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(54) Title: METHODS FOR PREVENTING MITOCHONDRIAL PERMEABILITY TRANSITION

(57) Abrégé/Abstract:
The invention provides a method of reducing or preventing mitochondrial permeability transitioning. The method comprises administering an effective amount of an aromatic-cationic peptide having at least one net positive charge; a minimum of four amino acids; a maximum of about twenty amino acids; a relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) wherein $3p_m$ is the largest number that is less than or equal to r + 1; and a relationship between the minimum number of aromatic groups (a) and the total number of net positive charges (p_t) wherein 2a is the largest number that is less than or equal to p_t + 1, except that when a is 1, p_t may also be 1.

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(54) Title: METHODS FOR PREVENTING MITOCHONDRIAL PERMEABILITY TRANSITION

(57) Abstract: The invention provides a method of reducing or preventing mitochondrial permeability transitioning. The method comprises administering an effective amount of an aromatic-cationic peptide having at least one net positive charge; a minimum of four amino acids; a maximum of about twenty amino acids; a relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) wherein $3p_m$ is the largest number that is less than or equal to r + 1; and a relationship between the minimum number of aromatic groups (a) and the total number of net positive charges (p_t) wherein 2a is the largest number that is less than or equal to p_t + 1, except that when a is 1, p_t may also be 1.

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METHODS FOR PREVENTING MITOCHONDRIAL PERMEABILITY TRANSITION

[0002] This invention was made with government support from the National Institute on Drug Abuse under Grant No. PO1 DA08924-08. The U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Mitochondria exist in virtually all eukaryotic cells, and are essential to cell survival by producing adenosine triphosphate (ATP) via oxidative phosphorylation. Interruption of this vital function can lead to cell death.

[0004] Mitochondria also play a major role in intracellular calcium regulation by accumulating calcium (Ca^{2+}). Accumulation of calcium occurs in the mitochondrial matrix through a membrane potential-driven uniporter.

[0005] The uptake of calcium activates mitochondrial dehydrogenases, and may be important in sustaining energy production and oxidative phosphorylation. In addition, the mitochondria serve as a sink for excessive cytosolic Ca^{2+}, thus protecting the cell from Ca^{2+} overload and necrotic death.

[0006] Ischemia or hypoglycemia can lead to mitochondrial dysfunction, including ATP hydrolysis and Ca^{2+} overload. The dysfunction causes mitochondrial permeability transition (MPT). MPT is characterized by uncoupling of oxidative phosphorylation, loss of mitochondrial membrane potential, increased permeability of the inner membrane and swelling.

[0007] In addition, the mitochondria intermembrane space is a reservoir of apoptogenic proteins. Therefore, the loss of mitochondrial potential and MPT can lead to release of apoptogenic proteins into the cytoplasm. Not surprisingly, there is accumulating evidence that MPT is involved in necrotic and apoptotic cell death (Crompton, *Biochem J.* 341:233-249, 1999). Milder forms of cellular insult may lead to apoptosis rather than necrosis.

[0008] Cyclosporin can inhibit MPT. Blockade of MPT by cyclosporin A can inhibit apoptosis in several cell types, including cells undergoing ischemia, hypoxia, Ca^{2+} overload and oxidative stress (Kroemer et al., *Annu Rev Physiol.* 60:619-642, 1998).

[0009] Cyclosporin A, however, is less than optimal as a treatment drug against necrotic and apoptotic cell death. For example, cyclosporin A does not specifically target the mitochondria. In addition, it is poorly delivered to the brain. Furthermore, the utility of cyclosporin A is reduced by its immunosuppressant activity.

[0010] The tetrapeptide [Dmt^1]DALDA (2',6'-dimethyltyrosine-D-Arg-Phe-Lys-NH_2; SS-02) has a molecular weight of 640 and carries a net three positive charge at physiological pH. [Dmt^1]DALDA readily penetrates the plasma membrane of several mammalian cell types in an energy-independent manner (Zhao et al., *J Pharmacol Exp Ther*. 304:425-432, 2003) and penetrates the blood-brain barrier (Zhao et al., *J Pharmacol Exp Ther*. 302:188-196, 2002). Although [Dmt^1]DALDA has been shown to be a potent mu-opioid receptor agonist, its utility has not been expanded to include the inhibition of MPT.

[0011] Thus, there is a need to inhibit MPT in conditions such as ischemia-reperfusion, hypoxia, hypoglycemia, and other diseases and conditions which result in pathological changes as a result of the permeability transitioning of the mitochondrial membranes. Such diseases and conditions include many of the common neurodegenerative diseases.

SUMMARY OF THE INVENTION

[0012] These and other objectives have been met by the present invention which provides a method for reducing the number of mitochondria undergoing a mitochondrial permeability transition (MPT), or preventing mitochondrial permeability transitioning in any mammal that has need thereof. The method comprises administering to the mammal an effective amount of an aromatic-cationic peptide having:

(a) at least one net positive charge;

(b) a minimum of three amino acids;

(c) a maximum of about twenty amino acids;

(d) a relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) wherein $3p_m$ is the largest number that is less than or equal to r + 1; and

(e) a relationship between the minimum number of aromatic groups (a) and the total number of net positive charges (p_t) wherein 2a is the largest number that is less than or equal to p_t + 1, except that when a is 1, p_t may also be 1.

[0013] In another embodiment, the invention provides a method for reducing the number of mitochondria undergoing a mitochondrial permeability transition (MPT), or preventing mitochondrial permeability transitioning in a removed organ of a mammal. The method comprises administering to the removed organ an effective amount of an aromatic-cationic peptide having:

(a) at least one net positive charge;

(b) a minimum of three amino acids;

(c) a maximum of about twenty amino acids;

(d) a relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) wherein $3p_m$ is the largest number that is less than or equal to r + 1; and

(e) a relationship between the minimum number of aromatic groups (a) and the total number of net positive charges (p_t) wherein 2a is the largest number that is less than or equal to p_t + 1, except that when a is 1, p_t may also be 1.

[0014] In yet another embodiment, the invention provides a method of reducing the number of mitochondria undergoing mitochondrial permeability transition (MPT), or preventing mitochondrial permeability transitioning in a mammal in need thereof. The method comprises administering to the mammal an effective amount of an aromatic-cationic peptide having:

(a) at least one net positive charge;

(b) a minimum of three amino acids;

(c) a maximum of about twenty amino acids;

(d) a relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) wherein $3p_m$ is the largest number that is less than or equal to r + 1; and

(e) a relationship between the minimum number of aromatic groups (a) and the total number of net positive charges (p_t) wherein 3a is the largest number that is less than or equal to p_t + 1, except that when a is 1, p_t may also be 1.

[0015] In a further embodiment, the invention provides a method of reducing the number of mitochondria undergoing mitochondrial permeability transition (MPT), or preventing mitochondrial permeability transitioning in a removed organ of a mammal. Tthe method comprises administering to the removed organ an effective amount of an aromatic-cationic peptide having:

(a) at least one net positive charge;

(b) a minimum of three amino acids;

(c) a maximum of about twenty amino acids;

(d) a relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) wherein $3p_m$ is the largest number that is less than or equal to r + 1; and

(e) a relationship between the minimum number of aromatic groups (a) and the total number of net positive charges (p_t) wherein 3a is the largest number that is less than or equal to p_t + 1, except that when a is 1, p_t may also be 1.

[0015a] In a further embodiment, the invention relates to an aromatic-cationic peptide for reducing the number of mitochondria undergoing mitochondrial permeability transition (MPT), or preventing mitochondrial permeability transitioning in a mammal in need thereof, wherein the aromatic cationic-peptide has the formula D-Arg-Dmt-Lys-Phe-NH_2.

[0015b] In a further embodiment, the invention relates to a peptide having the sequence D-Arg-Dmt-Lys-Phe-NH_2, D-Arg-Dmt-Phe-Lys-NH_2, D-Arg-Phe-Lys-Dmt-NH_2, D-Arg-Phe-Dmt-Lys-NH_2, D-Arg-Lys-Dmt-Phe-NH_2, D-Arg-Lys-Phe-Dmt-NH_2, Phe-Lys-Dmt-D-Arg-NH_2, Phe-Lys-D-Arg-Dmt-NH_2, Phe-D-Arg-Dmt-Lys-NH_2, Phe-D-Arg-Lys-Dmt-NH_2, Phe-D-Arg-Phe-Lys-NH_2, Dmp-D-Arg-Phe-Lys-NH_2, Phe-Dmt-D-Arg-Lys-NH_2, Phe-Dmt-Lys-D-Arg-NH_2, Lys-Phe-Dmt-D-Arg-NH_2, Lys-Dmt-D-Arg-Phe-NH_2, Lys-Dmt-Phe-D-Arg-NH_2, Lys-D-Arg-Phe-Dmt-NH_2, Lys-D-Arg-Dmt-Phe-NH_2, D-Arg-Dmt-D-Arg-Phe-NH_2, D-Arg-Dmt-D-Arg-Dmt-NH_2, D-Arg-Dmt-D-Arg-Tyr-NH_2, D-Arg-Dmt-D-Arg-Trp-NH_2, Trp-D-Arg-Phe-Lys-NH_2, Trp-D-Arg-Tyr-Lys-NH_2, Trp-D-Arg-Trp-Lys-NH_2, Trp-D-Arg-Dmt-Lys-NH_2, D-Arg-Trp-Lys-Phe-NH_2, D-Arg-Trp-Phe-Lys-NH_2, D-Arg-Trp-Lys-Dmt-NH_2, D-Arg-Trp-Dmt-Lys-NH_2, D-Arg-Lys-Trp-Phe-NH_2, D-Arg-Lys-Trp-Dmt-NH_2, Cyclohexyl-D-Arg-Phe-Lys-NH_2, or Ala-D-Arg-Phe-Lys-NH_2.

[0015c] In a further embodiment, the invention relates to a composition comprising a peptide having the sequence as described above and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE FIGURES

[0016] Figure 1: Cellular internalization and accumulation of [Dmt^1]DALDA (SS-02) in mitochondria. (A) Mitochondrial uptake of SS-19 was determined using fluorescence spectrophotometry (ex/em = 320/420 nm). Addition of isolated mouse liver mitochondria (0.35 mg/ml) resulted in immediate quenching of SS-19 fluorescence intensity. Pretreatment of mitochondria with FCCP (1.5 μM) reduced quenching by <20%. (B) Isolated mitochondria were incubated with [^{3}H]SS-02 at 37°C for 2 min. Uptake was stopped by centrifugation (16000 x g) for 5 min at 4°C, and radioactivity determined in the pellet. Pretreatment of mitochondria with FCCP inhibited [^{3}H]SS-02 uptake by ~20%. Data are shown as mean ± s.e.; n = 3. *, P<0.05 by Student's *t*-test. (C) Uptake of TMRM by isolated mitochondria is lost upon mitochondrial swelling induced by alamethicin, while uptake of SS-19 is retained to a large extent. (D) Addition of SS-02 (200 μM) to isolated mitochondria did not alter mitochondrial potential, as measured by TMRM fluorescence. Addition of FCCP (1.5 μM) caused immediate depolarization while Ca^{2+} (150 μM) resulted in depolarization and progressive onset of MPT.

[0017] Figure 2. [Dmt^1]DALDA (SS-02) protects against mitochondrial permeability transition (MPT) induced by Ca^{2+} overload and 3-nitroproprionic acid (3NP). (A) Pretreatment of isolated mitochondria with 10 μM SS-02 (addition indicated by down arrow) prevented onset of MPT caused by Ca^{2+} overload (up arrow). (B) Pretreatment of isolated mitochondria with SS-02 increased mitochondrial tolerance of multiple Ca^{2+} additions prior to onset of MPT. Arrow indicates addition of buffer or SS-02. *Line 1*, buffer; *line 2*, 50 μM SS-02; *line 3*, 100 μM SS-02. (C) SS-02 dose-dependently delayed the onset of MPT caused by 1 mM 3NP. Arrow indicates addition of buffer or SS-02. *Line 1*, buffer; *line 2*, 0.5 μM SS-02; *line 3*, 5 μM SS-02; *line 4*, 50 μM SS-02.

[0018] Figure 3. [Dmt^1]DALDA (SS-02) inhibits mitochondrial swelling and cytochrome c release. (A) Pretreatment of isolated mitochondria with SS-02 dose-dependently inhibited mitochondrial swelling induced by 200 μM Ca^{2+} in a dose-

dependent manner. Swelling was measured by absorbance at 540 nm. (B) SS-02 inhibited Ca^{2+}-induced release of cytochrome c from isolated mitochondria. The amount of cytochrome c released was expressed as percent of total cytochrome c in mitochondria. Data are presented as mean ± s.e., n = 3. (C) SS-02 also inhibited mitochondrial swelling induced by MPP^+ (300 μM).

[0019] Figure 4. D-Arg-Dmt-Lys-Phe-NH_2 (SS-31) inhibits mitochondrial swelling and cytochrome c release. (A) Pretreatment of isolated mitochondria with SS-31 (10 μM) prevents onset of MPT induced by Ca^{2+}.

(B) Pretreatment of mitochondria with SS-31 (50 μM) inhibited mitochondrial swelling induced by 200 mM Ca^{2+}. Swelling was measured by light scattering measured at 570 nm. (C). Comparison of SS-02 and SS-31 with cyclosporine (CsA) in inhibiting mitochondrial swelling and cytochrome c release induced by Ca^{2+}. The amount of cytochrome c released was expressed as percent of total cytochrome c in mitochondria. Data are presented as mean ± s.e., n = 3.

[0020] Figure 5. [Dmt^1]DALDA (SS-02) and D-Arg-Dmt-Lys-Phe-NH_2 (SS-31) protects myocardial contractile force during ischemia-reperfusion in the isolated perfused guinea pig heart. Hearts were perfused with buffer or buffer containing SS-02 (100 nM) or SS-31 (1 nM) for 30 min and then subjected to 30-min global ischemia. Reperfusion was carried out using the same perfusion solution. Significant differences were found among the three treatment groups (2-way ANOVA, $P<0.001$).

[0021] Figure 6. Addition of [Dmt^1]DALDA to cardioplegic solution significantly enhanced contractile function after prolonged ischemia in the isolated perfused guinea pig heart. After 30 min stabilization, hearts were perfused with St. Thomas cardioplegic solution (CPS) or CPS containing [Dmt^1]DALDA at 100 nM for 3 min. Global ischemia was then induced by complete interruption of coronary perfusion for 90 min. Reperfusion was subsequently carried out for 60 min. with oxygenated Krebs-Henseleit solution. Post-ischemic contractile force was significantly improved in the group receiving [Dmt^1]DALDA ($P<0.001$).

DETAILED DESCRIPTION OF THE INVENTION

[0022] The invention is based on the surprising discovery by the inventors that certain aromatic-cationic peptides significantly reduce the number of mitochondria undergoing, or even completely preventing, mitochondrial permeability transition (MPT). Reducing the number of mitochondria undergoing, and preventing, MPT is important, since MPT is associated with several common diseases and conditions in mammals. In addition, a removed organ of a mammal is susceptible to MPT. These diseases and conditions are of particular clinical importance as they afflict a large proportion of the human population at some stage during their lifetime.

Peptides

[0023] The aromatic-cationic peptides useful in the present invention are water-soluble and highly polar. Despite these properties, the peptides can readily penetrate cell membranes.

[0024] The aromatic-cationic peptides useful in the present invention include a minimum of three amino acids, and preferably include a minimum of four amino acids, covalently joined by peptide bonds.

[0025] The maximum number of amino acids present in the aromatic-cationic peptides of the present invention is about twenty amino acids covalently joined by peptide bonds. Preferably, the maximum number of amino acids is about twelve, more preferably about nine, and most preferably about six. Optimally, the number of amino acids present in the peptides is four.

[0026] The amino acids of the aromatic-cationic peptides useful in the present invention can be any amino acid. As used herein, the term "amino acid" is used to refer to any organic molecule that contains at least one amino group and at least one carboxyl group. Preferably, at least one amino group is at the α position relative to the carboxyl group.

[0027] The amino acids may be naturally occurring. Naturally occurring amino acids include, for example, the twenty most common levorotatory (L) amino acids normally found in mammalian proteins, i.e., alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Glu), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ileu), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan, (Trp), tyrosine (Tyr), and valine (Val).

[0028] Other naturally occurring amino acids include, for example, amino acids that are synthesized in metabolic processes not associated with protein synthesis. For example, the amino acids ornithine and citrulline are synthesized in mammalian metabolism during the production of urea.

[0029] The peptides useful in the present invention can contain one or more non-naturally occurring amino acids. The non-naturally occurring amino acids may be L-, dextrorotatory (D), or mixtures thereof. Optimally, the peptide has no amino acids that are naturally occurring.

[0030] Non-naturally occurring amino acids are those amino acids that typically are not synthesized in normal metabolic processes in living organisms, and do not naturally occur in proteins. In addition, the non-naturally occurring amino acids useful in the present invention preferably are also not recognized by common proteases.

