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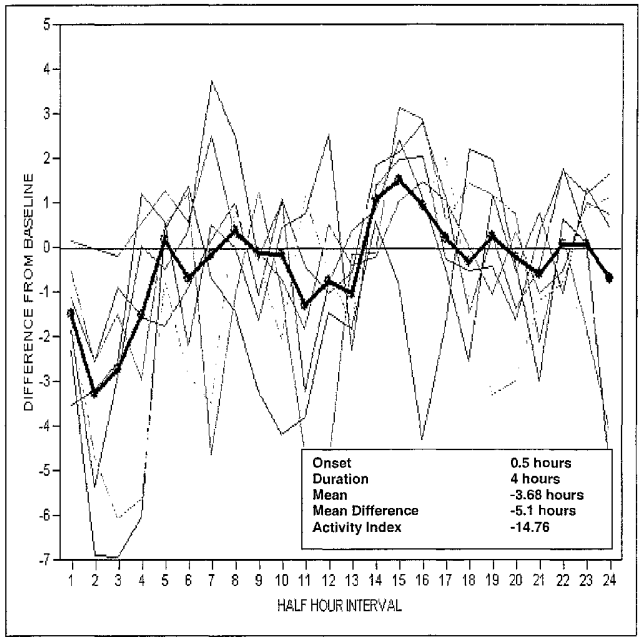
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(54) Title: METHOD FOR TREATING NERVOUS SYSTEM DISORDERS AND CONDITIONS



(57) Abstract: The present invention is directed to selective dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4methylphenyl)-3-azabicyclo[3.1.0]hexane, mazindol, methylphenidate, and 1-[2[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine, and methods of their use for treating certain nervous system disorders and conditions, including, *inter alla,* vasomotor symptoms (VMS), chronic pain, and Shy Drager syndrome.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

METHOD FOR TREATING NERVOUS SYSTEM DISORDERS AND CONDITIONS

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Application Nos. 60/590,103 and 60/590,203, both filed July 22, 2004, the entire disclosures of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to selective dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909), and methods of their use for treating certain nervous system disorders and conditions, including, *inter alia*, vasomotor symptoms (VMS) and chronic pain.

BACKGROUND OF THE INVENTION

[0003] It is well recognized that vasomotor instability and hot flushes are caused by fluctuations of sex steroid levels and can be disruptive and disabling to both females and males. The hot flush attacks can last up to thirty minutes and vary in their frequency from several times a week to multiple attacks per day. The patient experiences a hot flush as a sudden feeling of heat that spreads quickly from the face to the chest and back and then over the rest of the body. These attacks are usually accompanied by outbreaks of profuse sweating. They sometimes occur several times a day, and they often occur at night. Hot flushes and outbreaks of sweats occurring during the night can cause sleep deprivation. Psychological and emotional symptoms observes, such as nervousness, fatigue, irritability, insomnia, depression, memory loss, headache, anxiety, nervousness, and/or inability to concentrate are considered to be caused by the sleep deprivation following hot flush and night sweats (Kramer, *et al.*, In: Murphy, *et al.*, 3rd Int'l Symposium on Recent Advances in Urological Cancer Diagnosis and Treatment Proceedings, Paris, France: SCI: 3-7 (**1992**)).

[0004] Hot flushes may be even more severe in women treated for breast cancer for the following reasons:

(1) many survivors of breast cancer are given tamoxifen, the most prevalent side effect of which is hot flush;

(2) many women treated for breast cancer undergo premature menopause from chemotherapy; and

(3) women with a history of breast cancer have generally been denied estrogen therapy because of concerns about potential recurrence of breast cancer.

(Loprizini, *et al.*, *Lancet*, **2000**, 356 (9247), 2059-2063).

[0005] Men also experience hot flushes following steroid hormone (androgen) withdrawal. This is true in cases of age-associated androgen decline (Katovich, *et al., Proceedings of the Society for Experimental Biology & Medicine*, **1990**, 193(2), 129-135), as well as in extreme cases of hormone deprivation associated with treatments for prostate cancer (Berendsen, *et al., European Journal of Pharmacology*, **2001**, 419(1), 47-54). As many as one-third of these patients will experience persistent and frequent symptoms severe enough to cause significant discomfort and inconvenience.

[0006] The precise mechanism of these symptoms is unknown but generally is thought to represent disturbances to normal homeostatic mechanisms controlling thermoregulation and vasomotor activity (Kronenberg, *et al.*, *Can. J. Physiol. Pharmacol.*, **1987**, 65: 1312-1324).

[0007] The fact that estrogen treatment (*e.g.,* estrogen replacement therapy) relieves the symptoms establishes the link between these symptoms and an estrogen deficiency. For example, the menopausal stage of life is associated with a wide range of other acute symptoms, as described above, and these symptoms are generally estrogen responsive.

[0008] It has been suggested that estrogen may stimulate the activity of the norepinephrine (NE) system (Panek, *et al.*, *J. Pharmacology & Experimental Therapeutics*, **1986**, 236(3), 646-652), serotoninergic (5-HT) system (McEwen,

Recent Progress in Hormone Research, **2002**, 57: 357-384), and dopamine (Bosse, *et al.*, *Cellular and Molecular Neurobiology*, **1996**, 16:199-212.; Datla, *et al.*, *Neuroreport*, **2003**, 14:47-50.; DeMarinis, *et al.*, *Hormone and Metabolic Research*, **1991**, 23:30-4) system and provide a balance between these neurotransmitters that maintain the normal activity of the thermoregulatory center in the hypothalamus. The descending pathways from the hypothalamus via brainstem/spinal cord and the adrenals to the skin contribute to the maintenance of normal skin temperature.

[0009] Mechanisms by which increasing dopamine transmission could result in restoration of temperature regulation are multifaceted. Dopamine is involved with homeostatic processes involved in circadian rhythms (Wisor, *et al.*, *Journal of Neuroscience*, **1987**, 21:1787-94). Temperature regulation is tightly regulated by circadian rhythms. Dopamine and norepinephrine play an interconnected role in the maintenance of circadian rhythms. Therefore, it is conceivable that restoration of circadian rhythms will restore normal temperature regulation. Alternatively, although monoamine transport inhibitors are very selective these transporters are not selective for their substrates (Eshleman, *et al.*, *Journal of Pharmacology & Experimental Therapeutics*, **1999**, 289:877-85; Horn, *British Journal of Pharmacology*, **1973**, 47:332-8.; Raiteri, *et al.*, *European Journal of Pharmacology*, **1977**, 41:133-43.). The norepinephrine transporter (NET) can transport dopamine as well as norepinephrine and has a greater affinity for dopamine than the dopamine transporter (DAT) (Giros, *et al.*, *J. Biol. Chem*, **1994**, 269, 15985-15988). Thus, the use of dopamine reuptake inhibitors that result in region specific elevation of dopamine can affect the neurotransmission of the adrenergic system. Therefore, it is conceivable that the use of a dopamine reuptake inhibitor to treat vasomotor symptoms may work both by a direct effect on the dopamine transporter in maintenance of homeostasis (Gainetdinov, *et al.*, *Brain Research Brain Research Reviews*, **1998**, 26:148-53), as well as an indirect effect, mediated via the norepinephrine transporter.

[0010] Given the multifaceted nature of thermoregulation, multiple therapies and approaches can be developed to target vasomotor instability. The present invention focuses on methods directed to recovery of reduced activity of dopamine by the use of dopamine reuptake inhibitors to restore circadian temperature regulation or

indirectly by modulation of the noradrenergic system. The present invention is directed to these and other important uses for treating nervous system disorders and conditions, including, *inter alia*, vasomotor symptoms (VMS), chronic pain, and Shy Drager syndrome.

SUMMARY OF THE INVENTION

[0011] The present invention is directed to selective dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, mazindol, methylphenidate, and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909), and methods of their use for treating nervous system disorders or conditions, including, *inter alia*, vasomotor symptoms (VMS) and chronic pain.

[0012] In one embodiment, the present invention is directed to methods for treating at least one nervous system disorder or condition in a subject in need thereof, comprising the step of:

administering to said subject a composition comprising an effective amount of at least one selective dopamine reuptake inhibitor;

wherein said nervous system disorder or condition is a vasomotor symptom, chronic pain, Shy Drager syndrome, or a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The invention can be more fully understood from the following detailed description and the accompanying drawings that form a part of this application.

[0014] **FIGURE 1** shows the results of the administration of (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane at 1 dose (30 mg/kg, sc) in telemetry rat model of ovariectomy-induced thermoregulatory dysfunction (referred to in EXAMPLE 2).

[0015] **FIGURE 2** shows the results of the administration of 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909; also known

as vanoxerine) at 1 dose (30 mg/kg, sc) in telemetry rat model of ovariectomy-induced thermoregulatory dysfunction (referred to in EXAMPLE 2).

