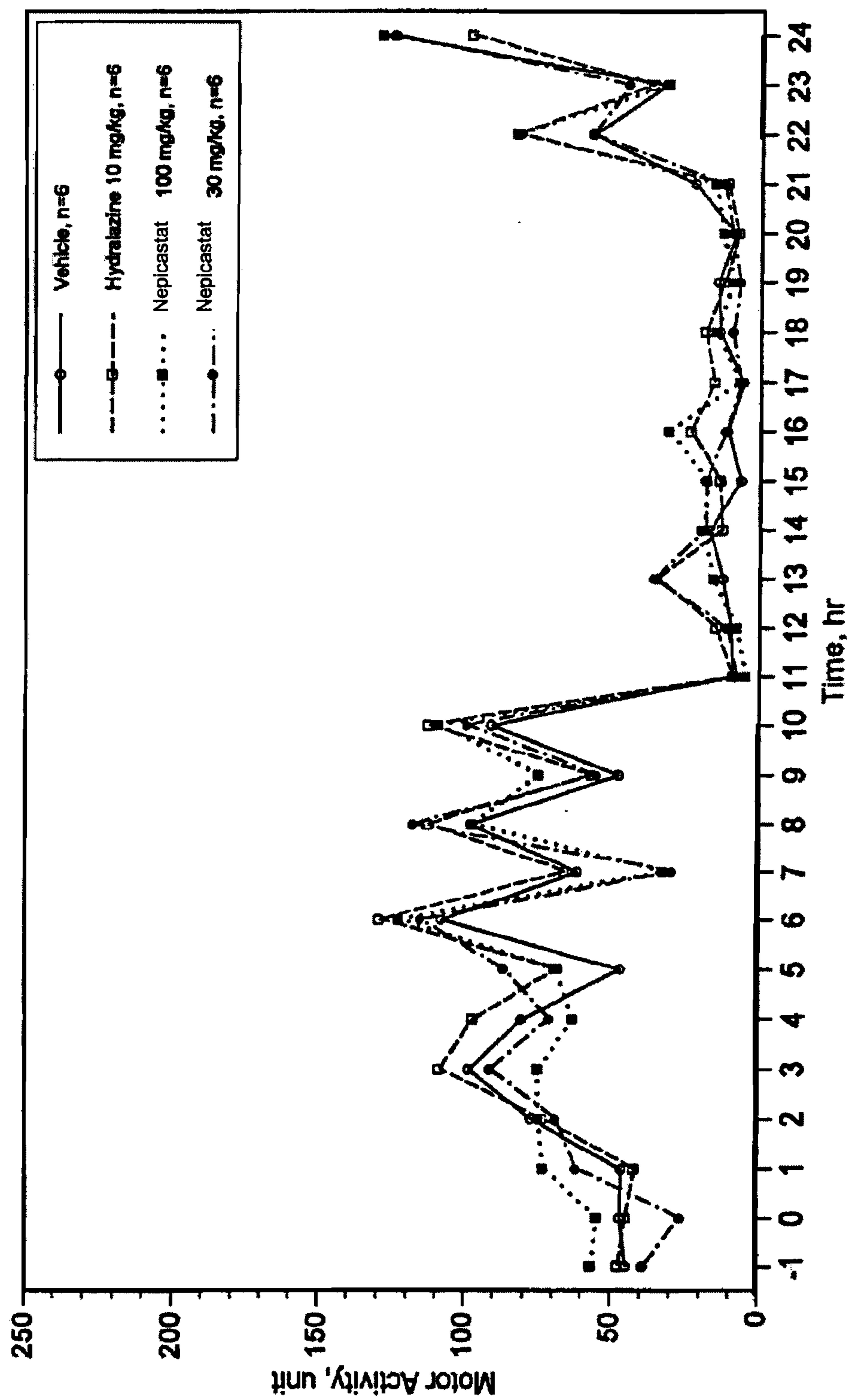
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Figure 177



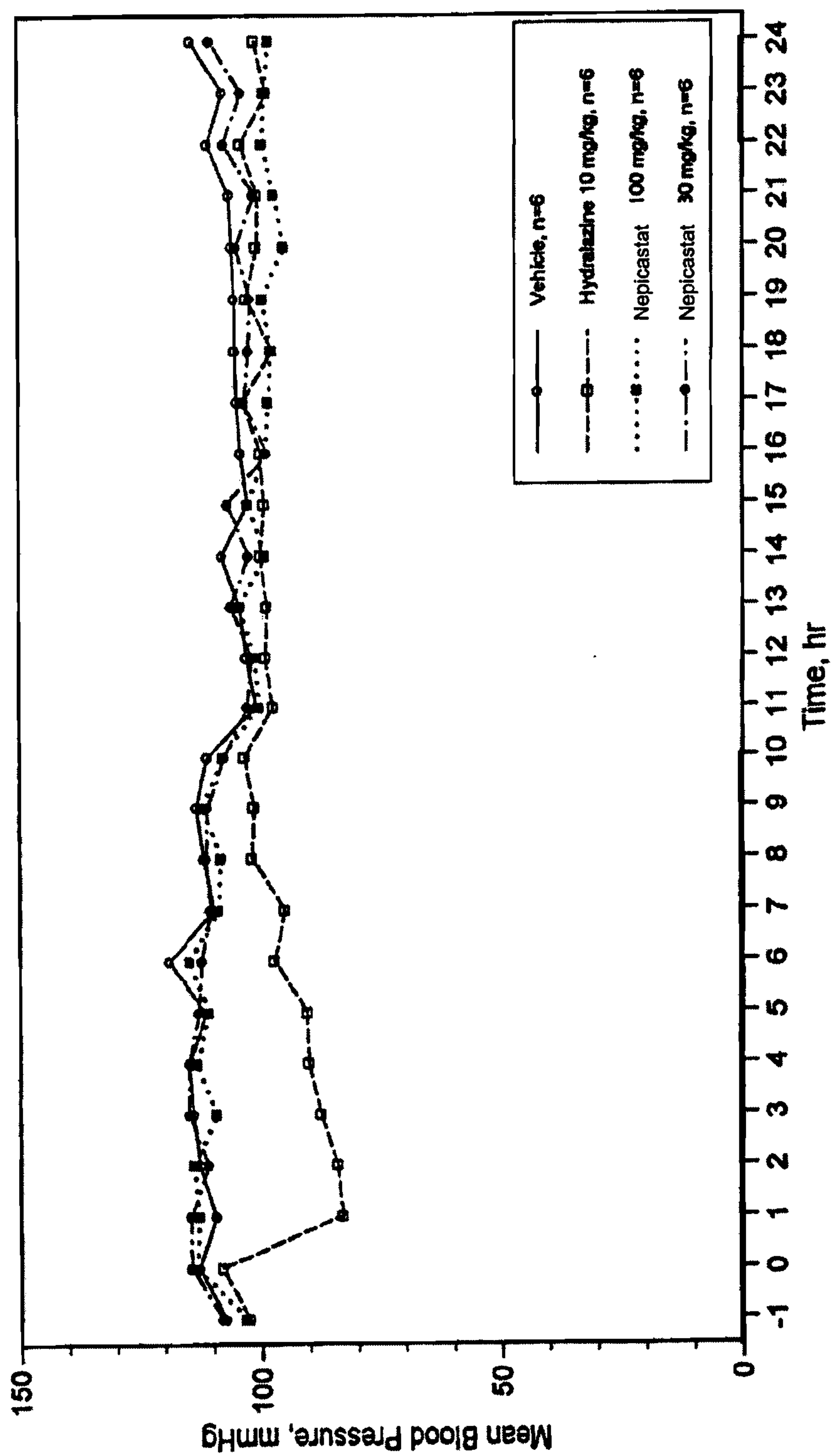
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Figure 178