[0031] The non-naturally occurring amino acid can be present at any position in the peptide. For example, the non-naturally occurring amino acid can be at the N-terminus, the C-terminus, or at any position between the N-terminus and the C-terminus.

[0032] The non-natural amino acids may, for example, comprise alkyl, aryl, or alkylaryl groups. Some examples of alkyl amino acids include α-aminobutyric acid, β-aminobutyric acid, γ-aminobutyric acid, δ-aminovaleric acid, and ε-aminocaproic acid. Some examples of aryl amino acids include ortho-, meta, and para-aminobenzoic acid. Some examples of alkylaryl amino acids include ortho-, meta-, and para-aminophenylacetic acid, and γ-phenyl-β-aminobutyric acid.

[0033] Non-naturally occurring amino acids also include derivatives of naturally occurring amino acids. The derivatives of naturally occurring amino acids may, for example, include the addition of one or more chemical groups to the naturally occurring amino acid.

[0034] For example, one or more chemical groups can be added to one or more of the 2', 3', 4', 5', or 6' position of the aromatic ring of a phenylalanine or tyrosine residue, or the 4', 5', 6', or 7' position of the benzo ring of a tryptophan residue. The group can be any chemical group that can be added to an aromatic ring. Some examples of such groups include branched or unbranched C_1-C_4 alkyl, such as methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, or t-butyl, C_1-C_4 alkyloxy (i.e., alkoxy), amino, C_1-C_4 alkylamino and C_1-C_4 dialkylamino (e.g., methylamino, dimethylamino), nitro, hydroxyl, halo (i.e., fluoro, chloro, bromo, or iodo). Some specific examples of non-naturally occurring derivatives of naturally occurring amino acids include norvaline (Nva), norleucine (Nle), and hydroxyproline (Hyp).

[0035] Another example of a modification of an amino acid in a peptide useful in the methods of the present invention is the derivatization of a carboxyl group of an aspartic acid or a glutamic acid residue of the peptide. One example of derivatization is amidation with ammonia or with a primary or secondary amine, e.g. methylamine, ethylamine, dimethylamine or diethylamine. Another example of derivatization includes esterification with, for example, methyl or ethyl alcohol.

[0036] Another such modification includes derivatization of an amino group of a lysine, arginine, or histidine residue. For example, such amino groups can be acylated. Some suitable acyl groups include, for example, a benzoyl group or an alkanoyl group comprising any of the C_1-C_4 alkyl groups mentioned above, such as an acetyl or propionyl group.

[0037] The non-naturally occurring amino acids are preferably resistant, and more preferably insensitive, to common proteases. Examples of non-naturally occurring amino acids that are resistant or insensitive to proteases include the dextrorotatory (D-) form of any of the above-mentioned naturally occurring L-amino acids, as well as L- and/or D-

non-naturally occurring amino acids. The D-amino acids do not normally occur in proteins, although they are found in certain peptide antibiotics that are synthesized by means other than the normal ribosomal protein synthetic machinery of the cell. As used herein, the D-amino acids are considered to be non-naturally occurring amino acids.

[0038] In order to minimize protease sensitivity, the peptides useful in the methods of the invention should have less than five, preferably less than four, more preferably less than three, and most preferably, less than two contiguous L-amino acids recognized by common proteases, irrespective of whether the amino acids are naturally or non-naturally occurring. Optimally, the peptide has only D-amino acids, and no L-amino acids.

[0039] If the peptide contains protease sensitive sequences of amino acids, at least one of the amino acids is preferably a non-naturally-occurring D-amino acid, thereby conferring protease resistance. An example of a protease sensitive sequence includes two or more contiguous basic amino acids that are readily cleaved by common proteases, such as endopeptidases and trypsin. Examples of basic amino acids include arginine, lysine and histidine.

[0040] It is important that the aromatic-cationic peptides have a minimum number of net positive charges at physiological pH in comparison to the total number of amino acid residues in the peptide. The minimum number of net positive charges at physiological pH will be referred to below as (p_m). The total number of amino acid residues in the peptide will be referred to below as (r).

[0041] The minimum number of net positive charges discussed below are all at physiological pH. The term "physiological pH" as used herein refers to the normal pH in the cells of the tissues and organs of the mammalian body. For instance, the physiological pH of a human is normally approximately 7.4, but normal physiological pH in mammals may be any pH from about 7.0 to about 7.8.

[0042] "Net charge" as used herein refers to the balance of the number of positive charges and the number of negative charges carried by the amino acids present in the

peptide. In this specification, it is understood that net charges are measured at physiological pH. The naturally occurring amino acids that are positively charged at physiological pH include L-lysine, L-arginine, and L-histidine. The naturally occurring amino acids that are negatively charged at physiological pH include L-aspartic acid and L-glutamic acid.

[0043] Typically, a peptide has a positively charged N-terminal amino group and a negatively charged C-terminal carboxyl group. The charges cancel each other out at physiological pH. As an example of calculating net charge, the peptide Tyr-Arg-Phe-Lys-Glu-His-Trp-Arg has one negatively charged amino acid (i.e., Glu) and four positively charged amino acids (i.e., two Arg residues, one Lys, and one His). Therefore, the above peptide has a net positive charge of three.

[0044] In one embodiment of the present invention, the aromatic-cationic peptides have a relationship between the minimum number of net positive charges at physiological pH (p_m) and the total number of amino acid residues (r) wherein $3p_m$ is the largest number that is less than or equal to r + 1. In this embodiment, the relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) is as follows:

(r)	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(p_m)	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	7

[0045] In another embodiment, the aromatic-cationic peptides have a relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) wherein $2p_m$ is the largest number that is less than or equal to r + 1. In this embodiment, the relationship between the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) is as follows:

(r)	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(p_m)	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10

[0046] In one embodiment, the minimum number of net positive charges (p_m) and the total number of amino acid residues (r) are equal. In another embodiment, the peptides have three or four amino acid residues and a minimum of one net positive charge, preferably, a minimum of two net positive charges and more preferably a minimum of three net positive charges.

[0047] It is also important that the aromatic-cationic peptides have a minimum number of aromatic groups in comparison to the total number of net positive charges (p_t). The minimum number of aromatic groups will be referred to below as (a).

[0048] Naturally occurring amino acids that have an aromatic group include the amino acids histidine, tryptophan, tyrosine, and phenylalanine. For example, the hexapeptide Lys-Gln-Tyr-Arg-Phe-Trp has a net positive charge of two (contributed by the lysine and arginine residues) and three aromatic groups (contributed by tyrosine, phenylalanine and tryptophan residues).

[0049] In one embodiment of the present invention, the aromatic-cationic peptides useful in the methods of the present invention have a relationship between the minimum number of aromatic groups (a) and the total number of net positive charges at physiological pH (p_t) wherein 3a is the largest number that is less than or equal to $p_t + 1$, except that when p_t is 1, a may also be 1. In this embodiment, the relationship between the minimum number of aromatic groups (a) and the total number of net positive charges (p_t) is as follows:

(p_t)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(a)	1	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	7

[0050] In another embodiment the aromatic-cationic peptides have a relationship between the minimum number of aromatic groups (a) and the total number of net positive charges (p_t) wherein 2a is the largest number that is less than or equal to $p_t + 1$. In this embodiment, the relationship between the minimum number of aromatic amino acid residues (a) and the total number of net positive charges (p_t) is as follows:

(p_t)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
(a)	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10

[0051] In another embodiment, the number of aromatic groups (a) and the total number of net positive charges (p_t) are equal.

[0052] Carboxyl groups, especially the terminal carboxyl group of a C-terminal amino acid, are preferably amidated with, for example, ammonia to form the C-terminal amide. Alternatively, the terminal carboxyl group of the C-terminal amino acid may be amidated with any primary or secondary amine. The primary or secondary amine may, for example, be an alkyl, especially a branched or unbranched C_1-C_4 alkyl, or an aryl amine. Accordingly, the amino acid at the C-terminus of the peptide may be converted to an amido, N-methylamido, N-ethylamido, N,N-dimethylamido, N,N-diethylamido, N-methyl-N-ethylamido, N-phenylamido or N-phenyl-N-ethylamido group.

[0053] The free carboxylate groups of the asparagine, glutamine, aspartic acid, and glutamic acid residues not occurring at the C-terminus of the aromatic-cationic peptides of the present invention may also be amidated wherever they occur within the

peptide. The amidation at these internal positions may be with ammonia or any of the primary or secondary amines described above.

[0054] In one embodiment, the aromatic-cationic peptide useful in the methods of the present invention is a tripeptide having two net positive charges and at least one aromatic amino acid. In a particular embodiment, the aromatic-cationic peptide useful in the methods of the present invention is a tripeptide having two net positive charges and two aromatic amino acids.

[0055] Aromatic-cationic peptides useful in the methods of the present invention include, but are not limited to, the following peptide examples:

Lys-D-Arg-Tyr-NH_2,

Phe-D-Arg-His,

D-Tyr-Trp-Lys-NH_2,

Trp-D-Lys-Tyr-Arg-NH_2,

Tyr-His-D-Gly-Met,

Phe-Arg-D-His-Asp,

Tyr-D-Arg-Phe-Lys-Glu-NH_2,

Met-Tyr-D-Lys-Phe-Arg,

D-His-Glu-Lys-Tyr-D-Phe-Arg,

Lys-D-Gln-Tyr-Arg-D-Phe-Trp-NH_2,

Phe-D-Arg-Lys-Trp-Tyr-D-Arg-His,

Gly-D-Phe-Lys-Tyr-His-D-Arg-Tyr-NH_2,

Val-D-Lys-His-Tyr-D-Phe-Ser-Tyr-Arg-NH_2,

Trp-Lys-Phe-D-Asp-Arg-Tyr-D-His-Lys,

Lys-Trp-D-Tyr-Arg-Asn-Phe-Tyr-D-His-NH_2,

Thr-Gly-Tyr-Arg-D-His-Phe-Trp-D-His-Lys,

Asp-D-Trp-Lys-Tyr-D-His-Phe-Arg- D-Gly-Lys-NH_2,

D-His-Lys-Tyr- D-Phe-Glu- D-Asp- D-His- D-Lys-Arg-Trp-NH_2,

Ala-D-Phe-D-Arg-Tyr-Lys-D-Trp-His-D-Tyr-Gly-Phe,

Tyr-D-His-Phe- D-Arg-Asp-Lys- D-Arg-His-Trp-D-His-Phe,

Phe-Phe-D-Tyr-Arg-Glu-Asp-D-Lys-Arg-D-Arg-His-Phe-NH_2,

Phe-Try-Lys-D-Arg-Trp-His-D-Lys-D-Lys-Glu-Arg-D-Tyr-Thr,

Tyr-Asp-D-Lys-Tyr-Phe- D-Lys- D-Arg-Phe-Pro-D-Tyr-His-Lys,

Glu-Arg-D-Lys-Tyr- D-Val-Phe- D-His-Trp-Arg-D-Gly-Tyr-Arg-D-Met-NH_2,

Arg-D-Leu-D-Tyr-Phe-Lys-Glu- D-Lys-Arg-D-Trp-Lys- D-Phe-Tyr-D-Arg-Gly,

D-Glu-Asp-Lys-D-Arg-D-His-Phe-Phe-D-Val-Tyr-Arg-Tyr-D-Tyr-Arg-His-Phe-NH_2,

Asp-Arg-D-Phe-Cys-Phe-D-Arg-D-Lys-Tyr-Arg-D-Tyr-Trp-D-His-Tyr-D-Phe-Lys-Phe,

His-Tyr-D-Arg-Trp-Lys-Phe-D-Asp-Ala-Arg-Cys-D-Tyr-His-Phe-D-Lys-Tyr-His-Ser-NH_2,

Gly-Ala-Lys-Phe-D-Lys-Glu-Arg-Tyr-His-D-Arg-D-Arg-Asp-Tyr-Trp-D-His-Trp-His-D-Lys-Asp

and

Thr-Tyr-Arg-D-Lys-Trp-Tyr-Glu-Asp-D-Lys-D-Arg-His-Phe-D-Tyr-Gly-Val-Ile-D-His-Arg-Tyr-NH_2.

[0056] In one embodiment, the peptides useful in the methods of the present invention have mu-opioid receptor agonist activity (i.e., activate the mu-opioid receptor). Activation of the mu-opioid receptor typically elicits an analgesic effect.

[0057] In certain instances, an aromatic-cationic peptide having mu-opioid receptor activity is preferred. For example, during short-term treatment, such as in an acute disease or condition, it may be beneficial to use an aromatic-cationic peptide that activates the mu-opioid receptor. Such acute diseases and conditions are often associated with moderate or severe pain. In these instances, the analgesic effect of the aromatic-cationic peptide may be beneficial in the treatment regimen of the patient or other mammal, although an aromatic-cationic peptide which does not activate the mu-opioid receptor may also be used with or without an analgesic according to clinical requirements.

[0058] Alternatively, in other instances, an aromatic-cationic peptide that does not have mu-opioid receptor activity is preferred. For example, during long-term treatment, such as in a chronic disease state or condition, the use of an aromatic-cationic peptide that activates the mu-opioid receptor may be contraindicated. In these instances the potentially adverse or addictive effects of the aromatic-cationic peptide may preclude

the use of an aromatic-cationic peptide that activates the mu-opioid receptor in the treatment regimen of a human patient or other mammal.

[0059] Potential adverse effects may include sedation, constipation and respiratory depression. In such instances an aromatic-cationic peptide that does not activate the mu-opioid receptor may be an appropriate treatment.

[0060] Examples of acute conditions include heart attack, stroke and traumatic injury. Traumatic injury may include traumatic brain and spinal cord injury.

[0061] Examples of chronic diseases or conditions include coronary artery disease and any neurodegenerative disorders, such as those described below.

[0062] Peptides useful in the methods of the present invention which have mu-opioid receptor activity are typically those peptides which have a tyrosine residue or a tyrosine derivative at the N-terminus (i.e., the first amino acid position). Preferred derivatives of tyrosine include 2'-methyltyrosine (Mmt); 2',6'-dimethyltyrosine (2'6'Dmt); 3',5'-dimethyltyrosine (3'5'Dmt); N,2',6'-trimethyltyrosine (Tmt); and 2'-hydroxy-6'-methyltryosine (Hmt).

[0063] In a particular preferred embodiment, a peptide that has mu-opioid receptor activity has the formula Tyr-D-Arg-Phe-Lys-NH_2 (for convenience represented by the acronym: DALDA, which is referred to herein as SS-01). DALDA has a net positive charge of three, contributed by the amino acids tyrosine, arginine, and lysine and has two aromatic groups contributed by the amino acids phenylalanine and tyrosine. The tyrosine of DALDA can be a modified derivative of tyrosine such as in 2',6'-dimethyltyrosine to produce the compound having the formula 2',6'-Dmt-D-Arg-Phe-Lys-NH_2 (i.e., Dmt^1-DALDA, which is referred to herein as SS-02).

[0064] Peptides that do not have mu-opioid receptor activity generally do not have a tyrosine residue or a derivative of tyrosine at the N-terminus (i.e., amino acid position one). The amino acid at the N-terminus can be any naturally occurring or non-naturally occurring amino acids other than tyrosine.

[0065] In one embodiment, the amino acid at the N-terminus is phenylalanine or its derivative. Preferred derivatives of phenylalanine include 2'-methylphenylalanine (Mmp), 2',6'-dimethylphenylalanine (Dmp), N,2',6'-trimethylphenylalanine (Tmp), and 2'-hydroxy-6'-methylphenylalanine (Hmp).

[0066] Other aromatic-cationic peptide that does not have mu-opioid receptor activity has the formula Phe-D-Arg-Phe-Lys-NH_2 (i.e., Phe^1-DALDA, which is referred to herein as SS-20). Alternatively, the N-terminal phenylalanine can be a derivative of phenylalanine such as 2',6'-dimethylphenylalanine (2'6'Dmp). DALDA containing 2',6'-dimethylphenylalanine at amino acid position one has the formula 2',6'-Dmp-D-Arg-Phe-Lys-NH_2 (i.e., 2'6'Dmp^1-DALDA).

[0067] In a preferred embodiment, the amino acid sequence of Dmt^1-DALDA (SS-02) is rearranged such that Dmt is not at the N-terminus. An example of such an aromatic-cationic peptide that does not have mu-opioid receptor activity has the formula D-Arg-2'6'Dmt-Lys-Phe-NH_2 (referred to in this specification as SS-31).

[0068] DALDA, Phe^1-DALDA, SS-31, and their derivatives can further include functional analogs. A peptide is considered a functional analog of DALDA, Phe^1-DALDA, or SS-31 if the analog has the same function as DALDA, Phe^1-DALDA, or SS-31. The analog may, for example, be a substitution variant of DALDA, Phe^1-DALDA, or SS-31, wherein one or more amino acid is substituted by another amino acid.

[0069] Suitable substitution variants of DALDA, Phe^1-DALDA, or SS-31 include conservative amino acid substitutions. Amino acids may be grouped according to their physicochemical characteristics as follows:

(a) Non-polar amino acids: Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);

(b) Acidic amino acids: Asn(N) Asp(D) Glu(E) Gln(Q);

(c) Basic amino acids: His(H) Arg(R) Lys(K);

(d) Hydrophobic amino acids: Met(M) Leu(L) Ile(I) Val(V); and

(e) Aromatic amino acids: Phe(F) Tyr(Y) Trp(W) His (H).