[0016] **FIGURE 3** is a plot of % reversal at 30, 60, 100, 180, and 300 minutes after administration of racemic 1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane (bicifadine), (+)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, gabapentin, and vehicle (referred to in EXAMPLE 3).

DETAILED DESCRIPTION OF THE INVENTION

[0017] The present invention is directed to selective dopamine reuptake inhibitors, (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane (also known as (1S,5R)-1-((4-methylphenyl)-3-azabicyclo[3.1.0]hexane measured as HCl salt), mazindol, methylphenidate, and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909), and methods of their use for treating nervous system disorders or conditions, including, *inter alia*, vasomotor symptoms (VMS), chronic pain, and Shy Drager syndrome.

[0018] The following definitions are provided for the full understanding of terms and abbreviations used in this specification.

[0019] As used herein and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context clearly indicates otherwise. Thus, for example, a reference to "an antagonist" includes a plurality of such antagonists, and a reference to "a compound" is a reference to one or more compounds and equivalents thereof known to those skilled in the art, and so forth.

[0020] The abbreviations in the specification correspond to units of measure, techniques, properties, or compounds as follows: "min" means minutes, "h" means hour(s), "µL" means microliter(s), "mL" means milliliter(s), "mM" means millimolar, "M" means molar, "mmole" means millimole(s), "cm" means centimeters, "SEM" means standard error of the mean and "IU" means International Units. "ED_{50} value" means dose which results in 50% alleviation of the observed condition or effect

(50% mean maximum endpoint). Optical rotations are measured for compounds in their HCl salt form, unless otherwise noted.

[0021] "Norepinephrine transporter" is abbreviated NET.
"Human norepinephrine transporter" is abbreviated hNET.
"Serotonin transporter" is abbreviated SERT.
"Human serotonin transporter" is abbreviated hSERT.
"Norepinephrine reuptake inhibitor" is abbreviated NRI.
"Selective norepinephrine reuptake inhibitor" is abbreviated SNRI.
"Serotonin reuptake inhibitor" is abbreviated SRI.
"Selective serotonin reuptake inhibitor" is abbreviated SSRI.
"Norepinephrine" is abbreviated NE.
"Serotonin is abbreviated 5-HT.
"Subcutaneous" is abbreviated sc.
"Intraperitoneal" is abbreviated ip.
"Oral" is abbreviated po.

[0022] As used herein, the term "treatment" includes preventative (*e.g.*, prophylactic), curative or palliative treatment and "treating" as used herein also includes preventative, curative and palliative treatment.

[0023] As used herein, the term "effective amount" refers to an amount effective, at dosages, and for periods of time necessary, to achieve the desired result with respect to the treatment of the nervous system disorder or condition. In particular, with respect to vasomotor symptoms, "effective amount" refers to the amount of compound or composition of compounds that would increase dopamine levels to compensate in part or total for the lack of steroid availability in subjects afflicted with a vasomotor symptom. Varying hormone levels will influence the amount of compound required in the present invention. For example, the pre-menopausal state may require a lower level of compound due to higher hormone levels than the peri-menopausal state.

[0024] It will be appreciated that the effective amount of components of the present invention will vary from patient to patient not only with the particular

compound, component or composition selected, the route of administration, and the ability of the components (alone or in combination with one or more combination drugs) to elicit a desired response in the individual, but also with factors such as the disease state or severity of the condition to be alleviated, hormone levels, age, sex, weight of the individual, the state of being of the patient, and the severity of the pathological condition being treated, concurrent medication or special diets then being followed by the particular patient, and other factors which those skilled in the art will recognize, with the appropriate dosage ultimately being at the discretion of the attendant physician. Dosage regimens may be adjusted to provide the improved therapeutic response. An effective amount is also one in which any toxic or detrimental effects of the components are outweighed by the therapeutically beneficial effects.

[0025] Preferably, the compounds useful in the methods of the present invention are administered at a dosage and for a time such that the number of VMS, particularly hot flush, is reduced as compared to the number of VMS before the start of treatment. Such treatment can also be beneficial to reduce the overall severity or intensity distribution of any VMS, especially, hot flushes still experienced, as compared to the severity of the VMS before the start of the treatment. With respect to chronic pain, and Shy Drager syndrome, the compounds useful in the methods of the present invention are administered at a dosage and for a time such that there is the prevention, alleviation, or elimination of the symptom or condition.

[0026] For example, for an afflicted patient, the selective dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, mazindol, methylphenidate, and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909), may be administered, preferably, at a dosage of from about 0.1 mg/day to about 200 mg/day, more preferably from about 1 mg/day to about 150 mg/day, even more preferably from about 1 mg/day to about 100 mg/day and most preferably from about 1 mg/day to 50 mg/day for a time sufficient to reduce and/or substantially eliminate the nervous system disorder or condition, for example, the number and/or severity of VMS and/or duration and/or severity of the chronic pain or Shy Drager syndrome.

[0027] As used herein, the terms "composition of compounds," "compound," "drug," "therapeutic agent," "pharmacologically active agent," "active agent," and "medicament" are used interchangeably herein to refer to a compound or compounds or composition of matter which, when administered to a subject (human or animal) induces a desired pharmacological and/or physiologic effect by local and/or systemic action.

[0028] As used herein, the term "modulation" refers to the capacity to either enhance or inhibit a functional property of a biological activity or process, for example, receptor binding or signaling activity. Such enhancement or inhibition may be contingent on the occurrence of a specific event, such as activation of a signal transduction pathway and/or may be manifest only in particular cell types. The modulator is intended to comprise any compound, *e.g.*, antibody, small molecule, peptide, oligopeptide, polypeptide, or protein, preferably small molecule, or peptide.

[0029] As used herein, the term "inhibitor" is intended to comprise any compound or agent, *e.g.*, antibody, small molecule, peptide, oligopeptide, polypeptide, or protein, preferably small molecule or peptide, that exhibits a partial, complete, competitive and/or inhibitory effect on mammal by inhibiting, suppressing, repressing, or decreasing a specific activity, such as serotonin reuptake activity or the norepinephrine reuptake activity. In certain embodiments, the term preferably refers to an inhibitor of human norepinephrine reuptake or both serotonin reuptake and norepinephrine reuptake, thus diminishing or blocking, preferably diminishing, some or all of the biological effects of endogenous norepinephrine reuptake or of both serotonin reuptake and the norepinephrine reuptake.

[0030] Within the present invention, the dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, mazindol, methylphenidate, and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909), may be prepared in the form of pharmaceutically acceptable salts. As used herein, the term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic acids, including inorganic salts, and organic salts. Suitable non-organic salts include inorganic and organic acids such as acetic,

benzenesulfonic, benzoic, camphorsulfonic, citric, ethenesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, malic, maleic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric acid, p-toluenesulfonic and the like. Particularly preferred are hydrochloric, hydrobromic, phosphoric, and sulfuric acids, and most preferably is the hydrochloride salt.

[0031] As used herein, term "administering" means either directly administering a compound or composition of the present invention, or administering a prodrug, derivative or analog which will form an equivalent amount of the active compound or substance within the body.

[0032] As used herein, the term "subject" or "patient" refers to an animal including the human species that is treatable with the compositions, and/or methods of the present invention. The term "subject" or "subjects" is intended to refer to both the male and female gender unless one gender is specifically indicated. Accordingly, the term "patient" comprises any mammal which may benefit from treatment of a nervous system disorder or condition, including, *inter alia*, vasomotor symptoms and/or chronic pain, such as a human, especially if the mammal is female, either in the pre-menopausal, peri-menopausal, or post-menopausal period. Furthermore, the term patient includes female animals including humans and, among humans, not only women of advanced age who have passed through menopause but also women who have undergone hysterectomy or for some other reason have suppressed estrogen production, such as those who have undergone long-term administration of corticosteroids, suffer from Cushing's syndrome or have gonadal dysgenesis. However, the term "patient" is not intended to be limited to a female.

[0033] As used herein, the terms "vasomotor symptoms," "vasomotor instability symptoms" and "vasomotor disturbances" include, but are not limited to, hot flushes (flashes), insomnia, sleep disturbances, mood disorders, irritability, excessive perspiration, night sweats, fatigue, and the like, caused by, *inter alia*, thermoregulatory dysfunction.

[0034] As used herein, the terms "hot flush" or "hot flash" is an art-recognized term that refers to an episodic disturbance in body temperature typically consisting of a sudden skin flushing, usually accompanied by perspiration in a subject.

[0035] As used herein, the terms "premature menopause" or "artificial menopause" refer to ovarian failure of unknown cause that may occur before age 40. It may be associated with smoking, living at high altitude, or poor nutritional status. Artificial menopause may result from oophorectomy, chemotherapy, radiation of the pelvis, or any process that impairs ovarian blood supply.

[0036] As used herein, the term "pre-menopausal" means before the menopause, the term "peri-menopausal" means during the menopause and the term "post-menopausal" means after the menopause. "Ovariectomy" means removal of an ovary or ovaries and can be effected according to Merchenthaler *et al., Maturitas*, **1998**, 30(3): 307-316.