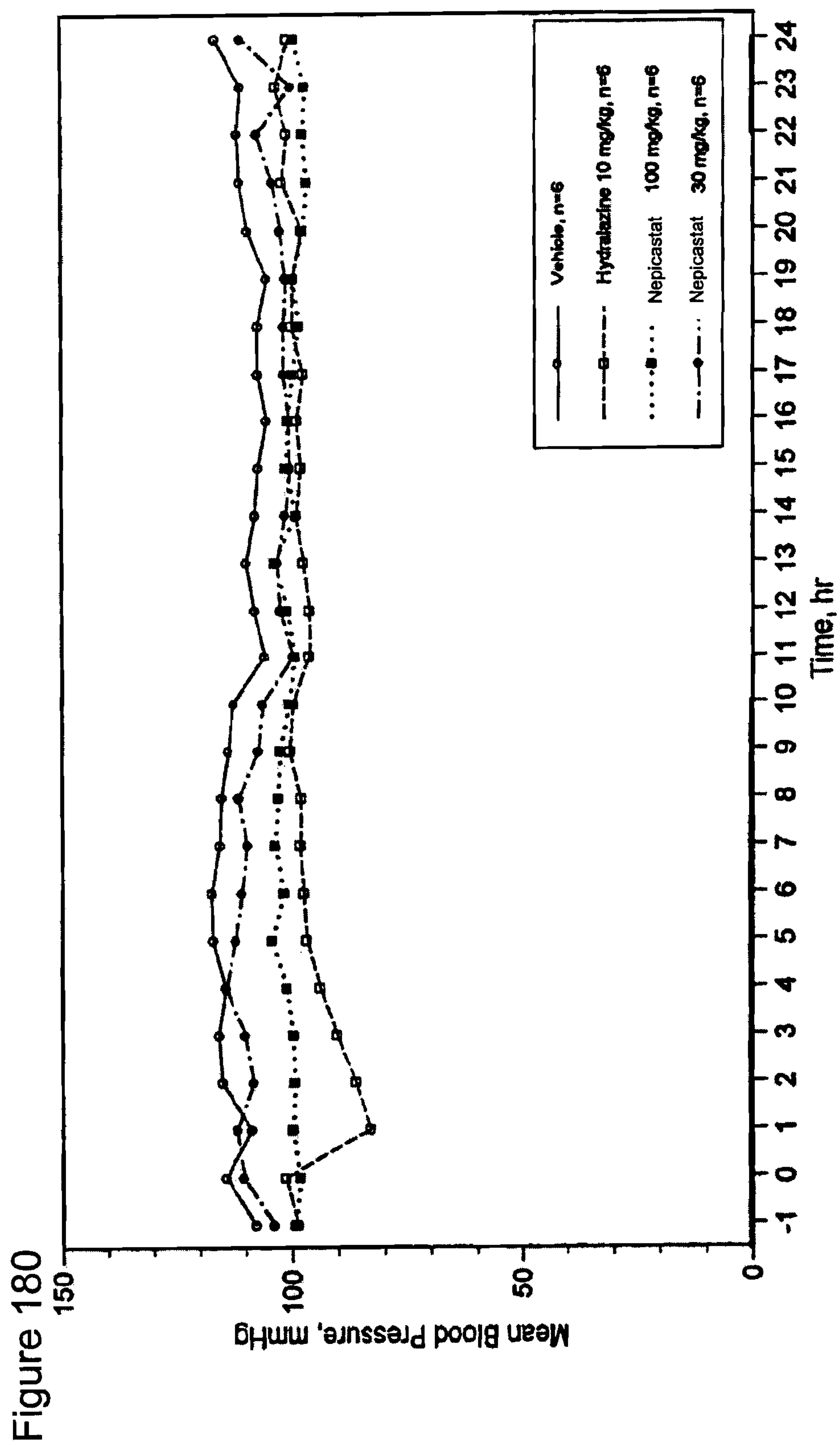


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Figure 179

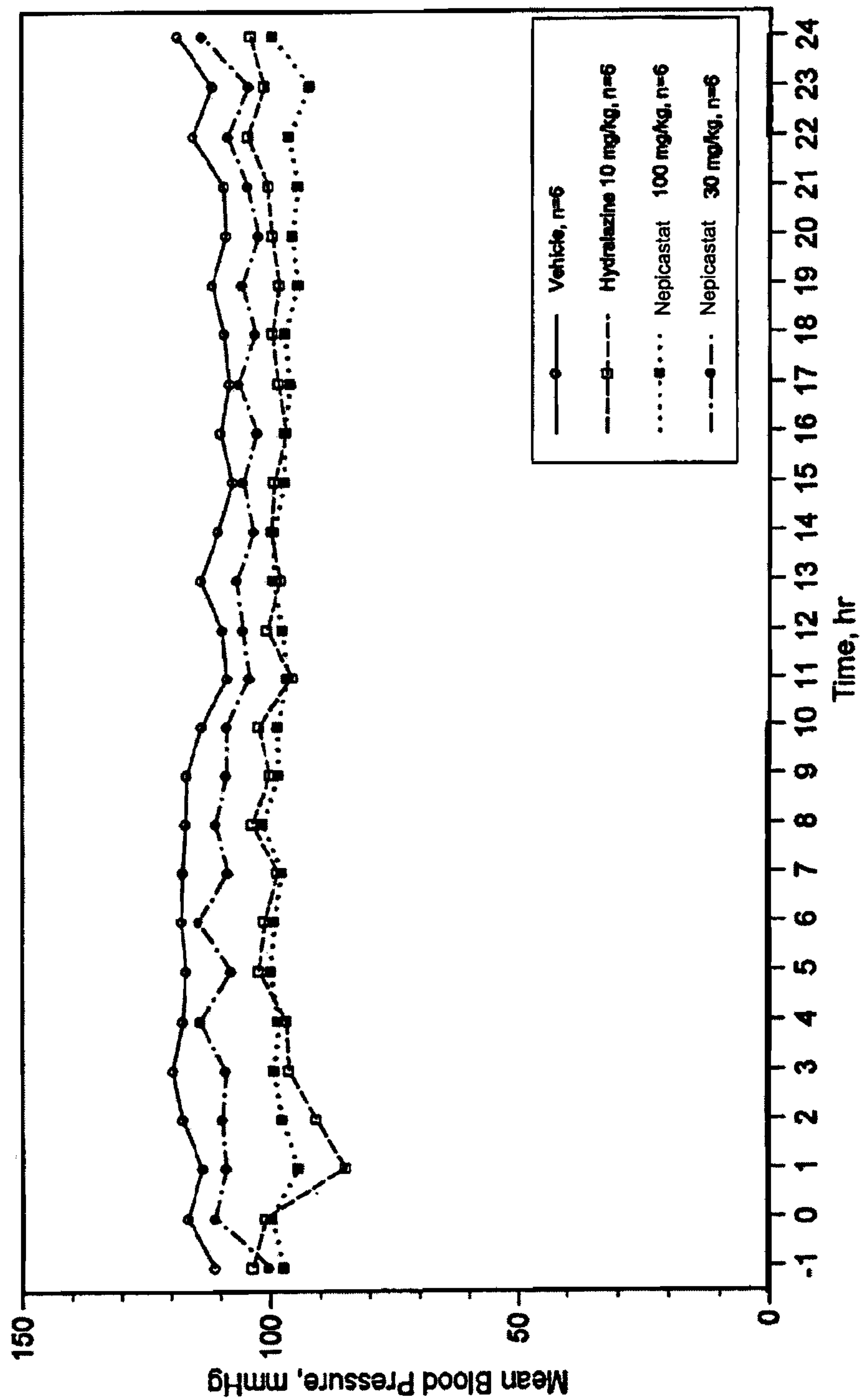


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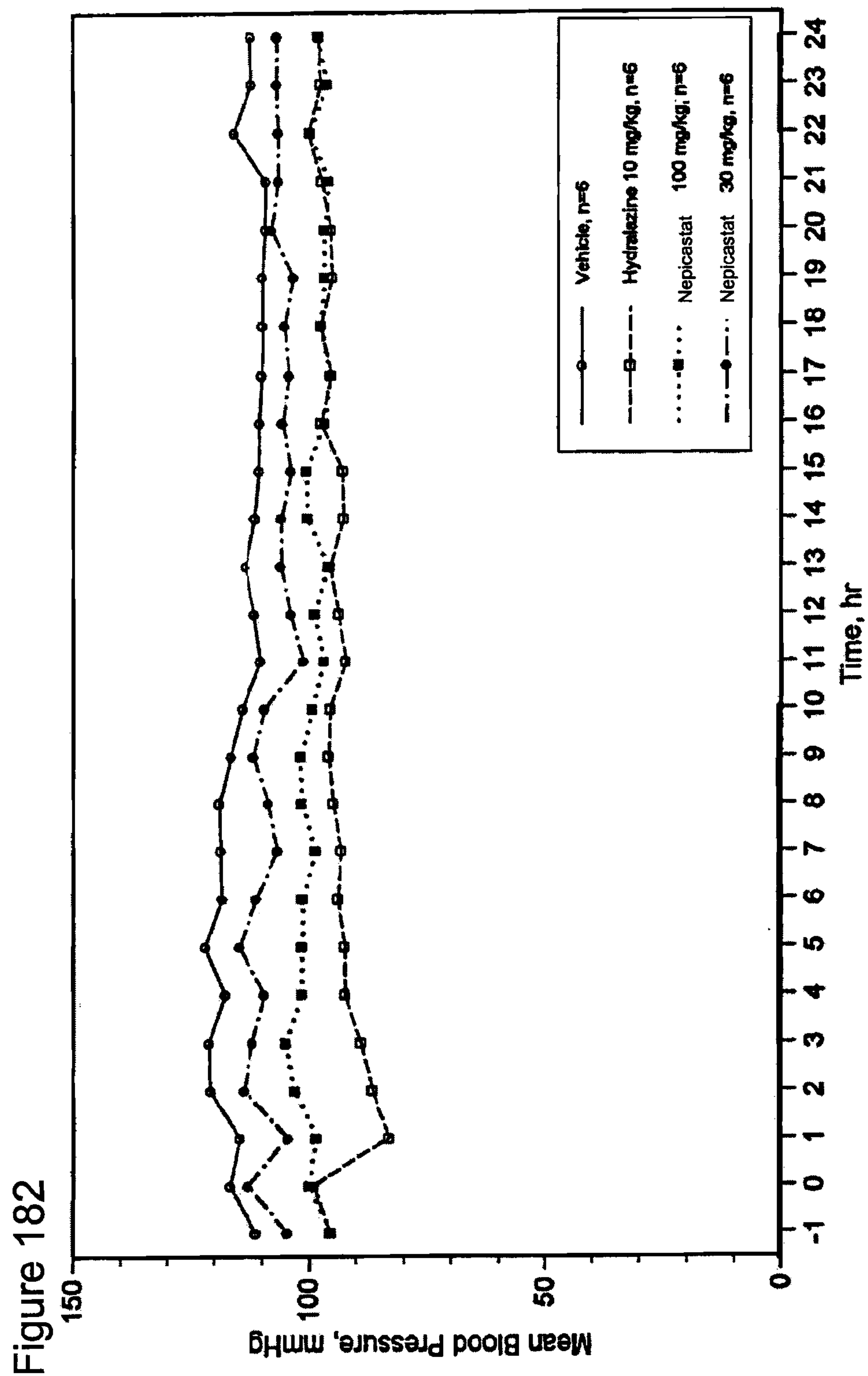