[0070] Substitutions of an amino acid in a peptide by another amino acid in the same group is referred to as a conservative substitution and may preserve the physicochemical characteristics of the original peptide. In contrast, substitutions of an amino acid in a peptide by another amino acid in a different group is generally more likely to alter the characteristics of the original peptide.

[0071] Examples of analogs useful in the practice of the present invention that activate mu-opioid receptors include, but are not limited, to the aromatic-cationic peptides shown in Table 1.

TABLE 1

Amino Acid Position 1	Amino Acid Position 2	Amino Acid Position 3	Amino Acid Position 4	Amino Acid Position 5 (if present)	C-Terminal Modification
Tyr	D-Arg	Phe	Lys		NH_2
Tyr	D-Arg	Phe	Orn		NH_2
Tyr	D-Arg	Phe	Dab		NH_2
Tyr	D-Arg	Phe	Dap		NH_2
2'6'Dmt	D-Arg	Phe	Lys		NH_2
2'6'Dmt	D-Arg	Phe	Lys	Cys	NH_2
2'6'Dmt	D-Arg	Phe	Lys-NH$(CH_2)_2$-NH-dns		NH_2
2'6'Dmt	D-Arg	Phe	Lys-NH$(CH_2)_2$-NH-atn		NH_2
2'6'Dmt	D-Arg	Phe	dnsLys		NH_2
2'6'Dmt	D-Cit	Phe	Lys		NH_2
2'6'Dmt	D-Cit	Phe	Ahp		NH_2
2'6'Dmt	D-Arg	Phe	Orn		NH_2
2'6'Dmt	D-Arg	Phe	Dab		NH_2
2'6'Dmt	D-Arg	Phe	Dap		NH_2
2'6'Dmt	D-Arg	Phe	Ahp(2-aminoheptanoic acid)		NH_2
Bio-2'6'Dmt	D-Arg	Phe	Lys		NH_2
3'5'Dmt	D-Arg	Phe	Lys		NH_2
3'5'Dmt	D-Arg	Phe	Orn		NH_2
3'5'Dmt	D-Arg	Phe	Dab		NH_2
3'5'Dmt	D-Arg	Phe	Dap		NH_2
Tyr	D-Arg	Tyr	Lys		NH_2
Tyr	D-Arg	Tyr	Orn		NH_2
Tyr	D-Arg	Tyr	Dab		NH_2
Tyr	D-Arg	Tyr	Dap		NH_2
2'6'Dmt	D-Arg	Tyr	Lys		NH_2
2'6'Dmt	D-Arg	Tyr	Orn		NH_2
2'6'Dmt	D-Arg	Tyr	Dab		NH_2
2'6'Dmt	D-Arg	Tyr	Dap		NH_2
2'6'Dmt	D-Arg	2'6'Dmt	Lys		NH_2
2'6'Dmt	D-Arg	2'6'Dmt	Orn		NH_2
2'6'Dmt	D-Arg	2'6'Dmt	Dab		NH_2

2'6'Dmt	D-Arg	2'6'Dmt	Dap	NH_2
3'5'Dmt	D-Arg	3'5'Dmt	Arg	NH_2
3'5'Dmt	D-Arg	3'5'Dmt	Lys	NH_2
3'5'Dmt	D-Arg	3'5'Dmt	Orn	NH_2
3'5'Dmt	D-Arg	3'5'Dmt	Dab	NH_2
Tyr	D-Lys	Phe	Dap	NH_2
Tyr	D-Lys	Phe	Arg	NH_2
Tyr	D-Lys	Phe	Lys	NH_2
Tyr	D-Lys	Phe	Orn	NH_2
2'6'Dmt	D-Lys	Phe	Dab	NH_2
2'6'Dmt	D-Lys	Phe	Dap	NH_2
2'6'Dmt	D-Lys	Phe	Arg	NH_2
2'6'Dmt	D-Lys	Phe	Lys	NH_2
3'5'Dmt	D-Lys	Phe	Orn	NH_2
3'5'Dmt	D-Lys	Phe	Dab	NH_2
3'5'Dmt	D-Lys	Phe	Dap	NH_2
3'5'Dmt	D-Lys	Phe	Arg	NH_2
Tyr	D-Lys	Tyr	Lys	NH_2
Tyr	D-Lys	Tyr	Orn	NH_2
Tyr	D-Lys	Tyr	Dab	NH_2
Tyr	D-Lys	Tyr	Dap	NH_2
2'6'Dmt	D-Lys	Tyr	Lys	NH_2
2'6'Dmt	D-Lys	Tyr	Orn	NH_2
2'6'Dmt	D-Lys	Tyr	Dab	NH_2
2'6'Dmt	D-Lys	Tyr	Dap	NH_2
2'6'Dmt	D-Lys	2'6'Dmt	Lys	NH_2
2'6'Dmt	D-Lys	2'6'Dmt	Orn	NH_2
2'6'Dmt	D-Lys	2'6'Dmt	Dab	NH_2
2'6'Dmt	D-Lys	2'6'Dmt	Dap	NH_2
2'6'Dmt	D-Arg	Phe	dnsDap	NH_2
2'6'Dmt	D-Arg	Phe	atnDap	NH_2
3'5'Dmt	D-Lys	3'5'Dmt	Lys	NH_2
3'5'Dmt	D-Lys	3'5'Dmt	Orn	NH_2
3'5'Dmt	D-Lys	3'5'Dmt	Dab	NH_2
3'5'Dmt	D-Lys	3'5'Dmt	Dap	NH_2
Tyr	D-Lys	Phe	Arg	NH_2
Tyr	D-Orn	Phe	Arg	NH_2
Tyr	D-Dab	Phe	Arg	NH_2
Tyr	D-Dap	Phe	Arg	NH_2
2'6'Dmt	D-Arg	Phe	Arg	NH_2
2'6'Dmt	D-Lys	Phe	Arg	NH_2
2'6'Dmt	D-Orn	Phe	Arg	NH_2
2'6'Dmt	D-Dab	Phe	Arg	NH_2
3'5'Dmt	D-Dap	Phe	Arg	NH_2
3'5'Dmt	D-Arg	Phe	Arg	NH_2
3'5'Dmt	D-Lys	Phe	Arg	NH_2
3'5'Dmt	D-Orn	Phe	Arg	NH_2
Tyr	D-Lys	Tyr	Arg	NH_2
Tyr	D-Orn	Tyr	Arg	NH_2
Tyr	D-Dab	Tyr	Arg	NH_2

Tyr	D-Dap	Tyr	Arg	NH_2
2'6'Dmt	D-Arg	2'6'Dmt	Arg	NH_2
2'6'Dmt	D-Lys	2'6'Dmt	Arg	NH_2
2'6'Dmt	D-Orn	2'6'Dmt	Arg	NH_2
2'6'Dmt	D-Dab	2'6'Dmt	Arg	NH_2
3'5'Dmt	D-Dap	3'5'Dmt	Arg	NH_2
3'5'Dmt	D-Arg	3'5'Dmt	Arg	NH_2
3'5'Dmt	D-Lys	3'5'Dmt	Arg	NH_2
3'5'Dmt	D-Orn	3'5'Dmt	Arg	NH_2
Mmt	D-Arg	Phe	Lys	NH_2
Mmt	D-Arg	Phe	Orn	NH_2
Mmt	D-Arg	Phe	Dab	NH_2
Mmt	D-Arg	Phe	Dap	NH_2
Tmt	D-Arg	Phe	Lys	NH_2
Tmt	D-Arg	Phe	Orn	NH_2
Tmt	D-Arg	Phe	Dab	NH_2
Tmt	D-Arg	Phe	Dap	NH_2
Hmt	D-Arg	Phe	Lys	NH_2
Hmt	D-Arg	Phe	Orn	NH_2
Hmt	D-Arg	Phe	Dab	NH_2
Hmt	D-Arg	Phe	Dap	NH_2
Mmt	D-Lys	Phe	Lys	NH_2
Mmt	D-Lys	Phe	Orn	NH_2
Mmt	D-Lys	Phe	Dab	NH_2
Mmt	D-Lys	Phe	Dap	NH_2
Mmt	D-Lys	Phe	Arg	NH_2
Tmt	D-Lys	Phe	Lys	NH_2
Tmt	D-Lys	Phe	Orn	NH_2
Tmt	D-Lys	Phe	Dab	NH_2
Tmt	D-Lys	Phe	Dap	NH_2
Tmt	D-Lys	Phe	Arg	NH_2
Hmt	D-Lys	Phe	Lys	NH_2
Hmt	D-Lys	Phe	Orn	NH_2
Hmt	D-Lys	Phe	Dab	NH_2
Hmt	D-Lys	Phe	Dap	NH_2
Hmt	D-Lys	Phe	Arg	NH_2
Mmt	D-Lys	Phe	Arg	NH_2
Mmt	D-Orn	Phe	Arg	NH_2
Mmt	D-Dab	Phe	Arg	NH_2
Mmt	D-Dap	Phe	Arg	NH_2
Mmt	D-Arg	Phe	Arg	NH_2
Tmt	D-Lys	Phe	Arg	NH_2
Tmt	D-Orn	Phe	Arg	NH_2
Tmt	D-Dab	Phe	Arg	NH_2
Tmt	D-Dap	Phe	Arg	NH_2
Tmt	D-Arg	Phe	Arg	NH_2
Hmt	D-Lys	Phe	Arg	NH_2
Hmt	D-Orn	Phe	Arg	NH_2
Hmt	D-Dab	Phe	Arg	NH_2
Hmt	D-Dap	Phe	Arg	NH_2

Hmt D-Arg Phe Arg NH_2

Dab = diaminobutyric

Dap = diaminopropionic acid

Dmt = dimethyltyrosine

Mmt = 2'-methyltyrosine

Tmt = N, 2',6'-trimethyltyrosine

Hmt = 2'-hydroxy,6'-methyltyrosine

dnsDap = β-dansyl-L-α,β-diaminopropionic acid

atnDap = β-anthraniloyl-L-α,β-diaminopropionic acid

Bio = biotin

[0072] Examples of analogs useful in the practice of the present invention that do not activate mu-opioid receptors include, but are not limited to, the aromatic-cationic peptides shown in Table 2.

TABLE 2

Amino Acid Position 1	Amino Acid Position 2	Amino Acid Position 3	Amino Acid Position 4	C-Terminal Modification
D-Arg	Dmt	Lys	Phe	NH_2
D-Arg	Dmt	Phe	Lys	NH_2
D-Arg	Phe	Lys	Dmt	NH_2
D-Arg	Phe	Dmt	Lys	NH_2
D-Arg	Lys	Dmt	Phe	NH_2
D-Arg	Lys	Phe	Dmt	NH_2
Phe	Lys	Dmt	D-Arg	NH_2
Phe	Lys	D-Arg	Dmt	NH_2
Phe	D-Arg	Dmt	Lys	NH_2
Phe	D-Arg	Lys	Dmt	NH_2
Phe	Dmt	D-Arg	Lys	NH_2
Phe	Dmt	Lys	D-Arg	NH_2
Lys	Phe	D-Arg	Dmt	NH_2
Lys	Phe	Dmt	D-Arg	NH_2
Lys	Dmt	D-Arg	Phe	NH_2
Lys	Dmt	Phe	D-Arg	NH_2
Lys	D-Arg	Phe	Dmt	NH_2
Lys	D-Arg	Dmt	Phe	NH_2
D-Arg	Dmt	D-Arg	Phe	NH_2
D-Arg	Dmt	D-Arg	Dmt	NH_2

D-Arg	Dmt	D-Arg	Tyr	NH_2
D-Arg	Dmt	D-Arg	Trp	NH_2
Trp	D-Arg	Phe	Lys	NH_2
Trp	D-Arg	Tyr	Lys	NH_2
Trp	D-Arg	Trp	Lys	NH_2
Trp	D-Arg	Dmt	Lys	NH_2
D-Arg	Trp	Lys	Phe	NH_2
D-Arg	Trp	Phe	Lys	NH_2
D-Arg	Trp	Lys	Dmt	NH_2
D-Arg	Trp	Dmt	Lys	NH_2
D-Arg	Lys	Trp	Phe	NH_2
D-Arg	Lys	Trp	Dmt	NH_2
Cha	D-Arg	Phe	Lys	NH_2
Ala	D-Arg	Phe	Lys	NH_2

Cha = cyclohexyl

[0073] The amino acids of the peptides shown in table 1 and 2 may be in either the L- or the D- configuration.

Methods of Treating

[0074] The peptides described above are useful in treating any disease or condition that is associated with MPT. Such diseases and conditions include, but are not limited to, ischemia and/or reperfusion of a tissue or organ, hypoxia and any of a number of neurodegenerative diseases. Mammals in need of treatment or prevention of MPT are those mammals suffering from these diseases or conditions.

[0075] Ischemia in a tissue or organ of a mammal is a multifaceted pathological condition which is caused by oxygen deprivation (hypoxia) and/or glucose (e.g., substrate) deprivation. Oxygen and/or glucose deprivation in cells of a tissue or organ leads to a reduction or total loss of energy generating capacity and consequent loss of function of active ion transport across the cell membranes. Oxygen and/or glucose deprivation also leads to pathological changes in other cell membranes, including permeability transition in the mitochondrial membranes. In addition other molecules, such as apoptotic proteins normally compartmentalized within the mitochondria, may leak out into the cytoplasm and cause apoptotic cell death. Profound ischemia can lead to necrotic cell death.

[0076] Ischemia or hypoxia in a particular tissue or organ may be caused by a loss or severe reduction in blood supply to the tissue or organ. The loss or severe reduction in blood supply may, for example, be due to thromboembolic stroke, coronary atherosclerosis, or peripheral vascular disease. The tissue affected by ischemia or hypoxia is typically muscle, such as cardiac, skeletal, or smooth muscle.

[0077] The organ affected by ischemia or hypoxia may be any organ that is subject to ischemia or hypoxia. Examples of organs affected by ischemia or hypoxia include brain, heart, kidney, and prostate. For instance, cardiac muscle ischemia or hypoxia is commonly caused by atherosclerotic or thrombotic blockages which lead to the reduction or loss of oxygen delivery to the cardiac tissues by the cardiac arterial and capillary blood supply. Such cardiac ischemia or hypoxia may cause pain and necrosis of the affected cardiac muscle, and ultimately may lead to cardiac failure.

[0078] Ischemia or hypoxia in skeletal muscle or smooth muscle may arise from similar causes. For example, ischemia or hypoxia in intestinal smooth muscle or skeletal muscle of the limbs may also be caused by atherosclerotic or thrombotic blockages.

[0079] Reperfusion is the restoration of blood flow to any organ or tissue in which the flow of blood is decreased or blocked. For example, blood flow can be restored to any organ or tissue affected by ischemia or hypoxia. The restoration of blood flow (reperfusion) can occur by any method known to those in the art. For instance, reperfusion of ischemic cardiac tissues may arise from angioplasty, coronary artery bypass graft, or the use of thrombolytic drugs.

[0080] The methods of the present invention can also be used in the treatment or prophylaxis of neurodegenerative diseases associated with MPT. Neurodegenerative diseases associated with MPT include, for instance, Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic Lateral Sclerosis (ALS, also known as Lou Gherig's disease). The methods of the present invention can be used to delay the onset or slow the progression of these and other neurodegenerative diseases associated with MPT. The methods of the present invention are particularly useful in the treatment

of humans suffering from the early stages of neurodegenerative diseases associated with MPT and in humans predisposed to these diseases.

[0081] The peptides useful in the present invention may also be used in preserving an organ of a mammal prior to transplantation. For example, a removed organ can be susceptible to MPT due to lack of blood flow. Therefore, the peptides can be used to prevent MPT in the removed organ.

[0082] The removed organ can be placed in a standard buffered solution, such as those commonly used in the art. For example, a removed heart can be placed in a cardioplegic solution containing the peptides described above. The concentration of peptides in the standard buffered solution can be easily determined by those skilled in the art. Such concentrations may be, for example, between about 0.1 nM to about 10 μM, preferably about 1 μM to about 10 μM.

[0083] The peptides may also be administered to a mammal taking a drug to treat a condition or disease. If a side effect of the drug includes MPT, mammals taking such drugs would greatly benefit from the peptides of the invention.

[0084] An example of a drug which induces cell toxicity by effecting MPT is the chemotherapy drug Adriamycin.

Synthesis of the Peptides

[0085] The peptides useful in the methods of the present invention may be chemically synthesized by any of the methods well known in the art. Suitable methods for synthesizing the protein include, for example those described by Stuart and Young in "Solid Phase Peptide Synthesis," Second Edition, Pierce Chemical Company (1984), and in "Solid Phase Peptide Synthesis," Methods Enzymol. 289, Academic Press, Inc, New York (1997).