[0037] As used herein, the term "chronic pain" refers to centralized or peripheral pain that is intense, localized, sharp, or stinging, and/or dull, aching, diffuse, or burning in nature and that occurs for extended periods of time (*i.e.*, persistent), including, for the purpose of the present invention, neuropathic pain and cancer pain. Chronic pain includes neuropathic pain, hyperalgesia, and/or allodynia.

[0038] As used herein, the term "neuropathic pain" refers to chronic pain caused by damage to or pathological changes in the peripheral or central nervous systems. Examples of pathological changes related to neuropathic pain include prolonged peripheral or central neuronal sensitization, central sensitization related damage to nervous system inhibitory and/or exhibitory functions and abnormal interactions between the parasympathetic and sympathetic nervous systems. A wide range of clinical conditions may be associated with or form the basis for neuropathic pain including for example diabetes, post traumatic pain of amputation, lower back pain, cancer, chemical injury, or toxins, other major surgeries, peripheral nerve damage due to traumatic injury compression, nutritional deficiencies, or infections such as shingles or human immunodeficiency virus (HIV). Neuropathic pain may be associated with, for example, diabetic neuropathy, peripheral neuropathy, post-

herpetic neuralgia, trigeminal neuralgia, lumbar or cervical radiculopathies, fibromyalgia, glossopharyngeal neuralgia, reflex sympathetic dystrophy, casualgia, thalamic syndrome, nerve root avulsion, or nerve damage caused by injury resulting in peripheral and/or central sensitization such as phantom limb pain, reflex sympathetic dystrophy or postthoracotomy pain, cancer, chemical injury, toxins, nutritional deficiencies, or viral or bacterial infections such as shingles or HIV, or combinations thereof. The methods of use for compounds of this invention further include treatments in which the neuropathic pain is a condition secondary to metastatic infiltration, adiposis dolorosa, burns or central pain conditions related to thalamic conditions, or combinations thereof.

[0039] As used herein, the term "hyperalgesia" refers to pain where there is an increase in sensitivity to a typically noxious stimulus.

[0040] As used herein, the term "allodynia" refers to an increase in sensitivity to a typically non-noxious stimulus.

[0041] As used herein, the term "side effect" refers to a consequence other than the one(s) for which an agent or measure is used, as the adverse effects produced by a drug, especially on a tissue or organ system other then the one sought to be benefited by its administration. In the case, for example, of high doses of NRIs or NRI/SRI compounds alone, the term "side effect" may refer to such conditions as, for example, vomiting, nausea, sweating, and flushes (Janowsky, *et al., Journal of Clinical Psychiatry*, **1984**, 45(10 Pt 2): 3-9).

[0042] As used herein, the phrase "substantially free of (+)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane or a pharmaceutically acceptable salt thereof" means a composition containing no more than about 5% by weight based on the total weight of the composition (w/w) of (+)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane or a pharmaceutically acceptable salt thereof, preferably less than about 2% w/w, and more preferably less than about 1% w/w.

[0043] As used herein, the phrase "substantially free of (+)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane or a pharmaceutically acceptable salt thereof" means a composition containing no more than about 5% by weight based on the total weight

of the composition (w/w) of (+)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane (also known as (1R,5S)-1-((4-methylphenyl)-3-azabicyclo[3.1.0]hexane) or a pharmaceutically acceptable salt thereof, preferably less than about 2% w/w, and more preferably less than about 1% w/w.

[0044] As used herein, the terms "selective dopamine reuptake inhibitor" and "selective DRI" mean a compound that alters the level of dopamine (DA) by inhibiting the uptake of DA through neurons of the central and/or peripheral nervous system and/or the peripheral system and that has a selectivity ratio of DAT:NET or DAT:SERT activity, as measured by the EC_{50} value or by % specific bound DA uptake for the human transporter, of at least about 1:1, preferably at least about 2:1, more preferably, at least about 5:1, even more preferably, at least about 10:1, yet even more preferably, at least 20:1, and even more preferably, at least about 50:1.

[0045] The present invention is directed to selective dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane (also known as (1R,5S)-1-((4-methylphenyl)-3-azabicyclo[3.1.0]hexane), mazindol, methylphenidate, and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909; also known as vanoxerine), and methods of their use for treating nervous system disorders or conditions, including, *inter alia*, vasomotor symptoms (VMS) and chronic pain, especially neuropathic pain, and even more especially neuropathic pain excluding chronic back pain. It is believed that the present invention described presents a substantial breakthrough in the field of treatment, alleviation, inhibition, and/or prevention of nervous system disorders and conditions, including, *inter alia*, vasomotor symptoms, chronic pain, especially neuropathic pain, and even more especially neuropathic pain excluding chronic back pain, Shy Drager syndrome, or a combination thereof.

[0046] In one embodiment, the present invention is directed to methods for treating at least one nervous system disorder or condition in a subject in need thereof, comprising the step of:

administering to said subject a composition comprising an effective amount of at least one selective dopamine reuptake inhibitor;

wherein said nervous system disorder or condition is a vasomotor symptom, chronic pain, Shy Drager syndrome, or a combination thereof.
In preferred embodiments, the nervous system disorder or condition is a vasomotor symptom or chronic pain, especially neuropathic pain, and even more especially neuropathic pain excluding chronic back pain.

[0047] Neuropathic pain may be associated with, for example, diabetic neuropathy, post-herpetic neuralgia, trigeminal neuralgia, complex regional pain syndrome, lumbar or cervical radiculopathies, fibromyalgia, glossopharyngeal neuralgia, reflex sympathetic dystrophy, causalgia, thalamic syndrome, nerve root avulsion, monoclonal gammopathy of undetermined significance (MGUS) neuropathy, sarcoid polyneuropathy, HIV-related neuropathy arising from a variety of causes such as from medication used to treat HIV, peripheral neuropathy such as peripheral neuropathy with connective tissue disease, paraneoplastic sensory neuropathy, familial amyloid polyneuropathy, acquired amyloid polyneuropathy, inherited neuropathy, neuropathy with renal failure, hereditary sensory autonomic neuropathy, Fabry's disease, Celiac disease or nerve damage cause by injury resulting in peripheral and/or central sensitization such as phantom limb pain, reflex sympathetic dystrophy or postthoracotomy pain, cancer including neuropathies caused by chemotherapy agents or other agents used to treat the disease, chemical injury, toxins such as arsenic neuropathy, nutritional deficiencies, or viral or bacterial infections such as shingles or HIV-related neuropathy, or combinations thereof. The methods of use for compounds of this invention further include treatments in which the neuropathic pain is a condition secondary to metastatic infiltration, adiposis dolorosa, burns, or central pain conditions related to thalamic conditions.

[0048] Neuropathic pains described above may also be, in some circumstances, classified as "painful small fiber neuropathies" such as idiopathic small-fiber painful sensory neuropathy, or "painful large fiber neuropathies" such as demylinating neuropathy or axonal neuropathy, or combinations thereof. Such neuropathies are described in more detail, for example, in the J. Mendell *et al., N. Engl. J. Med.* **2003**, 348:1243-1255, which is hereby incorporated by reference in its entirety.

[0049] In certain embodiments, the selective dopamine reuptake inhibitor has norepinephrine reuptake inhibitory activity of greater than about 0.5 μM activity in a functional norepinephrine reuptake bioassay, preferably, the selective dopamine reuptake inhibitor has essentially no norepinephrine reuptake inhibitory activity, that is, greater than about 1 μM activity in a functional norepinephrine reuptake bioassay.

[0050] In certain embodiments, the selective dopamine reuptake inhibitor has serotonin reuptake inhibitory activity of greater than about 0.5 μM activity in a functional serotonin reuptake bioassay, preferably, the selective dopamine reuptake inhibitor has essentially no serotonin reuptake inhibitory activity, that is, greater than about 1 μM activity in a functional serotonin reuptake bioassay.

[0051] In certain embodiments, the selective dopamine reuptake inhibitor has dual norepinephrine and serotonin reuptake inhibitory activity of greater than about 0.5 μM activity in both functional norepinephrine and serotonin reuptake bioassays, preferably, the selective dopamine reuptake inhibitor has essentially no dual norepinephrine and serotonin reuptake inhibitory activity, that is, greater than about 1 μM activity in both functional norepinephrine and serotonin reuptake bioassays.

[0052] Preferably, the selective dopamine reuptake inhibitor is (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, mazindol, methylphenidate, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909; also known as vanoxerine), or a pharmaceutically acceptable salt thereof. Selective dopamine reuptake inhibitor compounds may be identified using techniques known in the art, such as testing the compounds in assays including a dopamine uptake transporter binding assay, a membrane binding assay, and radioligand binding assay, such as those described in the Example section below.