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Figure 181

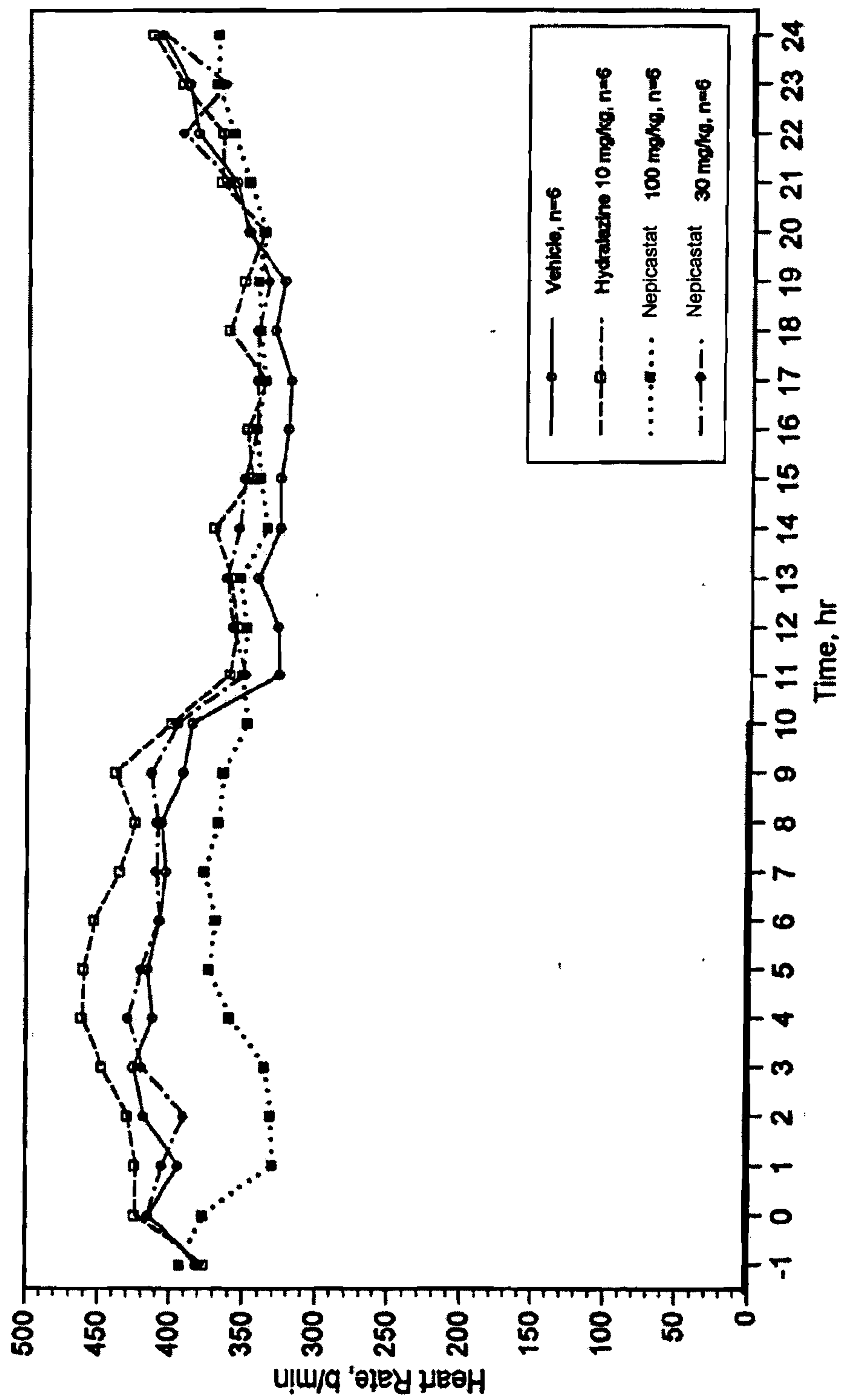


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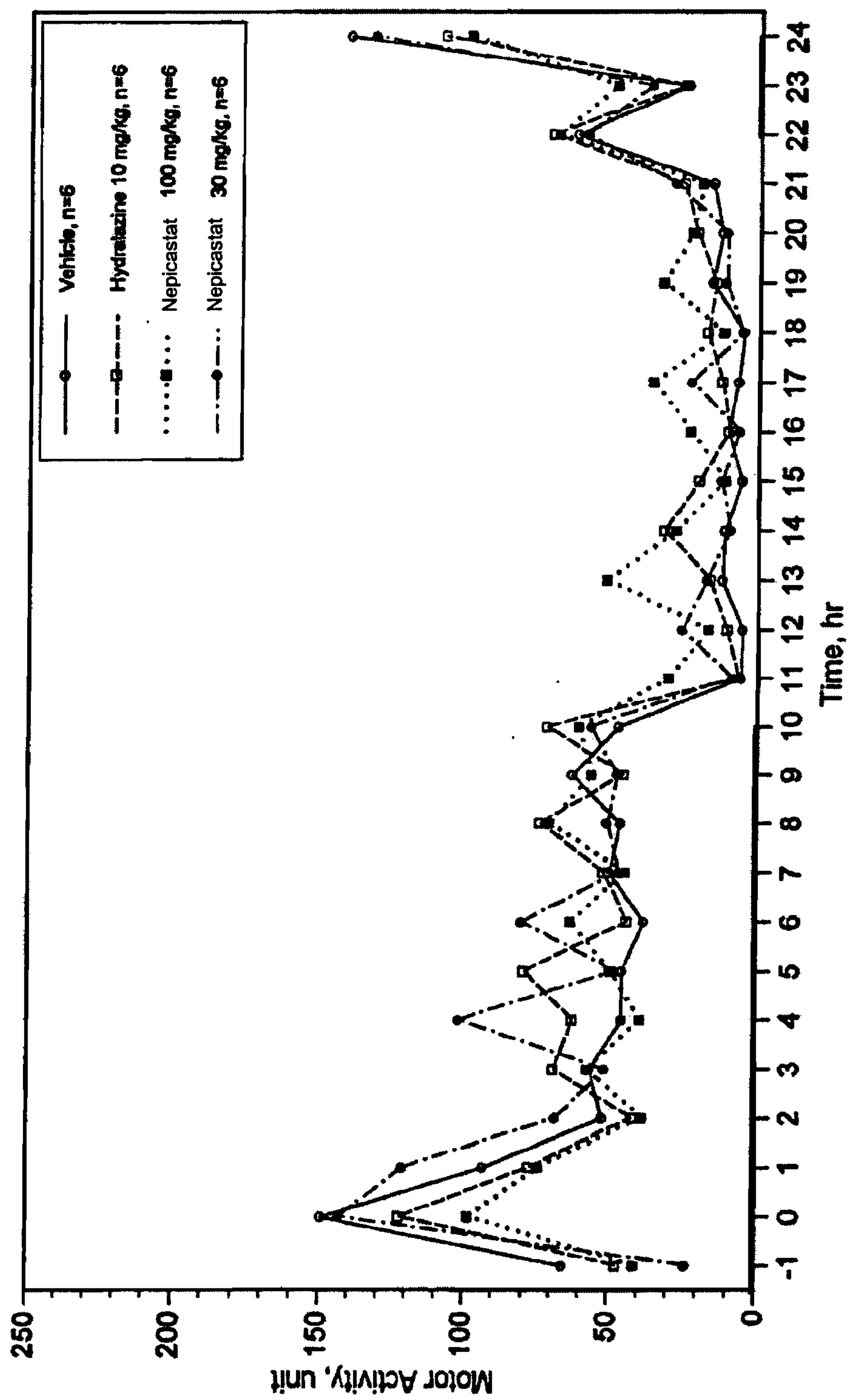