Modes of Administration

[0086] The peptide useful in the methods of the present invention is administered to a mammal in an amount effective in reducing the number of mitochondria undergoing, or preventing, MPT. The effective amount is determined during pre-clinical trials and clinical trials by methods familiar to physicians and clinicians.

[0087] An effective amount of a peptide useful in the methods of the present invention, preferably in a pharmaceutical composition, may be administered to a mammal in need thereof by any of a number of well-known methods for administering pharmaceutical compounds.

[0088] The peptide may be administered systemically or locally. In one embodiment, the peptide is administered intravenously. For example, the aromatic-cationic peptides useful in the methods of the present invention may be administered via rapid intravenous bolus injection. Preferably, however, the peptide is administered as a constant rate intravenous infusion.

[0089] The peptide can be injected directly into coronary artery during, for example, angioplasty or coronary bypass surgery, or applied onto coronary stents.

[0090] The peptide may also be administered orally, topically, intranasally, intramuscularly, subcutaneously, or transdermally. In a preferred embodiment, transdermal administration of the aromatic-cationic peptides by methods of the present invention is by iontophoresis, in which the charged peptide is delivered across the skin by an electric current.

[0091] Other routes of administration include intracerebroventricularly or intrathecally. Intracerebroventiculatly refers to administration into the ventricular system of the brain. Intrathecally refers to administration into the space under the arachnoid membrane of the spinal cord. Thus intracerebroventricular or intrathecal administration may be preferred for those diseases and conditions which affect the organs or tissues of the central nervous system. In a preferred embodiment, intrathecal administration is used for traumatic spinal cord injury.

[0092] The peptides useful in the methods of the invention may also be administered to mammals by sustained release, as is known in the art. Sustained release administration is a method of drug delivery to achieve a certain level of the drug over a particular period of time. The level typically is measured by serum or plasma concentration.

[0093] Any formulation known in the art of pharmacy is suitable for administration of the aromatic-cationic peptides useful in the methods of the present invention. For oral administration, liquid or solid formulations may be used. Some examples of formulations include tablets, gelatin capsules, pills, troches, elixirs, suspensions, syrups, wafers, chewing gum and the like. The peptides can be mixed with a suitable pharmaceutical carrier (vehicle) or excipient as understood by practitioners in the art. Examples of carriers and excipients include starch, milk, sugar, certain types of clay, gelatin, lactic acid, stearic acid or salts thereof, including magnesium or calcium stearate, talc, vegetable fats or oils, gums and glycols.

[0094] For systemic, intracerebroventricular, intrathecal, topical, intranasal, subcutaneous, or transdermal administration, formulations of the aromatic-cationic peptides useful in the methods of the present inventions may utilize conventional diluents, carriers, or excipients etc., such as are known in the art can be employed to deliver the peptides. For example, the formulations may comprise one or more of the following: a stabilizer, a surfactant, preferably a nonionic surfactant, and optionally a salt and/or a buffering agent. The peptide may be delivered in the form of an aqueous solution, or in a lyophilized form.

[0095] The stabilizer may, for example, be an amino acid, such as for instance, glycine; or an oligosaccharide, such as for example, sucrose, tetralose, lactose or a dextran. Alternatively, the stabilizer may be a sugar alcohol, such as for instance, mannitol; or a combination thereof. Preferably the stabilizer or combination of stabilizers constitutes from about 0.1% to about 10% weight for weight of the peptide.

[0096] The surfactant is preferably a nonionic surfactant, such as a polysorbate. Some examples of suitable surfactants include Tween™ 20, Tween™ 80; a polyethylene glycol

or a polyoxyethylene polyoxypropylene glycol, such as Pluronic™ F-68 at from about 0.001% (w/v) to about 10% (w/v).

[0097] The salt or buffering agent may be any salt or buffering agent, such as for example, sodium chloride, or sodium/potassium phosphate, respectively. Preferably, the buffering agent maintains the pH of the pharmaceutical composition in the range of about 5.5 to about 7.5. The salt and/or buffering agent is also useful to maintain the osmolality at a level suitable for administration to a human or an animal. Preferably the salt or buffering agent is present at a roughly isotonic concentration of about 150mM to about 300mM.

[0098] The formulations of the peptides useful in the methods of the present invention may additionally contain one or more conventional additive. Some examples of such additives include a solubilizer such as, for example, glycerol; an antioxidant such as for example, benzalkonium chloride (a mixture of quaternary ammonium compounds, known as "quats"), benzyl alcohol, chloretone or chlorobutanol; anaesthetic agent such as for example a morphine derivative; or an isotonic agent etc., such as described above. As a further precaution against oxidation or other spoilage, the pharmaceutical compositions may be stored under nitrogen gas in vials sealed with impermeable stoppers.

[0099] The mammal can be any mammal, including, for example, farm animals, such as sheep, pigs, cows, and horses; pet animals, such as dogs and cats; laboratory animals, such as rats, mice and rabbits. In a preferred embodiment, the mammal is a human.

EXAMPLES

Example 1: [Dmt^1]DALDA penetrates cell membrane.

[0100] The cellular uptake of [^{3}H][Dmt^1]DALDA was studied using a human intestinal epithelial cell line (Caco-2), and confirmed with SH-SY5Y (human neuroblastoma cell), HEK293 (human embryonic kidney cell) and CRFK cells (kidney epithelial cell). Monolayers of cells were grown on 12-well plates ($5x10^5$ cells/well) coated with collagen for 3 days. On day 4, cells were washed twice with pre-warmed HBSS, and then incubated with 0.2 ml of HBSS containing either 250nM [^{3}H][Dmt^1]DALDA at 37°C or 4°C for various times up to 1 h.

[0101] [^{3}H][Dmt^1]DALDA was observed in cell lysate as early as 5 min, and steady state levels were achieved by 30 min. The total amount of [^{3}H][Dmt^1]DALDA recovered in the cell lysate after 1 h incubation represented about 1% of the total drug. The uptake of [^{3}H][Dmt^1]DALDA was slower at 4°C compared to 37°C, but reached 76.5% by 45 min and 86.3% by 1 h. The internalization of [^{3}H][Dmt^1]DALDA was not limited to Caco-2 cells, but was also observed in SH-SY5Y, HEK293 and CRFK cells. The intracellular concentration of [Dmt^1]DALDA was estimated to be approximately 50 times higher than extracellular concentration.

[0102] In a separate experiment, cells were incubated with a range of [Dmt^1]DALDA concentrations (1 μM – 3 mM) for 1 h at 37°C. At the end of the incubation period, cells were washed 4 times with HBSS, and 0.2ml of 0.1N NaOH with 1% SDS was added to each well. The cell contents were then transferred to scintillation vials and radioactivity counted. To distinguish between internalized radioactivity from surface-associated radioactivity, an acid-wash step was included. Prior to cell lysis, cells were incubated with 0.2ml of 0.2 M acetic acid / 0.05 M NaCl for 5 min on ice.

[0103] The uptake of [Dmt^1]DALDA into Caco-2 cells was confirmed by confocal laser scanning microscopy (CLSM) using a fluorescent analog of

[Dmt^1]DALDA (Dmt-D-Arg-Phe-dnsDap-NH_2; where dnsDap = β-dansyl-l-α,β-diaminopropionic acid). Cells were grown as described above and were plated on (35 mm) glass bottom dishes (MatTek Corp., Ashland, MA) for 2 days. The medium was then removed and cells were incubated with 1 ml of HBSS containing 0.1 μM to 1.0 μM of the fluorescent peptide analog at 37°C for 1 h. Cells were then washed three times with ice-cold HBSS and covered with 200 μl of PBS, and microscopy was performed within 10 min at room temperature using a Nikon™ confocal laser scanning microscope with a C-Apochromat 63x/1.2W corr objective. Excitation was performed at 340 nm by means of a UV laser, and emission was measured at 520 nm. For optical sectioning in z-direction, 5-10 frames with 2.0 μm were made.

[0104] CLSM confirmed the uptake of fluorescent Dmt-D-Arg-Phe-dnsDap-NH_2 into Caco-2 cells after incubation with 0.1 μM [Dmt^1,DnsDap^4]DALDA for 1h at 37°C. The uptake of the fluorescent peptide was similar at 37°C and 4°C. The fluorescence appeared diffuse throughout the cytoplasm but was completely excluded from the nucleus.

Example 2: Targeting of [Dmt^1]DALDA to mitochondria.

[0105] To examine the subcellular distribution of [Dmt^1]DALDA, the fluorescent analog, [Dmt^1,AtnDap^4]DALDA (Dmt-D-Arg-Phe-atnDap-NH_2; where atn = β-anthraniloyl-l-α,β-diamino-propionic acid), was prepared. The analog contained β-anthraniloyl-l-α,β-diaminopropionic acid in place of the lysine reside at position 4. The cells were grown as described in Example 1 and were plated on (35 mm) glass bottom dishes (MatTek Corp., Ashland, MA) for 2 days. The medium was then removed and cells were incubated with 1 ml of HBSS containing 0.1 μM of [Dmt^1,AtnDap^4]DALDA at 37°C for 15 min to 1 h.

[0106] Cells were also incubated with tetramethylrhodamine methyl ester (TMRM, 25 nM), a dye for staining mitochondria, for 15 min at 37°C. Cells were then washed three times with ice-cold HBSS and covered with 200 μl of PBS, and microscopy

was performed within 10 min at room temperature using a Nikon confocal laser scanning microscope with a C-Apochromat 63x/1.2W corr objective.

[0107] For [Dmt^1,AtnDap^4]DALDA, excitation was performed at 350 nm by means of a UV laser, and emission was measured at 520 nm. For TMRM, excitation was performed at 536 nm, and emission was measured at 560 nm.

[0108] CLSM showed the uptake of fluorescent [Dmt^1,AtnDap^4]DALDA into Caco-2 cells after incubation for as little as 15 min at 37°C. The uptake of dye was completely excluded from the nucleus, but the blue dye showed a streaky distribution within the cytoplasm. Mitochondria were labeled red with TMRM. The distribution of [Dmt^1,AtnDap^4]DALDA to mitochondria was demonstrated by the overlap of the [Dmt^1,AtnDap^4]DALDA distribution and the TMRM distribution.

Example 3: Uptake of [Dmt^1]DALDA into mitochondria.

[0109] To isolate mitochondria from mouse liver, mice were sacrificed by decapitation. The liver was removed and rapidly placed into chilled liver homogenization medium. The liver was finely minced using scissors and then homogenized by hand using a glass homogenizer.

[0110] The homogenate was centrifuged for 10 min at 1000xg at 4°C. The supernatant was aspirated and transferred to polycarbonate tubes and centrifuged again for 10 min at 3000xg, 4°C. The resulting supernatant was removed, and the fatty lipids on the side-wall of the tube were carefully wiped off.

[0111] The pellet was resuspended in liver homogenate medium and the homogenization repeated twice. The final purified mitochondrial pellet was resuspended in medium. Protein concentration in the mitochondrial preparation was determined by the Bradford procedure.

[0112] Approximately 1.5 mg mitochondria in 400 µl buffer was incubated with [^{3}H][Dmt^1]DALDA for 5-30 min at 37°C. The mitochondria were then centrifuged down and the amount of radioactivity determined in the mitochondrial fraction and buffer fraction. Assuming a mitochondrial matrix volume of 0.7 µl/mg protein (Lim et al., J Physiol 545:961-974, 2002), the concentration of [^{3}H][Dmt^1]DALDA in mitochondria was found to be 200 times higher than in the buffer. Thus [Dmt^1]DALDA is concentrated in mitochondria.

[0113] Based on these data, the concentration of [Dmt^1]DALDA in mitochondria when the isolated guinea pig hearts were perfused with [Dmt^1]DALDA can be estimated:

Concentration of [Dmt^1]DALDA in coronary perfusate	0.1 µM
Concentration of [Dmt^1]DALDA in myocyte	5 µM
Concentration of [Dmt^1]DALDA in mitochondria	1.0 mM

Example 4: Accumulation of [Dmt^1]DALDA by isolated mitochondria (Fig. 1)

[0114] To further demonstrate that [Dmt^1]DALDA is selectively distributed to mitochondria, we examined the uptake of [Dmt^1,AtnDap^4]DALDA and [^{3}H][Dmt^1]DALDA into isolated mouse liver mitochondria. The rapid uptake of [Dmt^1,AtnDap^4]DALDA was observed as immediate quenching of its fluorescence upon addition of mitochondria (Figure 1A). Pretreatment of mitochondria with FCCP (carbonyl cyanide *p*-(trifluoromethoxy)-phenylhydrazone), an uncoupler that results in immediate depolarization of mitochondria, only reduced [Dmt^1,AtnDap^4]DALDA uptake by <20%. Thus uptake of [Dmt^1,AtnDap^4]DALDA was not potential-dependent.

[0115] To confirm that the mitochondrial targeting was not an artifact of the fluorophore, we also examined mitochondrial uptake of [^{3}H][Dmt^1]DALDA. Isolated mitochondria were incubated with [^{3}H][Dmt^1]DALDA and radioactivity determined in the mitochondrial pellet and supernatant. The amount of radioactivity in the pellet did not change from 2 min to 8 min. Treatment of mitochondria with FCCP only decreased

the amount of [^{3}H][Dmt^1]DALDA associated with the mitochondrial pellet by ~20% (Figure 1B).

[0116] The minimal effect of FCCP on [Dmt^1]DALDA uptake suggested that [Dmt^1]DALDA was likely to be associated with mitochondrial membranes or in the intermembrane space rather than in the matrix. We next examined the effect of mitochondrial swelling on the accumulation of [Dmt^1,AtnDap^4]DALDA in mitochondria by using alamethicin to induce swelling and rupture of the outer membrane. Unlike TMRM, the uptake of [Dmt^1,AtnDap^4]DALDA was only partially reversed by mitochondrial swelling (Fig. 1C). Thus, [Dmt^1]DALDA is associated with mitochondrial membranes.

Example 5: [Dmt^1]DALDA does not alter mitochondrial respiration or potential (Fig. 1D)

[0117] The accumulation of [Dmt^1]DALDA in mitochondria did not alter mitochondrial function. Incubating isolated mouse liver mitochondria with 100 μM [Dmt^1]DALDA did not alter oxygen consumption during state 3 or state 4, or the respiratory ratio (state 3/state 4) (6.2 versus 6.0). Mitochondrial membrane potential was measured using TMRM (Fig. 1D) Addition of mitochondria resulted in immediate quenching of the TMRM signal which was readily reversed by the addition of FCCP, indicating mitochondrial depolarization. The addition of Ca^{2+} (150 μM) resulted in immediate depolarization followed by progressive loss of quenching indicative of MPT. Addition of [Dmt^1]DALDA alone, even at 200 μM, did not cause mitochondrial depolarization or MPT.

Example 6: [Dmt^1]DALDA protects against MPT induced by Ca^{2+} and 3-nitropropionic acid. (Fig. 2)

[0118] In addition to having no direct effect on mitochondrial potential, [Dmt^1]DALDA was able to protect against MPT induced by Ca^{2+} overload. Pretreatment of isolated mitochondria with [Dmt^1]DALDA (10 μM) for 2 min prior to addition of Ca^{2+} resulted only in transient depolarization and prevented onset of MPT (Figure 2A). [Dmt^1]DALDA dose-dependently increased the tolerance of mitochondria to cumulative Ca^{2+} challenges. Figure 2B shows that [Dmt^1]DALDA increased the number of Ca^{2+} additions that isolated mitochondria could tolerate prior to MPT.

[0119] 3-Nitropropionic acid (3NP) is an irreversible inhibitor of succinate dehydrogenase in complex II of the electron transport chain. Addition of 3NP (1 mM) to isolated mitochondria caused dissipation of mitochondrial potential and onset of MPT (Figure 2C). Pretreatment of mitochondria with [Dmt^1]DALDA dose-dependently delayed the onset of MPT induced by 3NP (Figure 2C).

[0120] To demonstrate that [Dmt^1]DALDA can penetrate cell membranes and protect against mitochondrial depolarization elicited by 3NP, Caco-2 cells were treated with 3NP (10 mM) in the absence or presence of [Dmt^1]DALDA (0.1 μM) for 4 h, and then incubated with TMRM and examined under LSCM. In control cells, the mitochondria are clearly visualized as fine streaks throughout the cytoplasm. In cells treated with 3NP, the TMRM fluorescence was much reduced, suggesting generalized depolarization. In contrast, concurrent treatment with [Dmt^1]DALDA protected against mitochondrial depolarization caused by 3NP.

Example 7: [Dmt^1]DALDA protects against mitochondrial swelling and cytochrome c release.

[0121] MPT pore opening results in mitochondrial swelling. We examined the effects of [Dmt^1]DALDA on mitochondrial swelling by measuring reduction in absorbance at 540 nm (A_{540}). The mitochondrial suspension was then centrifuged and cytochrome c in the mitochondrial pellet and supernatant determined by a commercially-available ELISA™ kit. Pretreatment of isolated mitochondria with SS-02 inhibited

swelling (Fig. 3A) and cytochrome c release (Fig. 3B) induced by Ca^{2+} overload. Besides preventing MPT induced by Ca^{2+} overload, SS-02 also prevented mitochondrial swelling induced by MPP^+ (1-methyl-4-phenylpyridium ion), an inhibitor of complex I of the mitochondrial electron transport chain (Fig. 3C).