[0053] The present invention includes prodrugs of the dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, mazindol, methylphenidate, and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909). As

used herein, the term "prodrug" means a compound which is convertible *in vivo* by metabolic means (*e.g.* by hydrolysis) to a the selective dopamine reuptake inhibitors. Various forms of prodrugs are known in the art, for example, as discussed in Bundgaard, (ed.), *Design of Prodrugs*, Elsevier (1985); Widder, *et al.* (ed.), *Methods in Enzymology*, vol. 4, Academic Press (1985); Krogsgaard-Larsen, et al., (ed). "Design and Application of Prodrugs," *Textbook of Drug Design and Development*, Chapter 5, 113-191 (1991), Bundgaard, *et al.*, *Journal of Drug Deliver Reviews*, **1992**, 8:1-38, Bundgaard, *J. of Pharmaceutical Sciences*, **1988**, 77:285 *et seq.*; and Higuchi and Stella (eds.) *Prodrugs as Novel Drug Delivery Systems*, American Chemical Society (**1975**).

[0054] Further, the dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, mazindol, methylphenidate, and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909), may exist in unsolvated as well as in solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like.

[0055] The compounds useful in the methods of the present invention may be prepared in a number of ways well known to those skilled in the art. The compounds can be synthesized, for example, by the methods as described below, or variations thereon as appreciated by the skilled artisan. The reagents used in the preparation of the compounds of this invention can be either commercially obtained or can be prepared by standard procedures described in the literature. All processes disclosed in association with the present invention are contemplated to be practiced on any scale, including milligram, gram, multigram, kilogram, multikilogram or commercial industrial scale.

[0056] As will be readily understood, functional groups present may contain protecting groups during the course of synthesis. Protecting groups are known *per se* as chemical functional groups that can be selectively appended to and removed from functionalities, such as hydroxyl groups and carboxyl groups. These groups are present in a chemical compound to render such functionality inert to chemical reaction conditions to which the compound is exposed. Any of a variety of protecting

groups may be employed with the present invention. Protecting groups that may be employed in accordance with the present invention may be described in Greene, T.W. and Wuts, P.G.M., *Protective Groups in Organic Synthesis* 2d. Ed., Wiley & Sons, 1991.

[0057] The dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane and (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, or pharmaceutically acceptable salts thereof may be prepared as described, for example, in US-A-4,131,611, US-A-4,435,419, US-B-6,204,284, US-B-6,372,919, US-B-6,569,887, and US-B-6,716,868, the disclosures of which are incorporated herein by reference.

[0058] The compound 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909; vanoxerine), or pharmaceutically acceptable salts thereof, may be prepared by conventional syntheses and is commercially available from Sigma Chemical Co. (St. Louis, MO), for example. The compounds mazindol, methylphenidate are commercially available.

[0059] The dopamine reuptake inhibitors, including (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane and (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane useful in the method of the invention, may be isolated from their racemic mixtures by any method known to those skilled in the art, including high performance liquid chromatography (HPLC) and the formation and crystallization of chiral salts or prepared by methods described herein. See, for example, US-B-6,372,912; Jacques, *et al.*, *Enantiomers, Racemates and Resolutions* (Wiley Interscience, New York, 1981); Wilen, S.H., *et al., Tetrahedron*, 33:2725 (1977); Eliel, E.L. *Stereochemistry of Carbon Compounds*, (McGraw-Hill, NY, 1962); Wilen, S.H. *Tables of Resolving Agents and Optical Resolutions*, p. 268 (E.L. Eliel, Ed., University of Notre Dame Press, Notre Dame, IN 1972).

[0060] In some embodiments, the (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane or the (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane is obtained by resolving racemic 1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane or racemic 1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane using a chiral polysaccharide

stationary phase and an organic eluent. Preferably, the polysaccharide is starch or starch derivative. A chiral HPLC column may be used, such as, for example, a CHIRALPAK™ AD HPLC column manufactured by Diacel and commercially available from Chiral Technologies, Inc., Exton, Pennsylvania, more preferably a 1 cm x 25 cm CHIRALPAK™ AD HPLC column. The preferred eluent is a hydrocarbon solvent adjusted in polarity with a miscible polar organic solvent. Preferably, the organic eluent contains a non-polar, hydrocarbon solvent present in about 95% to about 99.5% (volume/volume) and a polar organic solvent present in about 5% to about 0.5% (volume/volume). In a preferred embodiment, the hydrocarbon solvent is hexane and the miscible polar organic solvent is isopropylamine.

[0061] The dopamine reuptake inhibitors, including the azabicyclohexanes, useful in the methods of the invention may be used as a neat composition or as a composition containing at least one pharmaceutically acceptable carrier. Generally, the dopamine reuptake inhibitors, including the azabicyclohexanes, or a pharmaceutically acceptable salt thereof, will be present at a level of from about 0.1%, by weight, to about 90% by weight, based on the total weight of the composition, based on the total weight of the composition. Preferably, the dopamine reuptake inhibitors, including the azabicyclohexanes, or a pharmaceutically acceptable salt thereof will be present at a level of at least about 1%, by weight, based on the total weight of the composition. More preferably, the dopamine reuptake inhibitors, including the azabicyclohexanes, or a pharmaceutically acceptable salt thereof will be present at a level of at least about 5%, by weight, based on the total weight of the composition. Even more preferably, the dopamine reuptake inhibitors, including the azabicyclohexanes, or a pharmaceutically acceptable salt thereof will be present at a level of at least about 10%, by weight, based on the total weight of the composition. Yet even more preferably, the dopamine reuptake inhibitors, including the azabicyclohexanes, or a pharmaceutically acceptable salt thereof, will be present at a level of at least about 25%, by weight, based on the total weight of the composition.

[0062] Such compositions are prepared in accordance with acceptable pharmaceutical procedures, such as described in *Remington's Pharmaceutical*

Sciences, 17th edition, ed. Alfonoso R. Gennaro, Mack Publishing Company, Easton, PA (1985). Pharmaceutically acceptable carriers are those that are compatible with the other ingredients in the formulation and biologically acceptable.

[0063] The compounds of this invention may be administered orally or parenterally, neat or in combination with conventional pharmaceutical carriers. Applicable solid carriers can include one or more substances that may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents or an encapsulating material. In powders, the carrier is a finely divided solid that is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes, and ion exchange resins.

[0064] Liquid carriers may be used in preparing solutions, suspensions, emulsions, syrups, and elixirs. The active ingredient of this invention can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fat. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers, or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (particularly containing additives as above, *e.g.* cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, *e.g.* glycols) and their derivatives, and oils (*e.g.* fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are used in sterile liquid form compositions for parenteral administration.

[0065] Liquid pharmaceutical compositions, which are sterile solutions or suspensions, can be administered by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. Oral administration may be either liquid or solid composition form.

[0066] Preferably the pharmaceutical composition is in unit dosage form, *e.g.* as tablets, capsules, powders, solutions, suspensions, emulsions, granules, or suppositories. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form.

[0067] In another embodiment of the present invention, the compounds useful in the methods of the present invention may be administered to a mammal with one or more other pharmaceutical active agents such as those agents being used to treat any other medical condition present in the mammal. Examples of such pharmaceutical active agents include pain relieving agents, anti-angiogenic agents, anti-neoplastic agents, anti-diabetic agents, anti-infective agents, or gastrointestinal agents, or combinations thereof.

[0068] The one or more other pharmaceutical active agents may be administered in a therapeutically effective amount simultaneously (such as individually at the same time, or together in a pharmaceutical composition), and/or successively with one or more compounds of the present invention.

[0069] The term "combination therapy" refers to the administration of two or more therapeutic agents or compounds to treat a therapeutic disorder or condition described in the present disclosure, for example hot flush, sweating, thermoregulatory-related condition or disorder, or other. Such administration includes use of each type of therapeutic agent in a concurrent manner. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the conditions or disorders described herein.

[0070] The route of administration may be any route, which effectively transports the active dopamine reuptake inhibitor, including the azabicyclohexanes, or a pharmaceutically acceptable salt thereof, to the appropriate or desired site of action, such as oral, nasal, pulmonary, transdermal, such as passive or iontophoretic delivery, or parenteral, *e.g.* rectal, depot, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic solution or an ointment. Furthermore, the administration of the dopamine reuptake inhibitor, including the azabicyclohexanes, or pharmaceutically acceptable salt thereof with other active ingredients may be concurrent or simultaneous.

[0071] The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

EXAMPLES

Cell Lines, Culture Reagents, and Assays

[0072] MDCK-Net6 cells, stably transfected with human hNET (Pacholczyk, T., R.D. Blakely, and S.G. Amara, *Nature*, **1991**, **350**(6316): p. 350-4) may be cultured in growth medium containing high glucose DMEM (Gibco, Cat. No. 11995), 10% FBS (dialyzed, heat-inactivated, US Bio-Technologies, Lot FBD1129HI) and 500 µg/ml G418 (Gibco, Cat. No. 10131). Cells may be plated at 300,000/T75 flask and cells were split twice weekly. The JAR cell line (human placental choriocarcinoma) may be purchased from ATCC (Cat. No. HTB-144). The cells may be cultured in growth medium containing RPMI 1640 (Gibco, Cat. No. 72400), 10% FBS (Irvine, Cat. No. 3000), 1% sodium pyruvate (Gibco, Cat. No. 1136) and 0.25% glucose. Cells may

be plated at 250,000 cells/T75 flask and split twice weekly. For all assays, cells may be plated in Wallac 96-well sterile plates (PerkinElmer, Cat. No. 3983498).