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Figure 183



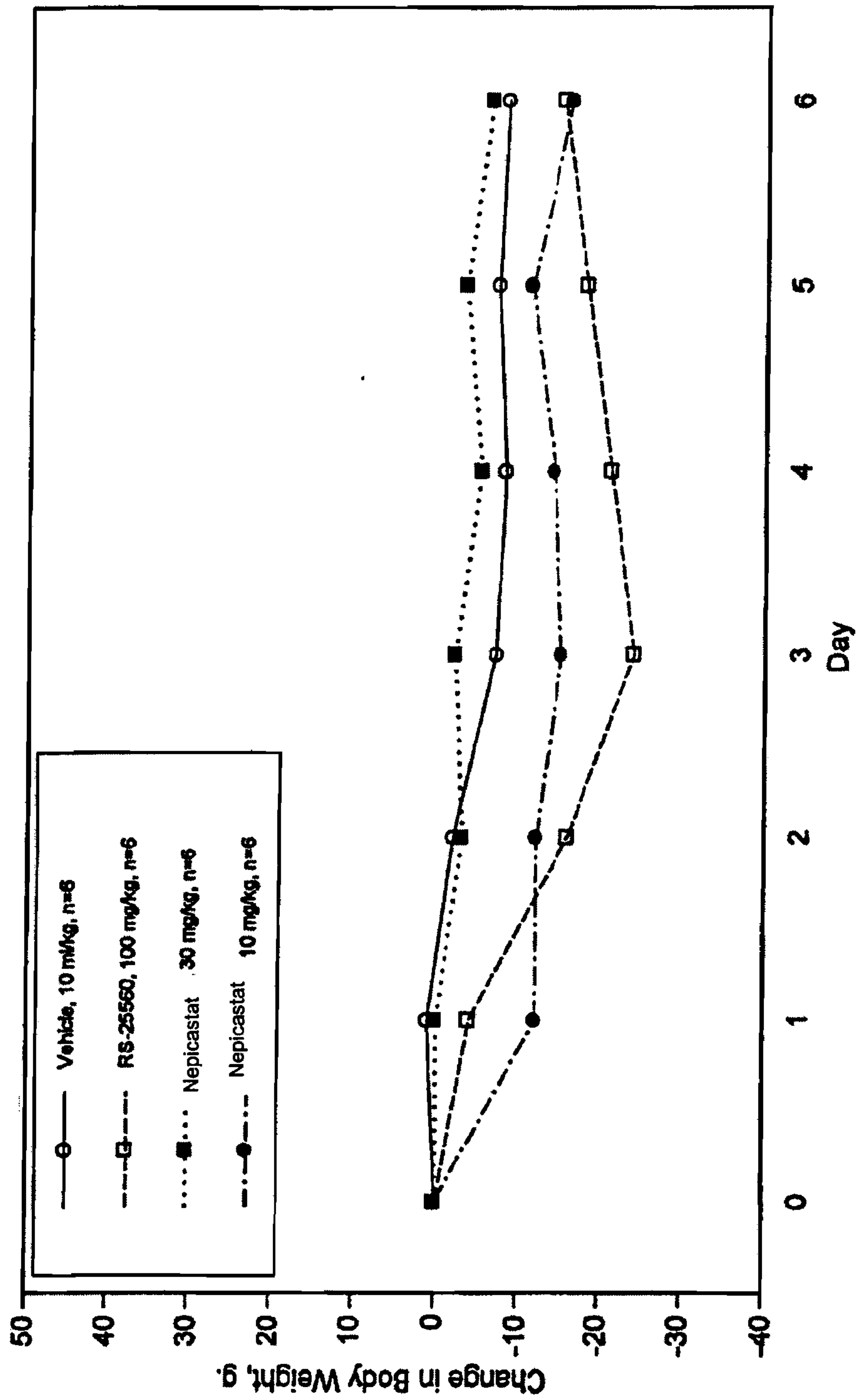
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Figure 184



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Figure 185



Day								