Example 8: D-Arg-Dmt-Lys-Phe-NH_2 (SS-31) can protect against MPT, mitochondrial swelling and cytochrome c release.

[0122] The non-opioid peptide SS-31 has the same ability to protect against MPT (Fig. 4A), mitochondrial swelling (Fig. 4B), and cytochrome c release (Fig. 4C), induced by Ca^{2+}. The methods for study are as described above for SS-02. In this example, mitochondrial swelling was measured using light scattering monitored at 570 nm.

Example 9: [Dmt^1]DALDA (SS-02) and D-Arg-Dmt-Lys-Phe-NH_2 (SS-31) protects against ischemia-reperfusion-induced myocardial stunning.

[0123] Guinea pig hearts were rapidly isolated, and the aorta was cannulated in situ and perfused in a retrograde fashion with an oxygenated Krebs-Henseleit solution (pH 7.4) at 34°C. The heart was then excised, mounted on a modified Langendorff perfusion apparatus, and perfused at constant pressure (40 cm H_2O). Contractile force was measured with a small hook inserted into the apex of the left ventricle and the silk ligature tightly connected to a force-displacement transducer. Coronary flow was measured by timed collection of pulmonary artery effluent.

[0124] Hearts were perfused with buffer, [Dmt^1]DALDA (SS-02) (100 nM) or D-Arg-Dmt-Lys-Phe-NH_2 (SS-31) (1 nM) for 30 min and then subjected to 30 min of global ischemia. Reperfusion was carried out with the same solution used prior to ischemia.

[0125] Two-way ANOVA revealed significant differences in contractile force ($P<0.001$), heart rate ($P=0.003$), and coronary flow ($P<0.001$) among the three treatment groups. In the buffer group, contractile force was significantly lower during reperfusion compared with before ischemia (Fig. 5). Both SS-02 and SS-31 treated hearts tolerated ischemia much better than buffer-treated hearts (Fig. 5). In particular, SS-31 provided complete inhibition of cardiac stunning. In addition, coronary flow is well-sustained throughout reperfusion and there was no decrease in heart rate.

Example 10: [Dmt^1]DALDA (SS-02) enhances organ preservation.

[0126] For heart transplantation, the donor heart is preserved in a cardioplegic solution during transport. The preservation solution contains high potassium which effectively stops the heart from beating and conserve energy. However, the survival time of the isolated heart is still quite limited.

[0127] We examined whether [Dmt^1]DALDA prolongs survival of organs. In this study, [Dmt^1]DALDA was added to a commonly used cardioplegic solution (St. Thomas) to determine whether [Dmt^1]DALDA enhances survival of the heart after prolonged ischemia (model of *ex vivo* organ survival).

[0128] Isolated guinea pig hearts were perfused in a retrograde fashion with an oxygenated Krebs-Henseleit solution at 34°C. After 30 min. of stabilization, the hearts were perfused with a cardioplegic solution CPS (St. Tohomas) with or withour [Dmt^1]DALDA at 100 nM for 3 min. Global ischemia was then induced by complete interruption of coronary perfusion for 90 min. Reperfusion was subsequently carried out for 60 min. with oxygenated Krebs-Henseleit solution. Contractile force, heart rate and coronary flow were monitored continuously throughout the experiment.

[0129] The addition of [Dmt^1]DALDA to cardioplegic solution significantly enhanced contractile function (Fig. 6) after prolonged ischemia.

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CLAIMS:

1. An aromatic-cationic peptide for reducing the number of mitochondria undergoing mitochondrial permeability transition (MPT), or preventing mitochondrial permeability transitioning in a mammal in need thereof, wherein the aromatic cationic-peptide has the formula D-Arg-Dmt-Lys-Phe-NH_2.

2. The peptide according to claim 1, wherein the peptide is formulated for oral, topical, intranasal, systemic, intravenous, subcutaneous, intramuscular, intracerebroventricular, intrathecal, or transdermal administration.

3. The peptide according to claim 1, wherein the mammal is suffering from ischemia or reperfusion injury.

4. The peptide according to claim 3, wherein the ischemia is due to stroke, intestinal ischemia or muscle tissue ischemia.

5. The peptide according to claim 4, wherein the muscle tissue is cardiac muscle tissue, skeletal muscle tissue, or smooth muscle tissue.

6. The peptide according to claim 1, wherein the mammal is suffering from hypoxia.

7. The peptide according to claim 1, wherein the mammal is suffering from a neurodegenerative disease or condition.

8. The peptide according to claim 7, wherein the neurodegenerative disease or condition is Parkinson's disease, Alzheimer's disease, Huntington's disease, or Amyotrophic Lateral Sclerosis (ALS).

9. The peptide according to claim 1, wherein the mammal is suffering from drug-induced MPT.

10. A peptide having the sequence

D-Arg-Dmt-Lys-Phe-NH_2,

D-Arg-Dmt-Phe-Lys-NH_2,

D-Arg-Phe-Lys-Dmt-NH_2,

D-Arg-Phe-Dmt-Lys-NH_2,

D-Arg-Lys-Dmt-Phe-NH_2,

D-Arg-Lys-Phe-Dmt-NH_2,

Phe-Lys-Dmt-D-Arg-NH_2,

Phe-Lys-D-Arg-Dmt-NH_2,

Phe-D-Arg-Dmt-Lys-NH_2,

Phe-D-Arg-Lys-Dmt-NH_2,

Phe-D-Arg-Phe-Lys-NH_2,

Dmp-D-Arg-Phe-Lys-NH_2,

Phe-Dmt-D-Arg-Lys-NH_2,

Phe-Dmt-Lys-D-Arg-NH_2,

Lys-Phe-Dmt-D-Arg-NH_2,

Lys-Dmt-D-Arg-Phe-NH_2,

Lys-Dmt-Phe-D-Arg-NH_2,

Lys-D-Arg-Phe-Dmt-NH_2,

Lys-D-Arg-Dmt-Phe-NH_2,

D-Arg-Dmt-D-Arg-Phe-NH_2,

D-Arg-Dmt-D-Arg-Dmt-NH_2,

D-Arg-Dmt-D-Arg-Tyr-NH_2,

D-Arg-Dmt-D-Arg-Trp-NH_2,

Trp-D-Arg-Phe-Lys-NH_2,

Trp-D-Arg-Tyr-Lys-NH_2,

Trp-D-Arg-Trp-Lys-NH_2,

Trp-D-Arg-Dmt-Lys-NH_2,

D-Arg-Trp-Lys-Phe-NH_2,

D-Arg-Trp-Phe-Lys-NH_2,

D-Arg-Trp-Lys-Dmt-NH_2,

D-Arg-Trp-Dmt-Lys-NH_2,

D-Arg-Lys-Trp-Phe-NH_2,

D-Arg-Lys-Trp-Dmt-NH_2,

Cyclohexyl-D-Arg-Phe-Lys-NH_2, or

Ala-D-Arg-Phe-Lys-NH_2.

11. A peptide having the sequence D-Arg-Dmt-Lys-Phe-NH_2.

12. A peptide having the sequence Phe-D-Arg-Phe-Lys-NH_2.

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13. A composition comprising a peptide having the sequence

D-Arg-Dmt-Lys-Phe-NH_2,

D-Arg-Dmt-Phe-Lys-NH_2,

D-Arg-Phe-Lys-Dmt-NH_2,

D-Arg-Phe-Dmt-Lys-NH_2,

D-Arg-Lys-Dmt-Phe-NH_2,

D-Arg-Lys-Phe-Dmt-NH_2,

Phe-Lys-Dmt-D-Arg-NH_2,

Phe-Lys-D-Arg-Dmt-NH_2,

Phe-D-Arg-Dmt-Lys-NH_2,

Phe-D-Arg-Lys-Dmt-NH_2,

Phe-Dmt-D-Arg-Lys-NH_2,

Phe-Dmt-Lys-D-Arg-NH_2,

Phe-D-Arg-Phe-Lys-NH_2,

Dmp-D-Arg-Phe-Lys-NH_2

Lys-Phe-Dmt-D-Arg-NH_2,

Lys-Dmt-D-Arg-Phe-NH_2,

Lys-Dmt-Phe-D-Arg-NH_2,

Lys-D-Arg-Phe-Dmt-NH_2,

Lys-D-Arg-Dmt-Phe-NH_2,

D-Arg-Dmt-D-Arg-Phe-NH_2,

D-Arg-Dmt-D-Arg-Dmt-NH_2,

D-Arg-Dmt-D-Arg-Tyr-NH_2,

D-Arg-Dmt-D-Arg-Trp-NH_2,

Trp-D-Arg-Phe-Lys-NH_2,

Trp-D-Arg-Tyr-Lys-NH_2,

Trp-D-Arg-Trp-Lys-NH_2,

Trp-D-Arg-Dmt-Lys-NH_2,

D-Arg-Trp-Lys-Phe-NH_2,

D-Arg-Trp-Phe-Lys-NH_2,

D-Arg-Trp-Lys-Dmt-NH_2,

D-Arg-Trp-Dmt-Lys-NH_2,

D-Arg-Lys-Trp-Phe-NH_2,

D-Arg-Lys-Trp-Dmt-NH_2,

Cyclohexyl-D-Arg-Phe-Lys-NH_2, or

Ala-D-Arg-Phe-Lys-NH_2,

and a pharmaceutically acceptable carrier.

14. A composition comprising a peptide having the sequence D-Arg-Dmt-Lys-Phe-NH_2 and a pharmaceutically acceptable carrier.

15. A composition comprising a peptide having the sequence Phe-D-Arg-Phe-Lys-NH_2 and a pharmaceutically acceptable carrier.

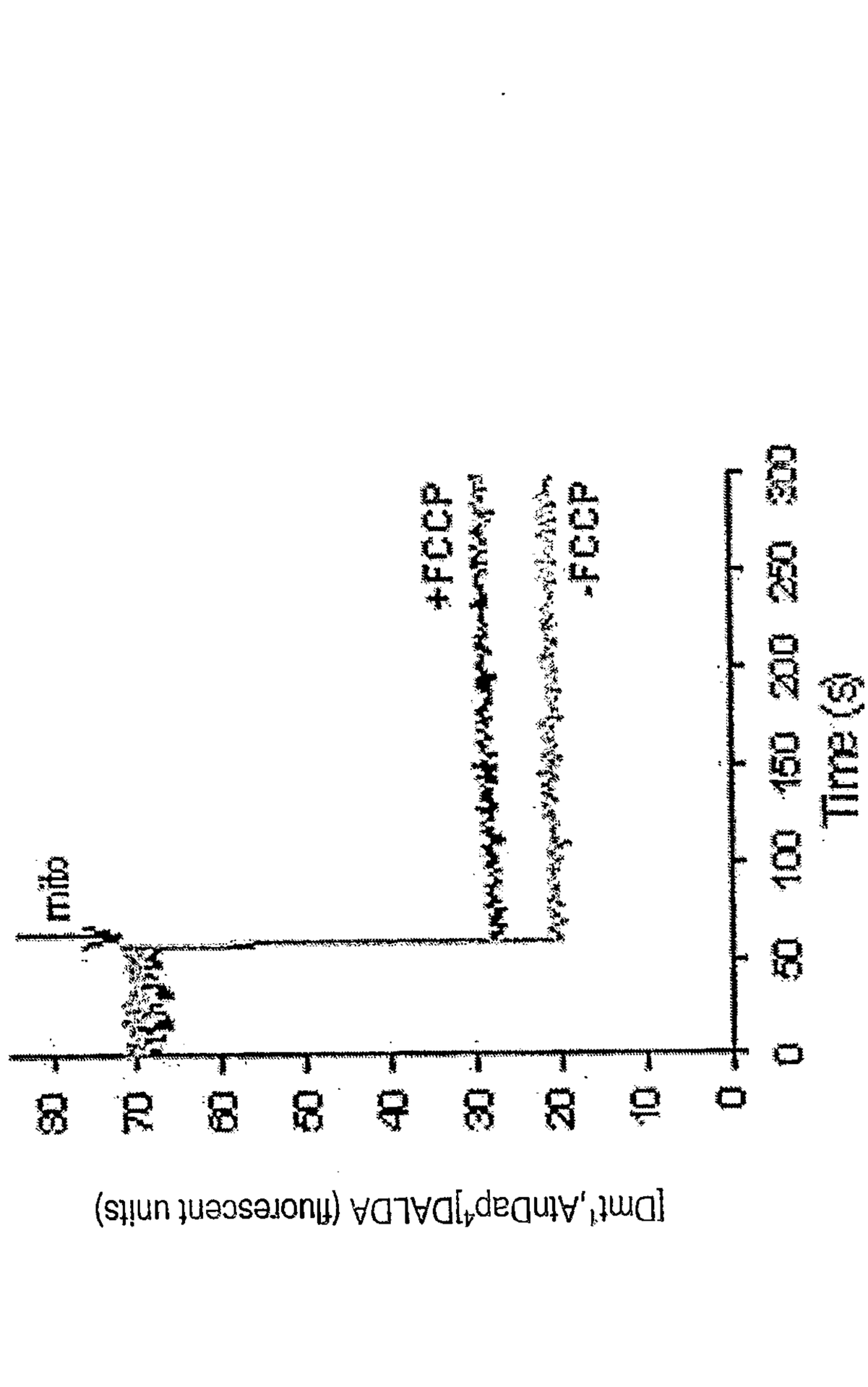

[Dmt¹,AtnDap⁴]DALDA (fluorescent units)
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (s)
mito
+FCCP
-FCCP