Dopamine Uptake Transporter Binding Assay

[0073] The dopamine uptake transporter binding assay using recombinant human dopamine transporter (hDAT) may be used to evaluate binding affinity to the dopamine transporter.

[0074] For single concentration screening, 10 mM compound solutions are prepared and serially diluted in solvent (*i.e.,* DMSO, 50% DMSO: water, ethanol, water) to a 1 mM concentration. The compounds are further diluted in binding buffer (2.1 µl of 1 mM stock to 100 µl in assay buffer) and are delivered in 5 µl aliquots to wells of a 96-well test plate containing hDAT membranes for a final test concentration of 1 µM. Compounds that inhibit ^{3}H-WIN 35,428 binding by at least 60% will be further evaluated to determine IC_{50} values.

[0075] For IC_{50} value determination, stocks at various concentrations are prepared by serial dilution from a 10 mM stock. The compounds are further diluted in binding buffer and are delivered in 5 µl aliquots to wells of a 96-well test plate for final concentrations that range from 1-1000 nM in whole or half-log increments.

[0076] The selective dopamine reuptake inhibitors (DRI's), S-514 (mazindol) and S-266 (methylphenidate), and the selective norepinephrine reuptake inhibitor (NRI), AHR-9543 (desipramine), are used as reference compounds to validate this assay. Selective DRI's and a selective NRI were chosen as reference compounds to demonstrate the ability of this assay to distinguish between the two classes of compounds. All reference compounds are commercially available.

[0077] Non-specific binding is determined in the presence of 10 µM mazindol. A 1 mM DMSO stock solution of mazindol is prepared, diluted in assay buffer, and delivered in 5 µl aliquots to wells of a 96-well test plate for a final concentration of 10 µM.

[0078] The radioligand used in this assay is ^{3}H-WIN 35,428 (PerkinElmer Cat. No. NET1033, SA 60-87 Ci/mmol) and is delivered at 15-20 nM final concentration in both single point testing and competition assays.

Membranes:

[0079] Membranes prepared from cells expressing the recombinant human dopamine transporter (hDAT) are purchased (PerkinElmer, Cat. No. RBHDATM) in tubes that contain protein for 100 assay wells. Each tube is diluted to 7.5 ml in binding buffer (50 mM Tris-HCl, pH 7.4; 100 mM NaCl), homogenized with a tissue-tearer (Polytron PT 1200C, Kinematica AG), and membranes are delivered at a volume of 75 µl to each well of a polypropylene 96-well test plate. Depending on the specific membrane lot, the resulting protein concentration is approximately 30 µg per well.

Radioligand Binding Assay:

[0080] This procedure is modified from the protocol information provided with the commercially purchased membrane (PerkinElmer Cat. No. RBHDATM) [1, 2]. A detailed description of the revised protocol follows.

[0081] Binding reactions are run in polypropylene 96-well plates (Costar Cat. Nos. 3359, 3930). Homogenized membrane preparation is delivered at a volume of 75 µl to each well of the test plate. Compound solutions at several concentrations are generated by serial dilution of 10 mM stocks. The compound solutions are further diluted in binding buffer to the desired delivery concentration, as described above. The compound dilution scheme is followed to standardize solvent solutions at each concentration and assay well. Test compounds are added in 5 µl aliquots to reaction wells containing homogenized membrane. Homogenized membrane and compounds are pre-incubated for 20 minutes at 4°C prior to initiation of the binding reaction. The binding reaction is initiated by addition of 25 µl of ^{3}H-WIN 35,428, diluted in binding buffer, for a final reaction concentration of 15-20 nM. The reaction

is incubated by shaking the test plates at low speed on a shaker platform for 2 hours at 4°C.

[0082] Millipore MultiScreen-FB opaque 96-well filter plates (Millipore glass fiber B, Cat. No. MAFBN0B) are used to separate free radioligand from bound. These filter plates are preincubated, prior to the start of the binding reaction, with 175 µl/ well of 0.5% polyethylenimine/H_20 (PEI; Sigma Cat. No. P-3143) for a minimum of 2 hours at room temperature to reduce non-specific radioligand binding. Prior to harvesting the reaction plates, the PEI solution is aspirated from the filter plates using a vacuum manifold (Millipore Multiscreen Filtration System, Cat. No. MAVM0960R). Aliquots of each reaction (90 µl of each 100 µl reaction well) are transferred from the reaction plates to the filter plates using a Zymark Rapid Plate-96 automated pipette station. The binding reaction is terminated by vacuum filtration (5-10 Hg) through the filter plate. The plates are washed 9x with 200 µl ice-cold wash buffer (50 mM Tris-HCl, 0.9% NaCl, pH 7.4) using a 12 channel aspirate/ wash system to remove unbound radioligand. The plastic bottom supports are removed from the filter plates, and the plates are placed in plastic holders. The plates are air dried for 10 minutes prior to addition of 50 µl/ well of scintillation fluid (Packard UltimaGold, Cat. No. 6013329). The top of each plate is sealed with adhesive film (Packard TopSeal-A, Cat. No. 6005185) and the plates are vigorously shaken for 10 to 15 minutes to ensure adequate dissolution of scintillant. The plates are counted using a Wallac Microbeta counter (PerkinElmer) and the cpms/well are collected as a data stream in Microsoft Excel format.

[0083] For compounds tested at a single concentration (1 µM), each test plate has a minimum of 3 wells to determine total binding (defined as binding in the presence of assay buffer alone) and a minimum of 3 wells to determine non-specific binding (defined as binding in the presence of 10 µM mazindol). Compound activity, expressed as percent inhibition (%I) of ^{3}H-WIN 35,428 binding, is calculated for each drug well using a Microsoft Excel spreadsheet applying the following formula:

percent inhibition (%I) = ((mean cpm for total binding wells – cpm for drug well)/ (mean cpm for total binding wells – mean cpm for non-specific binding wells)) * 100

Compounds that inhibit at least 60% of ^{3}H-WIN 35,428 binding when tested at 1 μM concentration will be further evaluated by determination of IC_{50} values.

[0084] For IC_{50} determination, raw cpm values are generated as a data stream from the Wallac Microbeta counter. The data is downloaded to the Microsoft Excel statistical application program, which calculates the estimated IC_{50} value. Calculations of IC_{50} values are performed using a logistic dose response program written by our Biometrics Department. The statistical program uses wells containing buffer only to determine the maximum binding value (total binding) and wells containing 10 μM mazindol to determine the minimum binding value (non-specific binding). Estimation of the IC_{50} value is derived from a log scale and the line is fit between the maximal and minimal binding values. If assessment of the data using Microsoft Excel indicates a better fit can be generated by log transformation of the data, the data will be log transformed prior to the line fit. In the event that the highest test concentration does not exceed 50% binding inhibition, data will be reported as percent maximal inhibition at the highest concentration tested.

1. Pristupa, Z.B., *et al.,* Pharmacological heterogeneity of the cloned and native human dopamine transporter: disassociation of [3H]WIN 35,428 and [3H]GBR 12,935 binding. *Molecular Pharmacology*, **1994**, 45(1):125-135.
2. Shimada, S., *et al.*, Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA [erratum appears in *Science* **1992** Mar 6; 255(5049):1195]. *Science*, **1991**, 254(5031):576-578.

Norepinephrine (NE) Uptake Assay

[0085] On day 1, cells are plated at 3,000 cells/well in growth medium and maintained in a cell incubator (37°C, 5% CO_2). On day 2, growth medium is replaced with 200 μl of assay buffer (25 mM HEPES; 120 mM NaCl; 5 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgSO_4$; 2 mg/ml glucose (pH 7.4, 37°C)) containing 0.2 mg/ml ascorbic acid and 10 μM pargyline. Plates containing cells with 200 μl of assay buffer is equilibrated for 10 minutes at 37°C prior to addition of compounds. A stock solution of desipramine is prepared in DMSO (10 mM) and delivered to triplicate

wells containing cells for a final test concentration of 1 μM. Data from these wells are used to define non-specific NE uptake (minimum NE uptake). Test compounds are prepared in DMSO (10 mM) and diluted in assay buffer according to test range (1 to 10,000 nM). Twenty-five microliters of assay buffer (maximum NE uptake) or test compound are added directly to triplicate wells containing cells in 200 μl of assay buffer. The cells in assay buffer with test compounds are incubated for 20 minutes at 37°C. To initiate the NE uptake, [^{3}H]NE diluted in assay buffer (120 nM final assay concentration) is delivered in 25 μl aliquots to each well and the plates is incubated for 5 minutes (37°C). The reaction is terminated by decanting the supernatant from the plate. The plates containing cells are washed twice with 200 μl assay buffer (37°C) to remove free radioligand. The plates are then inverted, left to dry for 2 minutes, then reinverted and air-dried for an additional 10 minutes. The cells are lysed in 25 μl of 0.25 N NaOH solution (4°C), placed on a shake table and vigorously shaken for 5 minutes. After cell lysis, 75 μl of scintillation cocktail is added to each well and the plates are sealed with film tape. The plates are returned to the shake table and vigorously shaken for a minimum of 10 minutes to ensure adequate partitioning of organic and aqueous solutions. The plates are counted in a Wallac Microbeta counter (PerkinElmer) to collect the raw cpm data.