Hour	1	2	3	4	5	6	7	
1	*							
2		*	*		**	**		
3		*	**	*	*	*		
4								
5		*	**		**	*	*	
6	**	**		*			*	
7			*		*			
8		*	**	*	**	*	**	
9	*	**	**	**	**	**	**	
10		**	**	**	**	**		
11		**	*	*	**	**	**	
12		*			**	**	*	
13		**	**	**	*	*	**	
14	*	*	*					
15		**			*	*	**	
16			*			*		
17		*			*	*		
18								
19			*				*	
20			*					
21					**			
22		*	*			*	**	
23		**	*					
24		*	*					

Figure 186

Figure 187

Day							
Hour	1	2	3	4	5	6	7
1		**	**	**	**	**	**
2		**	**	**	**	**	**
3		**	**	**	**	**	**
4		**	**	**	**	**	**
5		**	**	**	**	**	**
6		**	**	**	**	**	**
7		*	**		**	**	**
8		*	**	**	**	**	**
9		**	**	**	**	**	**
10		**	**	**	**	**	**
11			**	**	**	**	**
12		*	**	**	**	**	**
13			**	*	**	*	**
14	**	**	**	**		**	
15		*	**	**	**	**	**
16			**	**	**	**	**
17			*	**	**	**	**
18					**	*	
19			**	**	**		**
20	*	*	**		**		*
21				*	**	*	
22	**	**	**	**	**	**	**
23		*	**	**	**	**	
24	**	**	**	**	**	**	**