Figure 1A

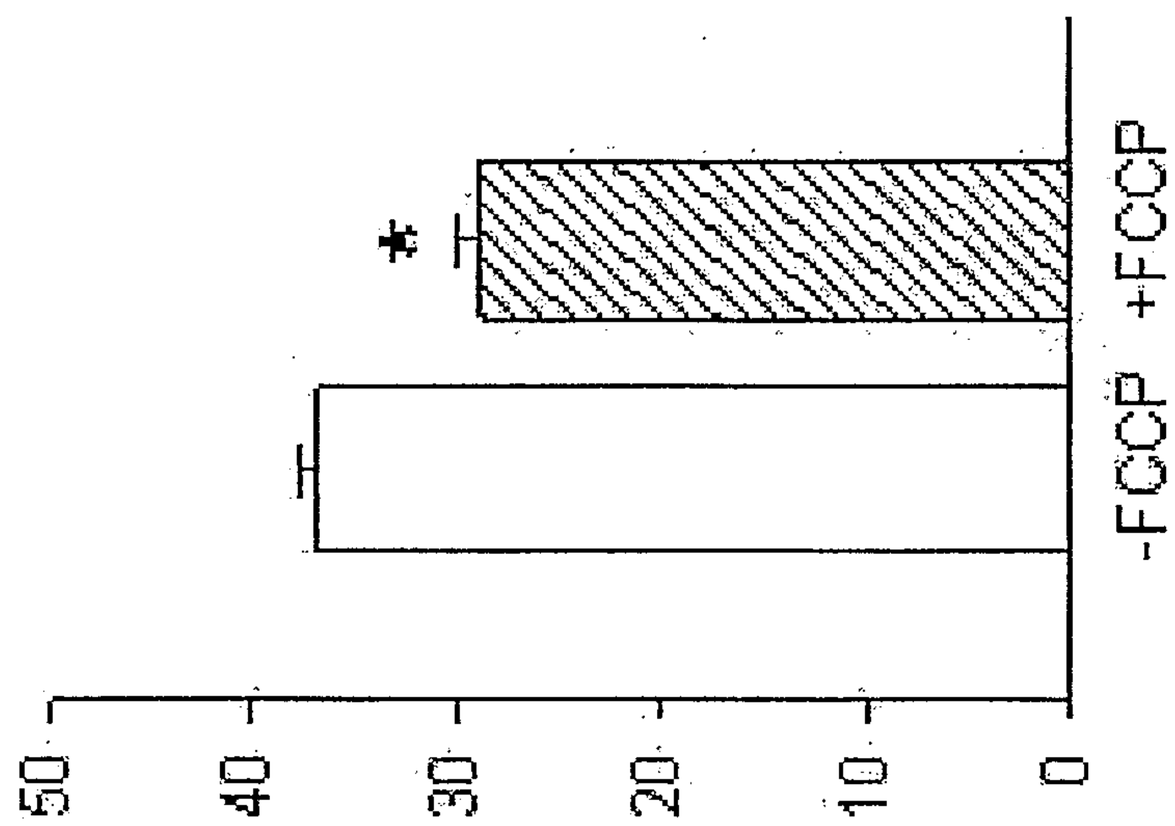
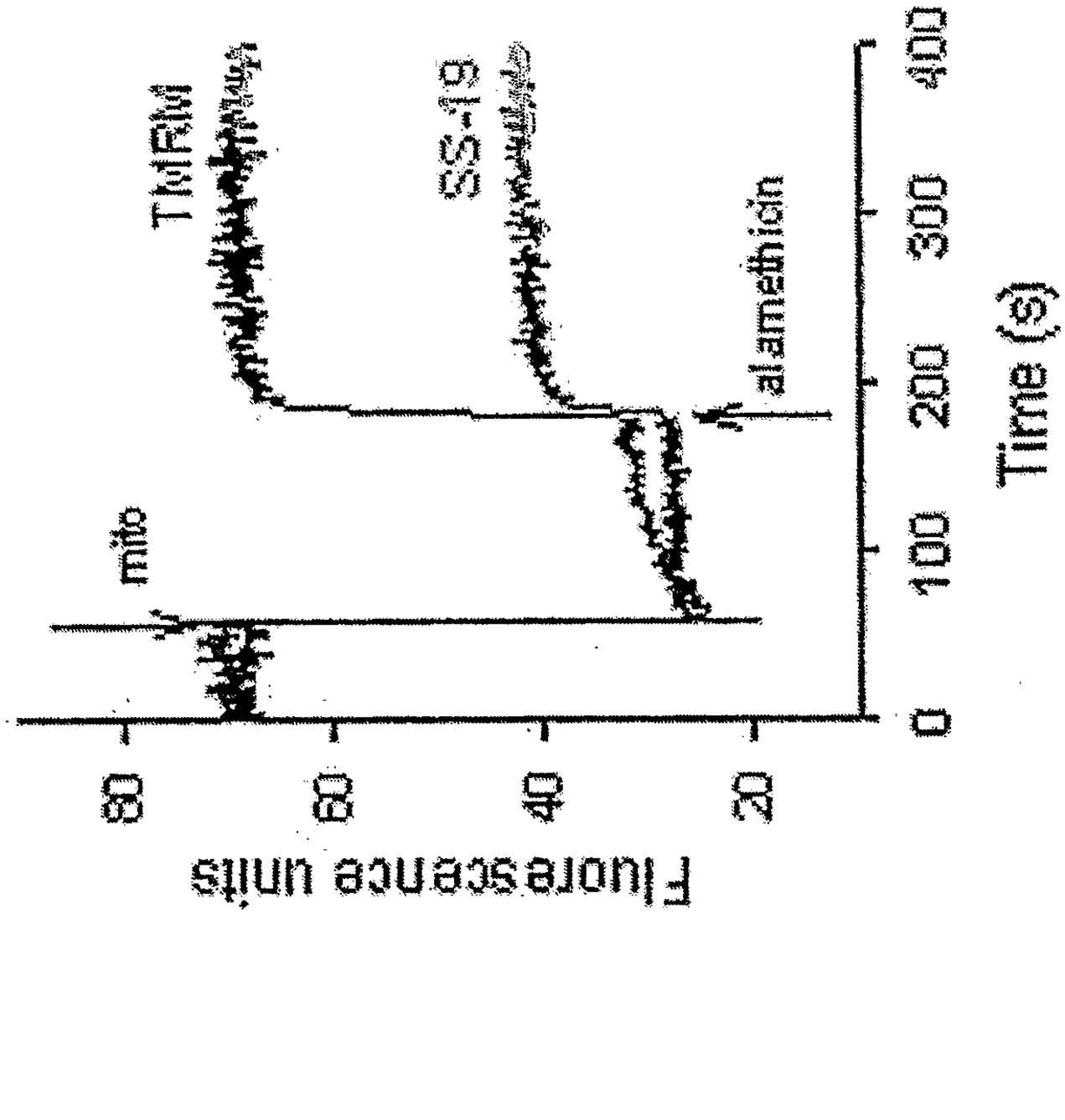


Figure 1B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SS-19
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mito
Fluorescence units
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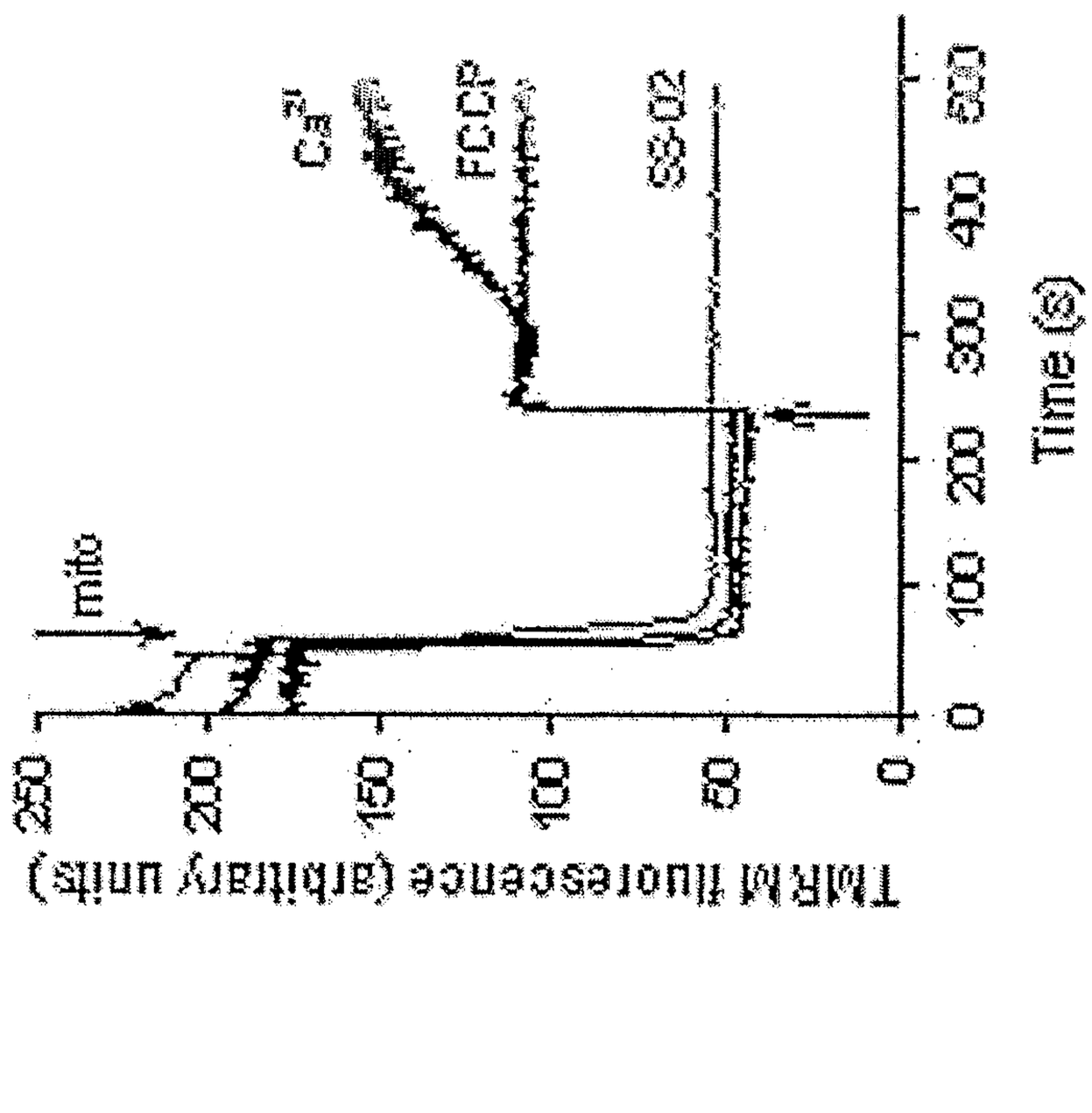
80
40
20
0
100
200
300
400
Time (s)

Figure 1C


TbRbl fluorescence (arbitrary units)
250
200
150
100
50
0
mito
0
100
200
300
400
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Time (s)
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FCCP
68-02

Figure 1D

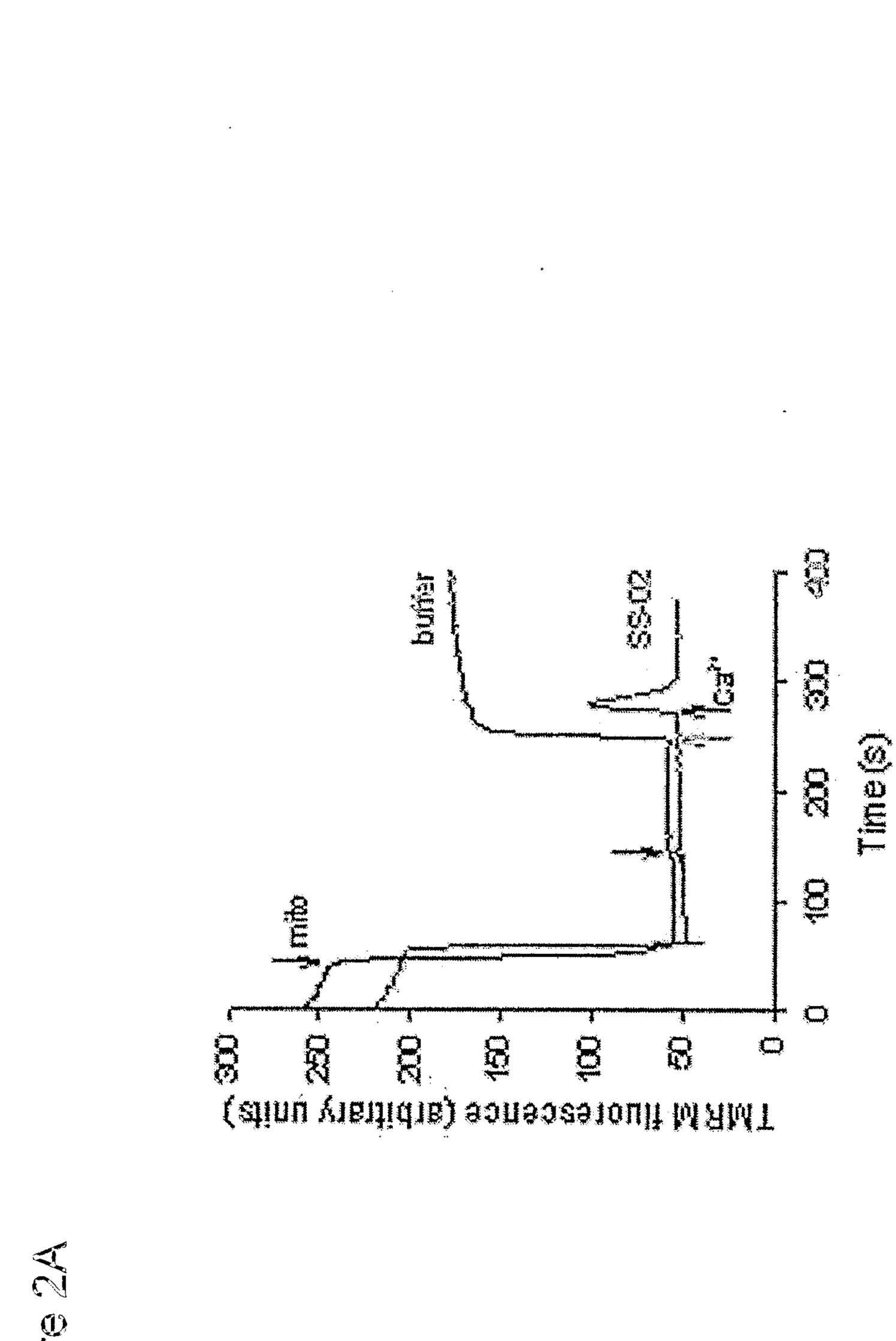
TMRM fluorescence (arbitrary units)
300
250
200
150
100
50
0
0
100
200
300
400
Time (s)
mito
buffer
SS-02
Ca2+

Figure 2A

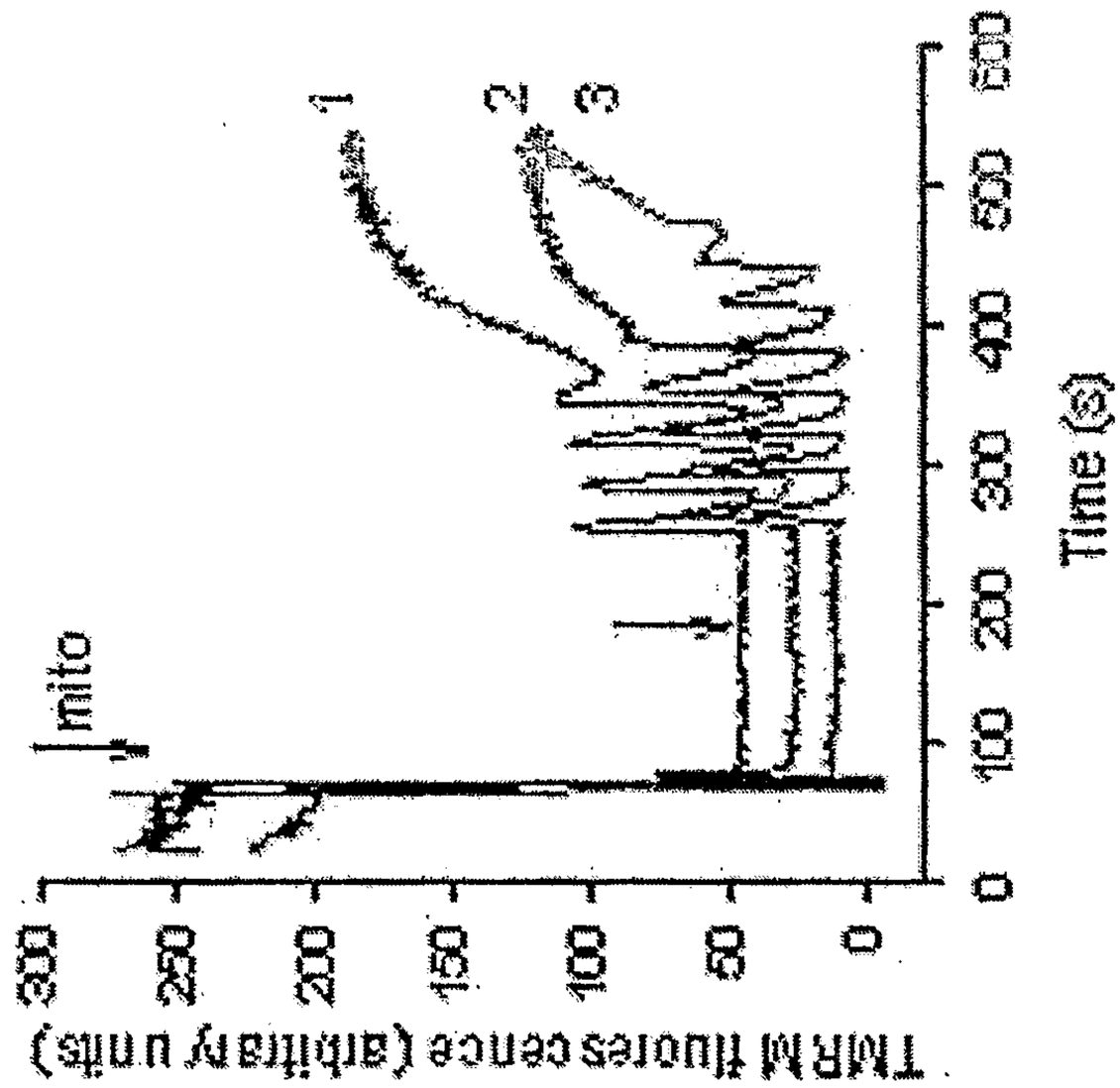
1
2
3
mito
TMRM fluorescence (arbitrary units)
300
250
200
150
100
50
0
0
100
200
300
400
500
600
Time (s)