Serotonin (5-HT) Uptake Assay

[0086] The methods for 5-HT functional reuptake using the JAR cell line is modified using a previous literature report (Prasad, *et al.*, *Placenta*, 1996. **17**(4): 201-7). On day 1, cells are plated at 15,000 cells/well in 96-well plates containing growth medium (RPMI 1640 with 10% FBS) and maintained in a cell incubator (37°C, 5% CO_2). On day 2, cells are stimulated with staurosporine (40 nM) to increase the expression of the 5-HT transporter. On day 3, cells are removed from the cell incubator two hours prior to assay and maintained at room temperature to equilibrate the growth medium to ambient oxygen concentration. Subsequently, the growth medium is replaced with 200 μl of assay buffer (25 mM HEPES; 120 mM NaCl; 5 mM KCl; 2.5 mM $CaCl_2$; 1.2 mM $MgSO_4$; 2 mg/ml glucose (pH 7.4, 37°C)) containing 0.2 mg/ml ascorbic acid and 10 μM pargyline. A stock solution of paroxetine (AHR-4389-1) is prepared in DMSO (10 mM) and delivered to triplicate wells containing

cells for a final test concentration of 1 μM. Data from these wells are used to define non-specific 5-HT uptake (minimum 5-HT uptake). Test compounds are prepared in DMSO (10 mM) and diluted in assay buffer according to test range (1 to 1,000 nM). Twenty-five microliters of assay buffer (maximum 5-HT uptake) or test compound are added directly to triplicate wells containing cells in 200 μl of assay buffer. The cells are incubated with the compound for 10 minutes (37°C). To initiate the reaction, [^{3}H]hydroxytryptamine creatinine sulfate diluted in assay buffer is delivered in 25 μl aliquots to each well for a final test concentration of 15 nM. The cells are incubated with the reaction mixture for 5 minutes at 37°C. The 5-HT uptake reaction is terminated by decanting the assay buffer. The cells are washed twice with 200 μl assay buffer (37°C) to remove free radioligand. The plates are inverted and left to dry for 2 minutes, then reinverted and air-dried for an additional 10 minutes. Subsequently, the cells are lysed in 25 μl of 0.25 N NaOH (4°C) then placed on a shaker table and shaken vigorously for 5 minutes. After cell lysis, 75 μl of scintillation cocktail are added to the wells, the plates are sealed with film tape and replaced on the shake table for a minimum of 10 minutes. The plates are counted in a Wallac Microbeta counter (PerkinElmer) to collect the raw cpm data.

Evaluation of Results

[0087] For each experiment, a data stream of cpm values collected from the Wallac Microbeta counter is downloaded to a Microsoft Excel statistical application program. Calculations of EC_{50} values are made using the transformed-both-sides logistic dose response program written by Wyeth Biometrics Department. The statistical program uses mean cpm values from wells representing maximum binding or uptake (assay buffer) and mean cpm values from wells representing minimum binding or uptake ((1 μM desipramine (hNET) or 1 μM paroxetine (hSERT)). Estimation of the EC_{50} value is completed on a log scale and the line is fit between the maximum and minimum binding or uptake values. All graphic data representation is generated by normalizing each data point to a mean percent based on the maximum and minimum binding or uptake values. The EC_{50} values reported from multiple experiments are calculated by pooling the raw data from each experiment and analyzing the pooled data as one experiment.

[0088] Telemetry model: This model has been modified from a previously reported protocol describing estrogen regulation of diurnal tail skin temperature (TST) patterns (Berendsen, *et al., European Journal of Pharmacology*, **2001**, 419(1): 47-54). Over a 24-hour period, intact cycling rats decrease TST during the active (dark) phase and TST remains elevated during the inactive (light) phase. In ovariectomized (OVX) rats, TST is elevated over the entire 24-hour period, thus the usual decrease in TST during the active (dark) phase is lost, thus, a compound's ability to restore this lowering of TST during the active phase was examined. A temperature and physical activity transmitter (PhysioTel TA10TA-F40, Data Sciences International) was implanted subcutaneously in the dorsal scapular region and the tip of the temperature probe was tunneled subcutaneously 2.5 cm beyond the base of the tail. After a 7-day recovery period, TST readings were continuously recorded for the remainder of the study. Tail skin temperature readings were collected from each animal every 5 minutes with values obtained over a 10 second sampling period. The day before test day, an average baseline TST value was calculated for each animal by averaging temperature readings recorded during the 12 hour active (dark) phase. In these studies, animals were dosed approximately 40 minutes prior to the onset of dark cycle.

[0089] Statistical analysis: Evaluation of a compound's ability to restore normal lowering of TST in the telemetry model was analyzed using hourly TST values calculated for each animal by averaging the 12 temperature readings obtained every 5 minutes over that recording time. To analyze ΔTST in the telemetry model, a two factors repeated measure ANOVA was performed. The model used for analysis was ΔTST = GRP (group) + HR (hours) + GRP*HR + BASELINE. Thus, the reported least squares means are the expected mean values as if both groups had the same baseline value. *Post-hoc* tests of hourly GRP*HR samples are t-tests of the difference between groups for each hour. To be conservative, a result was not considered significant unless the p-value was < 0.025. All analyses were performed using SAS PROC MIXED (SAS, Carey, NC).

EXAMPLE 1:

Functional Reuptake Bioassays of (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909)

[0090] IC_{50} for dopamine reuptake inhibition at the dopamine transport (DAT), norepinephrine reuptake inhibition at the norepinephrine transporter (NET), and serotonin reuptake inhibition at the serotonin transporter (SERT) were measured *in vitro* with homogenized rat brain for (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) (GBR12909). The results were as follows:

	(-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane	1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine
hNET function uptake (EC_{50} in nm)	1176 +/- 441	
hNET transporter (IC_{50} in nm)		440
hSERT function uptake (EC_{50} in nm)	977 +/- 131	
hSERT transporter (IC_{50} in nm)		170
hDAT transporter (IC_{50} in nm)		1
hDAT binding (%I at 10 μM)	55%	

EXAMPLE 2:

Telemetry Testing of (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909)

[0091] Rats were injected subcutaneously with vehicle (2%Tween/0.5% methylcellulose) or 30 mg/kg, sc test compound dissolved in 2%Tween/0.5% methylcellulose. The effect of test compound is measured by evaluating the following parameters in this model: onset of action, duration of effect on TST, maximal change in TST and mean change in TST over the duration of the compound effect.

[0092] (-)-1-(4-Methylphenyl)-3-azabicyclo[3.1.0]hexane and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909) restored normal TST in an OVX-induced thermoregulatory dysfunction telemetry model (telemetry model) 30 mg/kg, sc.

[0093] The results of the administration of (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane and 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine (GBR12909) at 1 dose (30 mg/kg, sc) in the telemetry rat model of ovariectomy-induced thermoregulatory dysfunction are shown in **FIGURE 1** and **FIGURE 2**, respectively.

EXAMPLE 3:

Evaluation of test compounds in the spinal nerve ligation (SNL) model of neuropathic pain

Materials and Methods

[0094] Animal maintenance and research were conducted in accordance with the National Research Council's policies and guidelines for the handling and use of laboratory animals outlined in the *Guide for the Care and Use of Laboratory Animals.* The laboratory facility was licensed by the United States Department of Agriculture and accredited by the American Association for Accreditation of Laboratory Animal Care. Research protocols were approved by the Wyeth Institutional Animal Care and Use Committee in accordance with the guidelines of the Committee for Research and Ethical Issues of IASP (Zimmermann, 1983).

[0095] Subjects. Male Sprague-Dawley rats (Indianapolis, IN) weighing 150 to 200 g at time of arrival, were individually housed in wire cages in a climate-controlled room. A 12-hour light/dark cycle (lights on at 0630) was in effect, and food and water were available *ad libitum.*

[0096] Surgery – Spinal Nerve Ligation. Rats were anesthetized with 3.5% halothane in O_2 at 1 L/min and maintained with 1.5% halothane in O_2 during surgery. Ligation of the L5 and L6 nerves was produced by an incision through the left paraspinal muscles. The left L5 and L6 spinal nerves were isolated adjacent to the vertebral column and ligated tightly with 6-0 silk suture just distal to the dorsal root ganglion. The wound was closed in layers using 4-0 silk suture and wound clips. Testing began 7 days after surgery.