Figure 2B

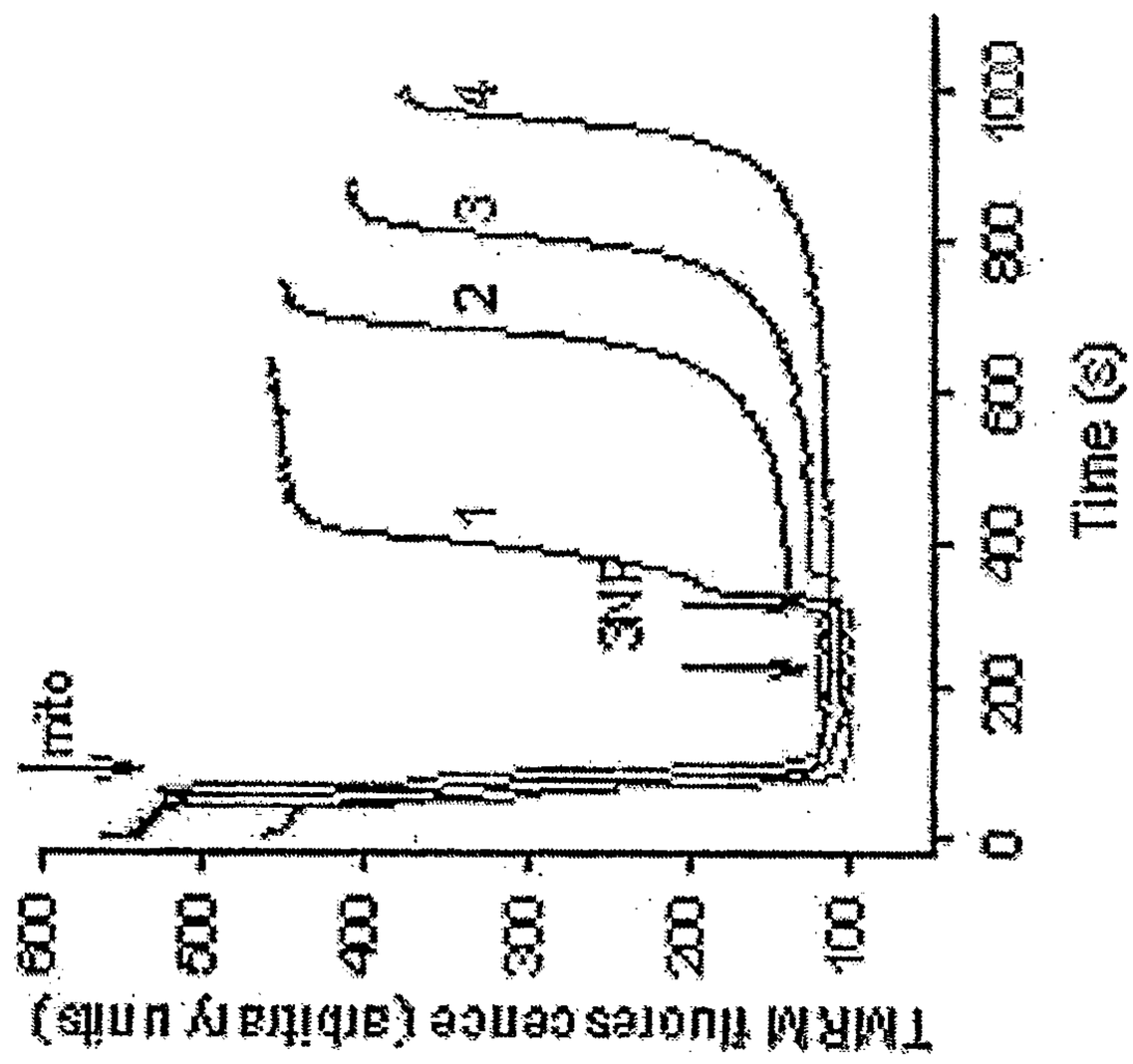
TMRM fluorescence (arbitrary units)
600
500
400
300
200
100
0
200
400
600
800
1000
Time (s)
mito
3NP
1
2
3

Figure 2C

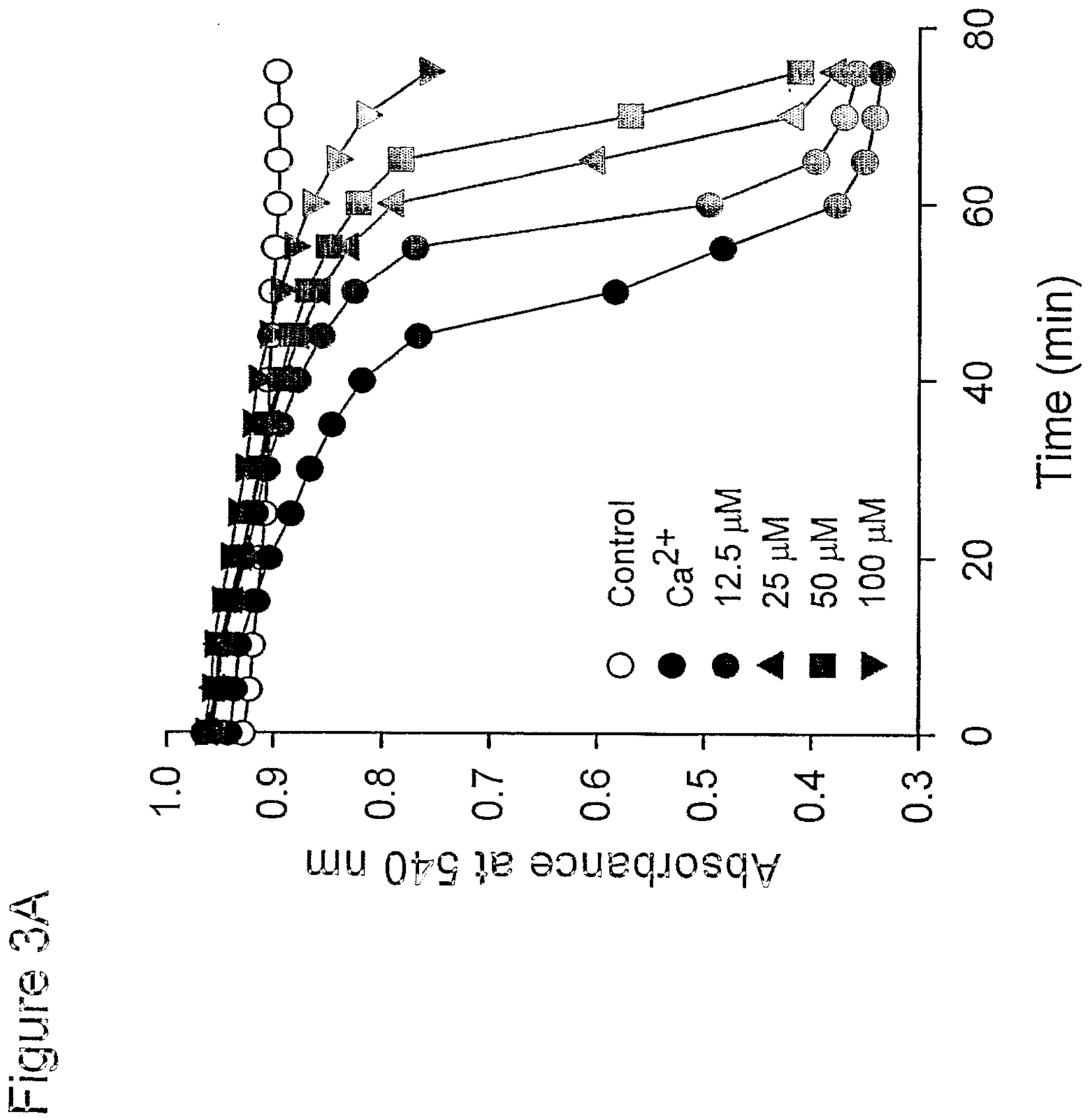
Absorbance at 540 nm
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0
20
40
60
80
Time (min)
Control
Ca2+
12.5 µM
25 µM
50 µM
100 µM

Figure 3A

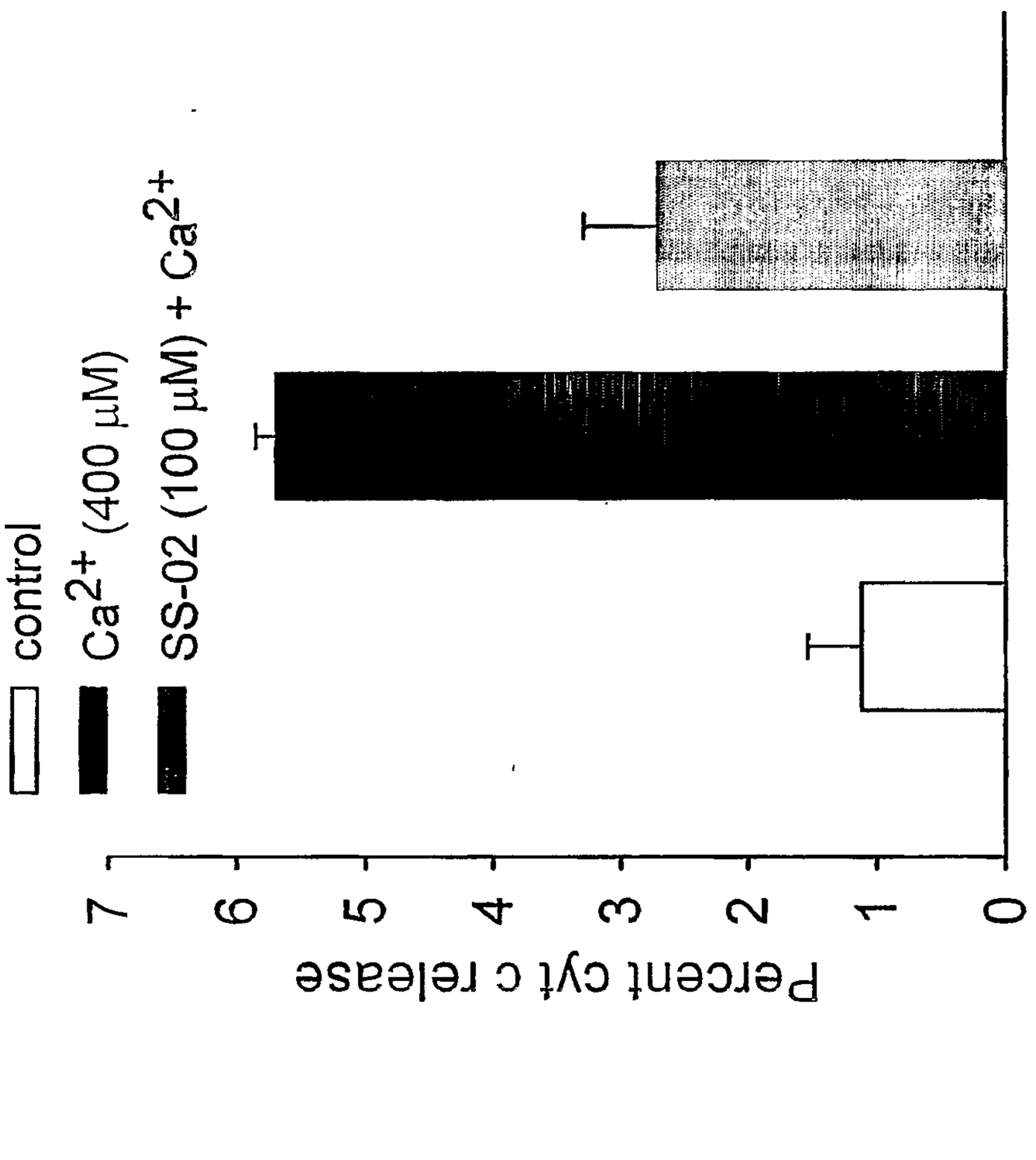
control
Ca2+ (400 µM)
SS-02 (100 µM) + Ca2+
Percent cyt c release
0
1
2
3
4
5
6
7

Figure 3B

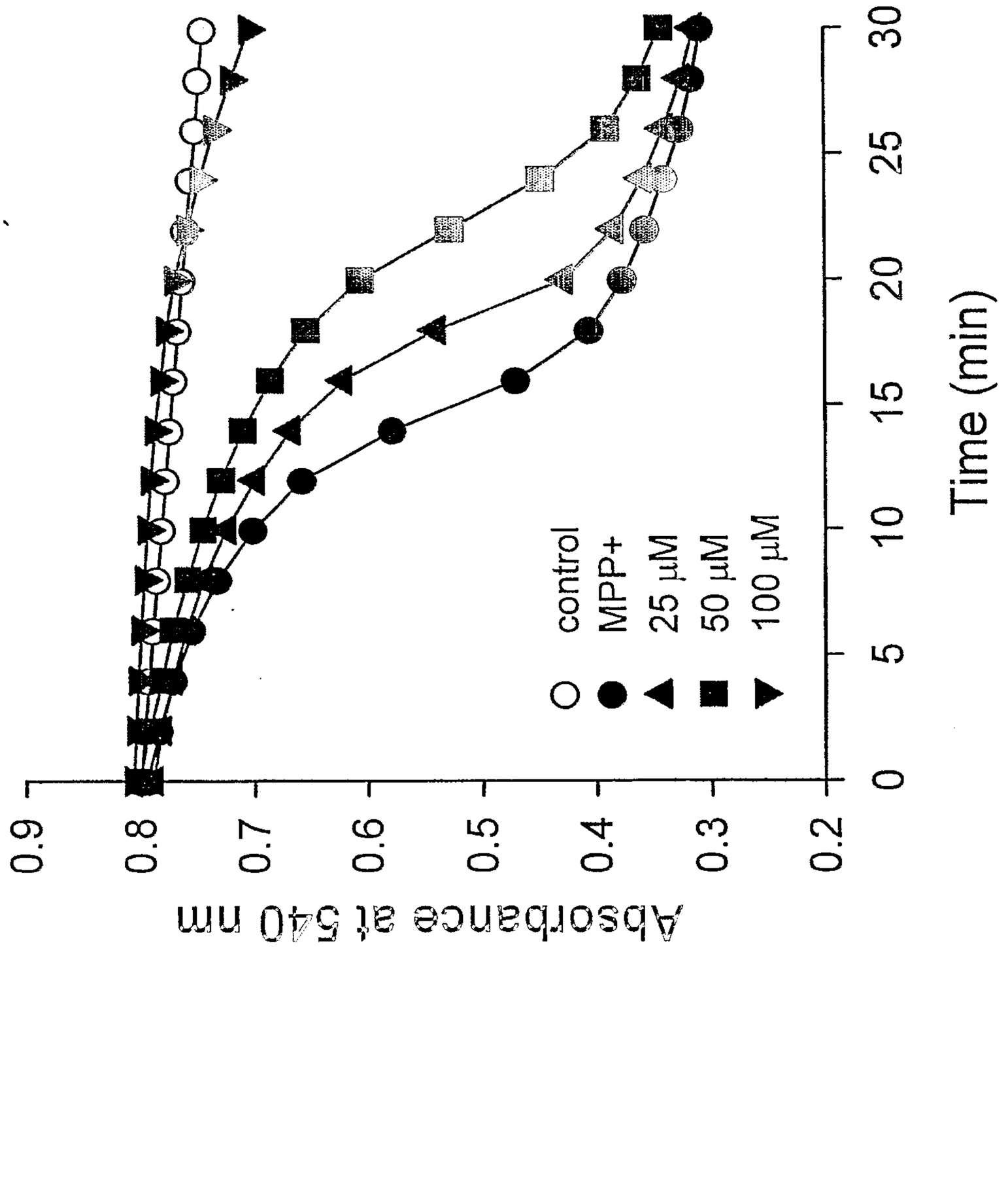
Absorbance at 540 nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
5
10
15
20
25
30
Time (min)
control
MPP+
25 µM
50 µM
100 µM

Figure 3C

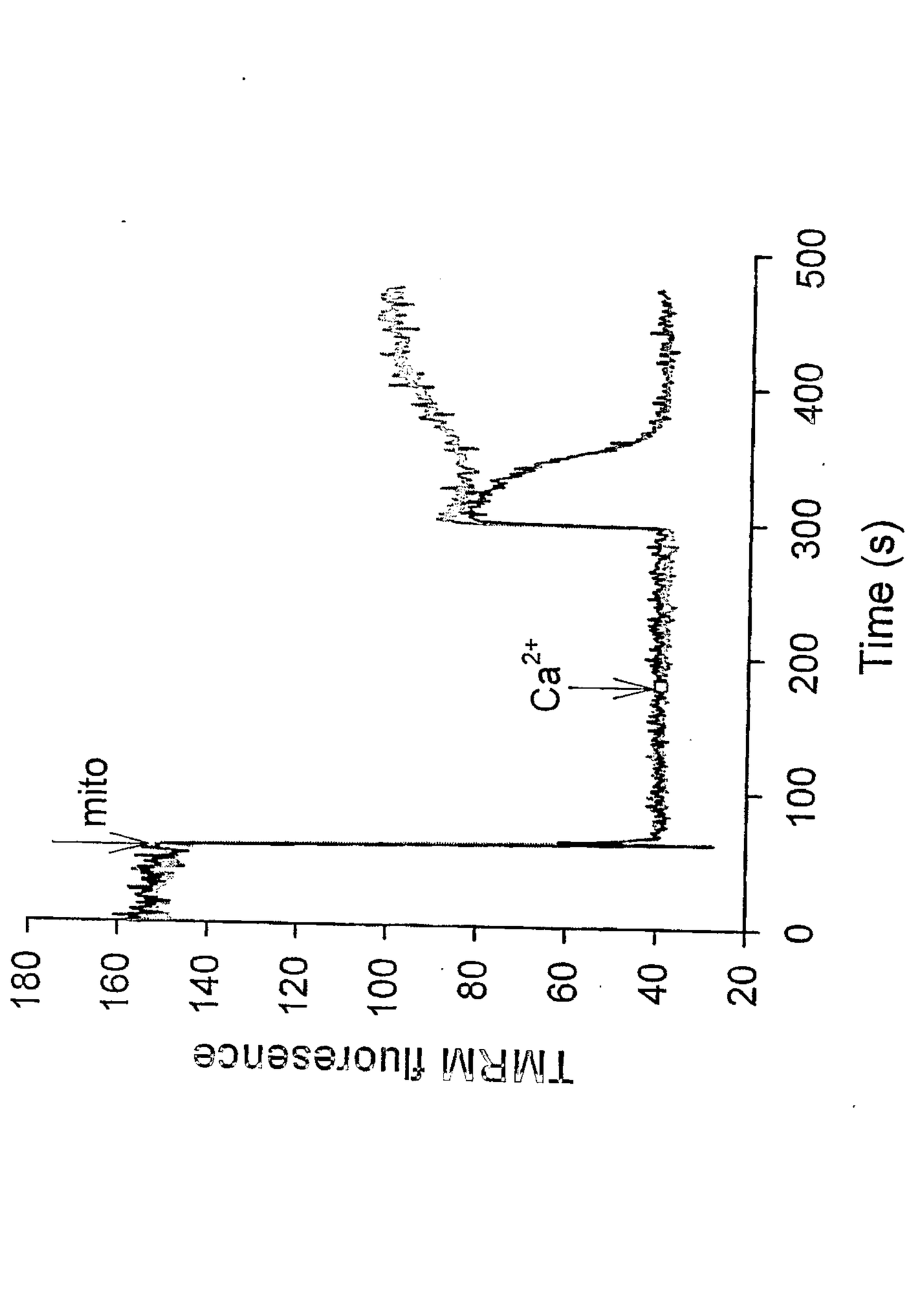
TMRM fluorescence
180
160
140
120
100
80
60
40
20
mito
Ca^{2+}
0
100
200
300
400
500
Time (s)