[0097] Assessment of tactile hypersensitivity. Animals were placed in elevated wire cages and allowed 45 to 60 minutes to acclimate to the testing room. Baseline tactile sensitivity was assessed using a series of calibrated von Frey monofilaments (Stoelting; Wood Dale, IL) 0 to 3 days before surgery. Von Frey monofilaments were applied to the mid-plantar hind paw in sequential ascending or descending order, as necessary, to hover as closely as possible to the threshold of responses. The

threshold was indicated by the lowest force that evoked a brisk withdrawal response to the stimuli. Thus, a withdrawal response led to the presentation of the next lighter stimulus and the lack of a withdrawal response led to the presentation of the next stronger stimulus. Rats with baseline thresholds < 10g force were excluded from the study. Three to four weeks following surgery, tactile sensitivities were reassessed, and animals that failed to exhibit subsequent tactile hypersensitivity (threshold ≥ 5g) were excluded from further testing. Subjects were pseudo-randomly divided into test groups of 7 so that average baseline and post-surgery sensitivities were similar among groups. The ability of a single dose of test compound to reverse established hypersensitivity was assessed using a time course procedure. Under this procedure, 30 mg/kg test compound or vehicle was administered IP and sensitivities were reassessed 30, 60, 100, 180 and 300 minutes after administration.

[0098] Results are presented as the 50% threshold values (50% threshold in g force) estimated by the Dixon non-parametric test. Fifteen-gram force was used as the maximal force. Individual tactile hypersensitivity threshold values were averaged to provide a mean response (± 1 SEM). Statistical analysis was done using a one-way analysis of variance (ANOVA). Significant main effects were analyzed further by subsequent least significant difference analysis. The criterion for significant differences was $p < 0.05$.

[0099] Reversal of tactile hypersensitivity was defined as a return to baseline of the tactile sensitivity and was calculated according to the following equation:

$$\% \text{ Reversal} = \frac{(50\% \text{ threshold}^{\text{drug + post surgery}}) - (50\% \text{ treshold}^{\text{post surgery}})}{(50\% \text{ threshold}^{\text{pre surgery}}) - (50\% \text{ threshold}^{\text{post surgery}})} \times 100$$

in which 50% threshold$^{\text{drug + post surgery}}$ is the 50% threshold in g force after drug in nerve injured subjects, 50% threshold$^{\text{post surgery}}$ is the 50% threshold in g force in nerve injured subjects, and 50% threshold$^{\text{pre surgery}}$ is the 50% threshold in g force before nerve injury. Maximal effect of 100% reversal represents a return to the mean pre-operative threshold value for subjects in that experimental condition.

[0100] The results for racemic 1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane (bicifadine), (+)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-

methylphenyl)-3-azabicyclo[3.1.0]hexane, gabapentin, and vehicle are shown in **FIGURE 3**, which is a plot of % reversal at 30, 60, 100, 180, and 300 minutes after administration of racemic 1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane (bicifadine), (+)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, gabapentin, and vehicle.

[0101] When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations and subcombinations of ranges specific embodiments therein are intended to be included.

[0102] The disclosures of each patent, patent application and publication cited or described in this document are hereby incorporated herein by reference, in its entirety.

[0103] Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

What is claimed is:

1. A method for treating at least one nervous system disorder or condition in a subject in need thereof, comprising the step of:

administering to said subject a composition comprising an effective amount of at least one selective dopamine reuptake inhibitor;

wherein said nervous system disorder or condition is a vasomotor symptom, chronic pain, Shy Drager syndrome, or a combination thereof.

2. A method according to claim 1,

wherein said selective dopamine reuptake inhibitor has norepinephrine reuptake inhibitory activity of greater than about 0.5 μM in a functional norepinephrine reuptake bioassay.

3. A method according to claim 2,

wherein said selective dopamine reuptake inhibitor has essentially no norepinephrine reuptake inhibitory activity.

4. A method according to claim 1,

wherein said selective dopamine reuptake inhibitor has serotonin reuptake inhibitory activity of greater than about 0.5 μM in a functional serotonin reuptake bioassay.

5. A method according to claim 4,

wherein said selective dopamine reuptake inhibitor has essentially no serotonin reuptake inhibitory activity.

6. A method according to claim 1,

wherein said selective dopamine reuptake inhibitor has dual norepinephrine reuptake inhibitory activity of greater than about 0.5 μM in a functional norepinephrine reuptake bioassay and serotonin reuptake inhibitory activity of greater than about 0.5 μM in a functional serotonin reuptake bioassay.

7. A method according to claim 6,

wherein said selective dopamine reuptake inhibitor has essentially no dual norepinephrine and serotonin reuptake inhibitory activity.

8. A method according to claim 1,

wherein said composition comprises a compound selected from the group consisting of (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, mazindol, methylphenidate, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine, or a pharmaceutically acceptable salt thereof, or a combination thereof.

9. A method according to claim 1,

wherein said composition comprises (-)-1-(3,4-dichlorophenyl)-3-azabicyclo[3.1.0]hexane, or a pharmaceutically acceptable salt thereof,

10. A method according to claim 1,

wherein said composition comprises (-)-1-(4-methylphenyl)-3-azabicyclo[3.1.0]hexane, or a pharmaceutically acceptable salt thereof.

11. A method according to claim 1,

wherein said composition comprises 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl) piperazine, or a pharmaceutically acceptable salt thereof.

12. A method according to any one of the preceding claims,

wherein said nervous system disorder or condition is a vasomotor symptom.

13. A method according to any one of the preceding claims,

wherein said vasomotor symptom is hot flush.

14. A method according to any one of the preceding claims,

wherein said subject is human.

15. A method according to claim 14,
wherein said human is a female.

16. A method according to claim 15,
wherein said female is pre-menopausal.

17. A method according to claim 15,
wherein said female is peri-menopausal.

18. A method according to claim 15,
wherein said female is post-menopausal.

19. A method according to claim 14,
wherein said human is a male.

20. A method according to claim 19,
wherein said male is naturally, chemically or surgically andropausal.

21. A method according to any one of Claims 1 to 11,
wherein said nervous system disorder or condition is chronic pain.

22. A method according to claim 21,
wherein said nervous system disorder or condition is neuropathic pain.

23. A method according to any one of Claims 1 to 11,
wherein said nervous system disorder or condition is Shy Drager syndrome.

24. Use of a selective dopamine reuptake inhibitor in the preparation of a medicament for the treatment of at least one nervous system disorder or condition in a subject in need thereof, wherein said nervous system disorder

or condition is a vasomotor symptom, chronic pain, Shy Drager syndrome, or a combination thereof.

FIGURE 1

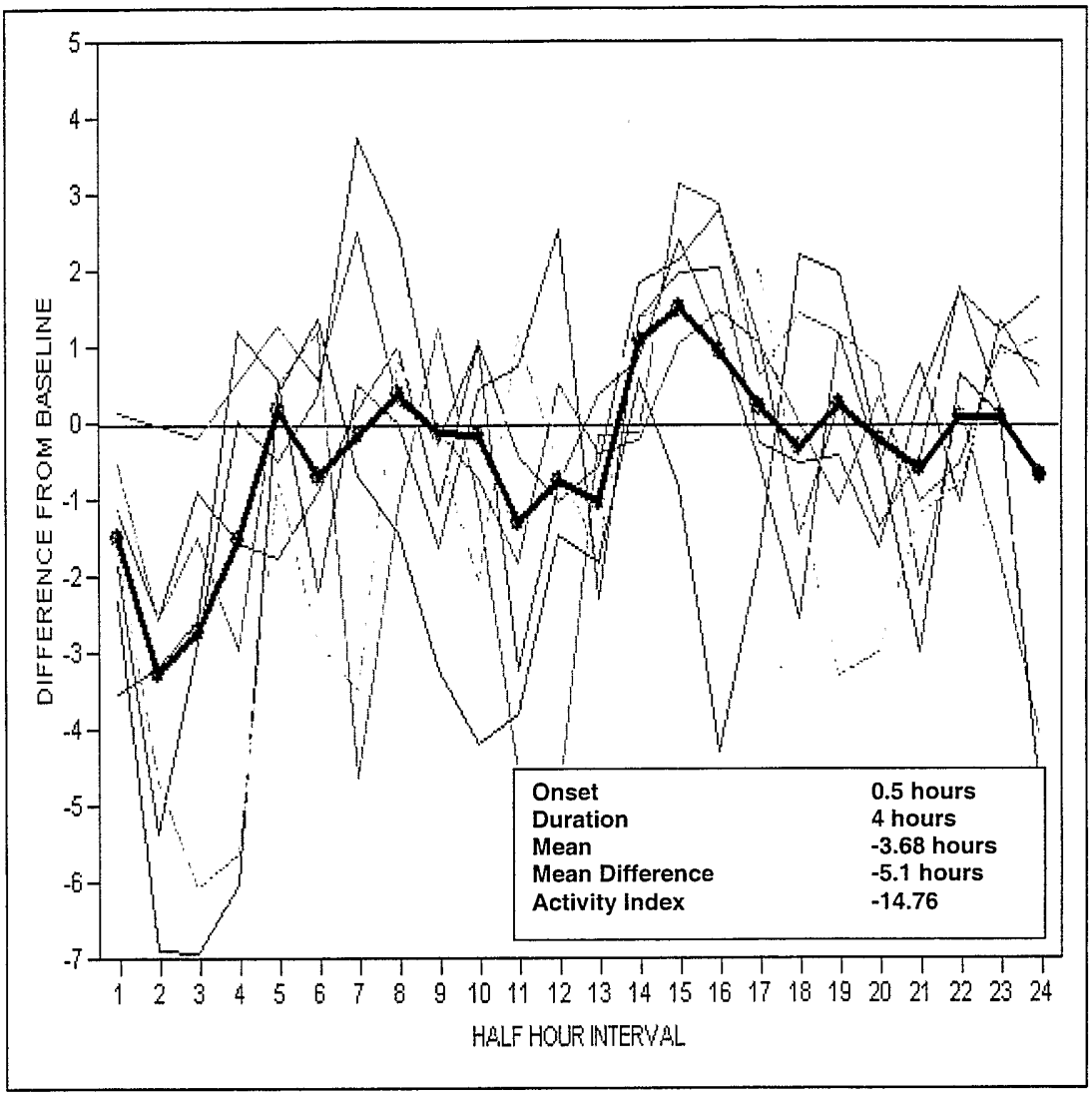

DIFFERENCE FROM BASELINE
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
HALF HOUR INTERVAL
Onset 0.5 hours
Duration 4 hours
Mean -3.68 hours
Mean Difference -5.1 hours
Activity Index -14.76

FIGURE 2

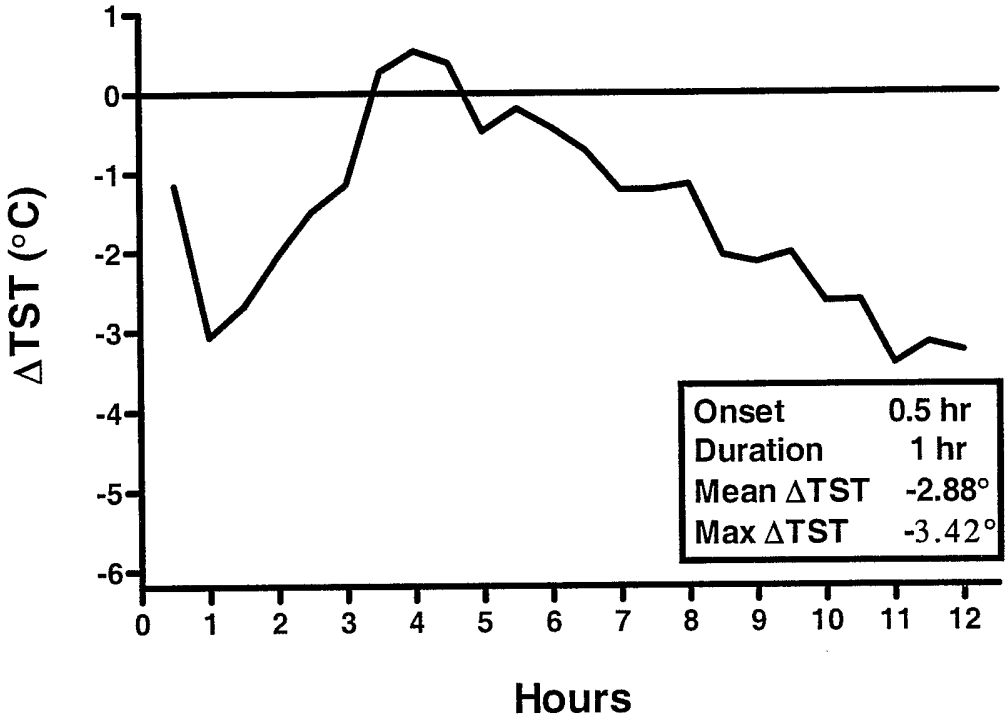
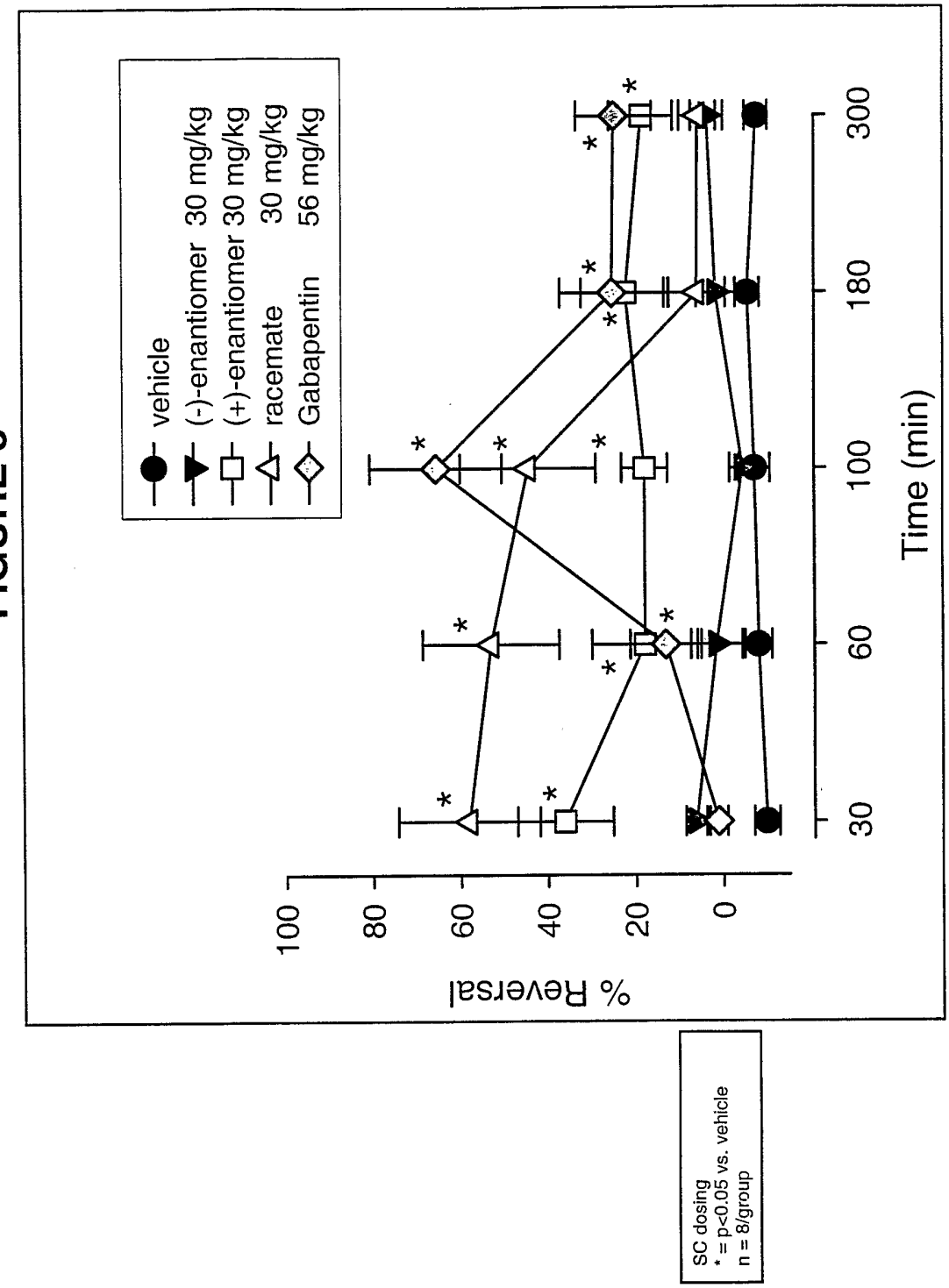

1
0
-1
-2
-3
-4
-5
-6
ΔTST (°C)
0 1 2 3 4 5 6 7 8 9 10 11 12
Hours
Onset 0.5 hr
Duration 1 hr
Mean ΔTST -2.88°
Max ΔTST -3.42°

FIGURE 3


vehicle
(-)-enantiomer 30 mg/kg
(+)-enantiomer 30 mg/kg
racemate 30 mg/kg
Gabapentin 56 mg/kg
% Reversal
100
80
60
40
20
0
30
60
100
180
300
Time (min)
SC dosing
* = p<0.05 vs. vehicle
n = 8/group