Figure 4A

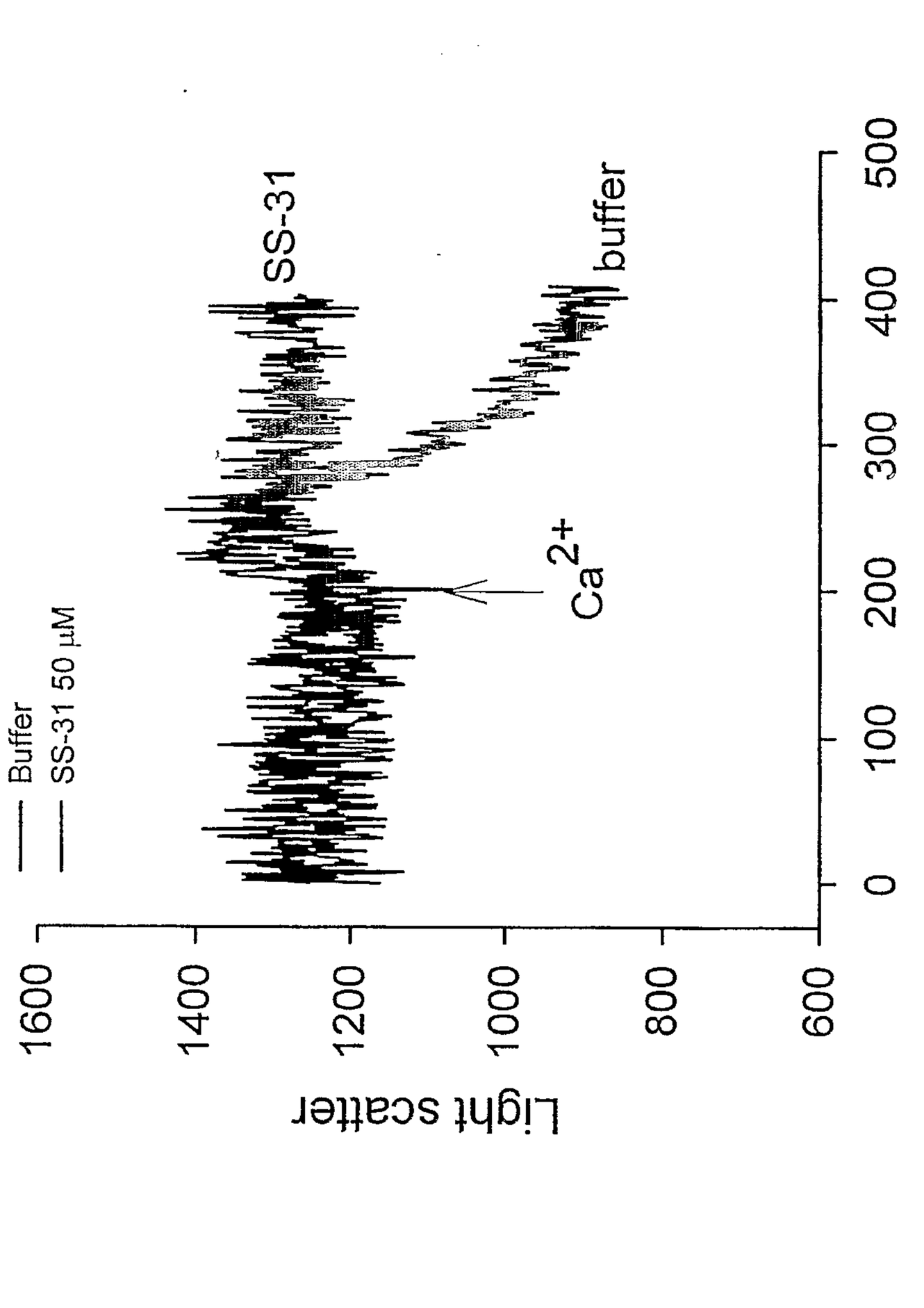
Buffer
SS-31 50 µM
Light scatter
1600
1400
1200
1000
800
600
0
100
200
300
400
500
Time (s)
Ca2+
SS-31
buffer

Figure 4B

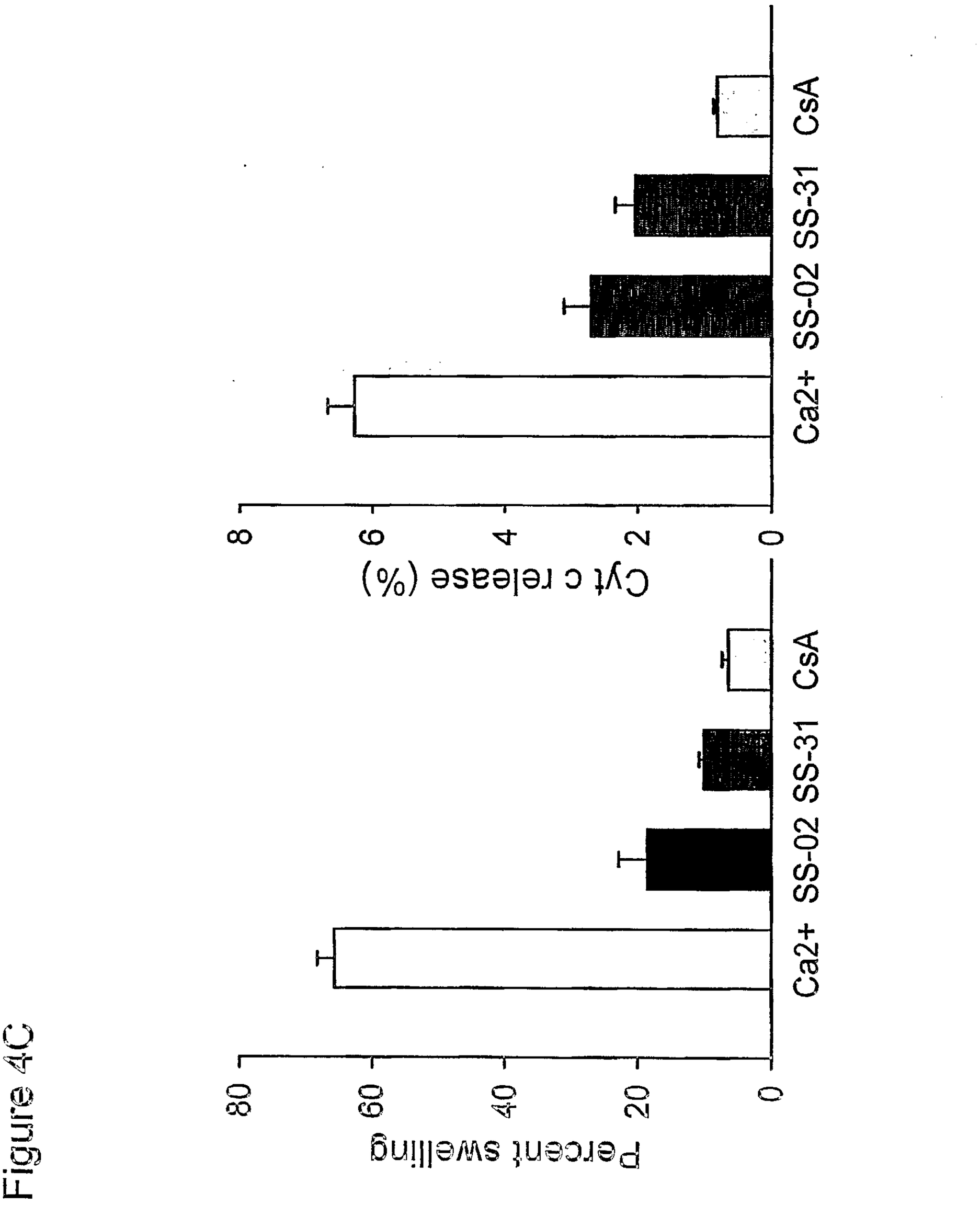
Percent swelling
0
20
40
60
80
Ca2+
SS-02
SS-31
CsA
Cyt c release (%)
0
2
4
6
8
Ca2+
SS-02
SS-31
CsA

Figure 4C

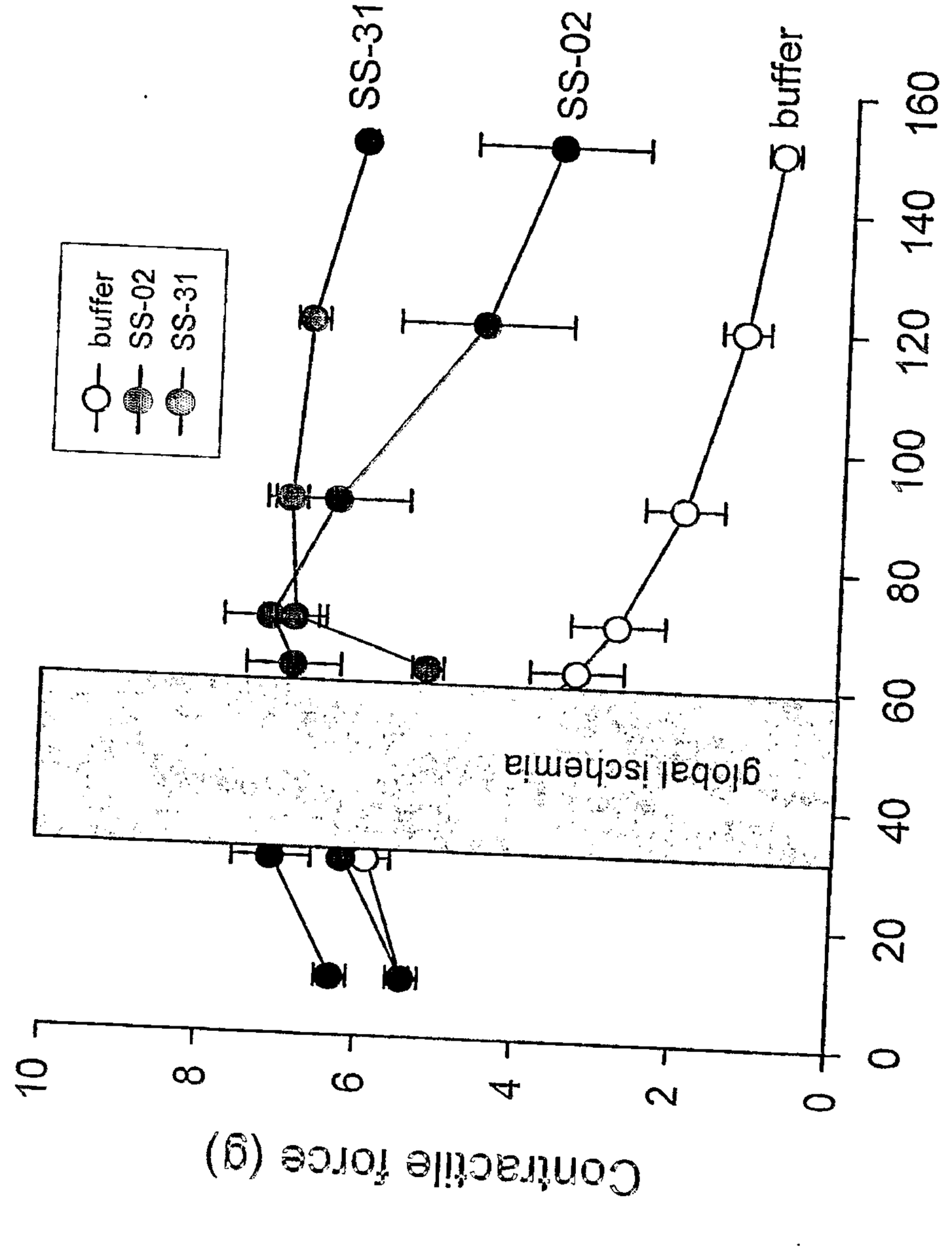
buffer
SS-02
SS-31
SS-31
SS-02
buffer
global ischemia
Contractile force (g)
Time (min)
0
2
4
6
8
10
0
20
40
60
80
100
120
140
160

Figure 5

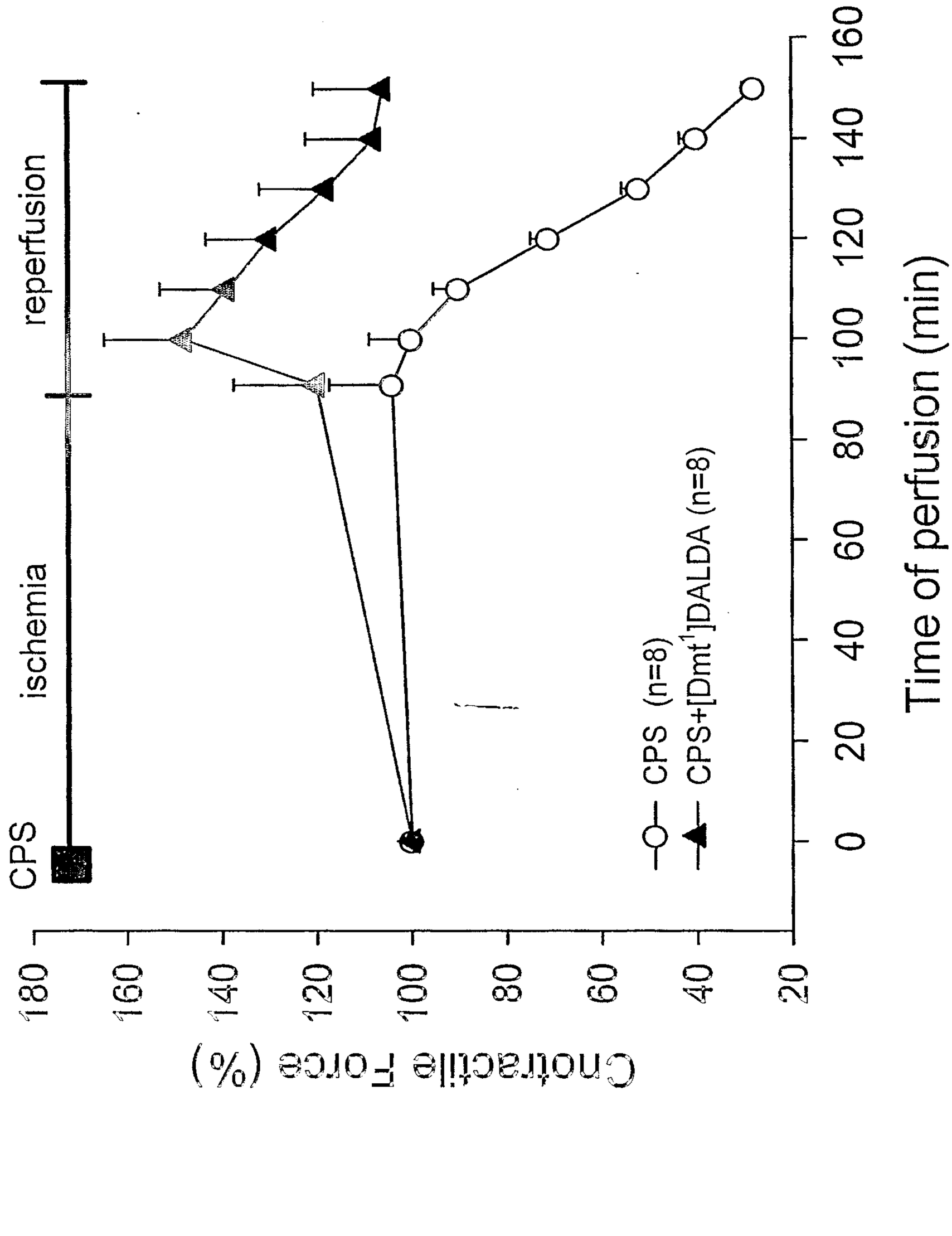
reperfusion
ischemia
CPS
CPS (n=8)
CPS+[Dmt1]DALDA (n=8)
Contractile Force (%)
Time of reperfusion (min)
0
20
40
60
80
100
120
140
160
180

Figure 6