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(54) Title: USE OF DELTA PKC PEPTIDES FOR MODULATION OF REACTIVE OXYGEN SPECIES

(57) Abstract: Methods for modulating the level of reactive oxygen species (ROS) in a cell or tissue by administering compounds that modulate the activity of δ PKC are described. In one embodiment, the level of ROS is decreased by administering a peptide effective to inhibit the activity of δ PKC. In another embodiment, the level of ROS is increased by administering a peptide having activity to agonize the activity of δ PKC. Also described are methods to treat or inhibit development of conditions preceded by or exacerbated by an increased level of ROS, such as arteriosclerosis and inflammatory diseases.



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USE OF DELTA PKC PEPTIDES FOR MODULATION OF REACTIVE OXYGEN SPECIES

Field of the Invention

5 The present invention relates to methods of treating disorders characterized by, preceded by, or exacerbated, at least in part, by reactive oxygen species. The method includes administering a compound, such as a delta protein kinase C (δ PKC) peptide, *in vivo* in an amount sufficient to modulate, and preferably, inhibit production of reactive oxygen species. The method finds particular use in
10 inhibiting disorders resulting from oxidative stress, such as arteriosclerosis, reperfusion injury, arthritis, and tissue damage in diabetic subjects.

Background of the Invention

 Species capable of independent existence that contain one or more
15 unpaired electrons are commonly referred to as free radicals. There are many types of free radicals, differing in their reactivity, origin, place of formation, degree of lipophilicity, and potential biological target. In recent years, the term "reactive oxygen species" (ROS) has been adopted to include molecules such as hypochlorous acid (HOCl), singlet oxygen ($^1\text{O}_2$), superoxide anion ($\cdot\text{O}_2^-$), hydroxyl
20 radical ($\cdot\text{OH}$), and hydrogen peroxide (H_2O_2), which are not radicals in nature but are capable of radical formation in the extra- and intracellular environments (Halliwell, B. and Gutteridge, J.M.C (Eds), FREE RADICAL IN BIOLOGY AND MEDICINE, 2nd Ed. Clarendon Press, Oxford, 1989).

 ROS are involved in many biological processes, including regulating
25 biochemical processes, assisting in the action of specific enzymes, and removing and destroying bacteria and damaged cells (Halliwell, B. and Gutteridge, J.M.C (Eds), FREE RADICAL IN BIOLOGY AND MEDICINE, 2nd Ed. Clarendon Press, Oxford, 1989). Free radicals are essential for the body and under normal circumstances there is a balance between oxidative and reductive compounds (redox state)
30 inside the cell. If the balance is impaired in favor of oxidative compounds, oxidative stress is said to occur (Parke *et al.*, *Int. J. Occup. Med. Environ. Health* 9:331-340 (1996); Knight, *Ann. Clin. Lab. Sci.* 27:11-25 (1997); Stohs, *J. Basic. Clin. Physiol. Pharmacol.* 6: 205-228 (1995)). Oxidative stress may occur as a

result of oxidative insults such as air pollution or the "oxidative burst" characteristic of activated neutrophils mediated by the immune response. A constant source of oxidative stress results from formation of superoxide anion via "electron leakage" in the mitochondria during production of adenosine triphosphate (ATP). Although
5 superoxide anion is not exceedingly reactive in and of itself, it can initiate a chain of events that eventually results in the formation of the highly reactive free radicals and other oxidants. If these reactive oxygen species are not controlled by enzymatic and/or non-enzymatic antioxidant systems, *in vivo* oxidation of critical cellular components such as membranes, DNA, and proteins will result, eventually
10 leading to tissue damage and dysfunction.

Reactive oxygen species (ROS) have been implicated in the development of many disorders. ROS are involved in arteriosclerotic lesions, in the evolution of various neurodegenerative diseases, and are also produced in association to epileptic episodes, in inflammation, in the mechanisms of action of various
15 neurotoxins, or as side effects of drugs. ROS also appear to play a role in reperfusion injury.

It is clear that the balance between oxidative and reductive compounds in biological systems is important. To preserve this balance the body has a number of protective antioxidant mechanisms that remove or prevent formation of ROS.
20 There are also mechanisms that repair damage caused by ROS *in vivo*. Defense systems include enzymatic as well as non-enzymatic antioxidant components. However, the development of methods and compounds to combat oxidative stress or toxicity associated with oxygen-related species has enjoyed limited success. Thus, there remains a need in the art for therapies that provide a defense against
25 damage due to reactive oxygen species.

Protein kinase C (PKC) is a key enzyme in signal transduction involved in a variety of cellular functions, including cell growth, regulation of gene expression and ion channel activity. The PKC family of isozymes includes at least eleven different protein kinases which can be divided into at least three subfamilies based
30 on their homology and sensitivity to activators. Members of the classical or cPKC subfamily, α , β_I , β_{II} and γ PKC, contain four homologous domains (C1, C2, C3 and C4) inter-spaced with isozyme-unique (variable or V) regions, and require calcium, phosphatidylserine (PS), and diacylglycerol (DG) or phorbol esters for activation. Members of the novel or nPKC subfamily, δ , ϵ , η , and θ PKC, lack the C2

homologous domain and do not require calcium for activation. Finally, members of the atypical or α PKC subfamily, ζ and λ PKC, lack both the C2 and one half of the C1 homologous domains and are insensitive to DG, phorbol esters and calcium.

Studies on the subcellular distribution of PKC isozymes demonstrate that activation of PKC results in its redistribution in the cells (also termed translocation), such that activated PKC isozymes associate with the plasma membrane, cytoskeletal elements, nuclei, and other subcellular compartments (Saito, N. *et al.*, *Proc. Natl. Acad. Sci. USA* 86:3409-3413 (1989); Papadopoulos, V. and Hall, P.F. *J. Cell Biol.* 108:553-567 (1989); Mochly-Rosen, D., *et al.*, *Molec. Biol. Cell* (formerly *Cell Reg.*) 1:693-706 (1990)).

The localization of different PKC isozymes to different areas of the cell in turn appears due to binding of the activated isozymes to specific anchoring molecules termed Receptors for Activated C-Kinase (RACKs). RACKs are thought to function by selectively anchoring activated PKC isozymes to their respective subcellular sites. RACKs bind only fully activated PKC and are not necessarily substrates of the enzyme. Translocation of PKC reflects binding of the activated enzyme to RACKs anchored to the cell particulate fraction and the binding to RACKs is required for PKC to produce its cellular responses (Mochly-Rosen, D., *et al.*, *Science* 268:247-251 (1995)).

Summary of the Invention

Accordingly, the invention provides a method of modulating the level of reactive oxygen species (ROS) in a cell or tissue.

The invention also provides a method of decreasing the level of ROS in a cell or tissue.

The invention also provides a method of increasing the level of ROS in a cell or tissue.

The invention also provides a method of reducing or protecting cells and tissue from damage due to ROS.

The invention also provides a method of increasing or decreasing the activity of δ PKC to modulate the level of ROS in a cell or tissue.

The invention also provides a method of treating a disorder associated with oxidative stress, i.e., and increased level of ROS in a particular tissue.

In one aspect, the invention includes a method of modulating production of reactive oxygen species in a cell or tissue by administering a compound effective to modulate the activity of δ PKC. The compound in one embodiment has activity to inhibit δ PKC activity to reduce production of reactive oxygen species in the cell or tissue. In another embodiment, the compound has activity to activate δ PKC activity to stimulate production of reactive oxygen species in the cell or tissue.

Compounds having inhibitory activity are, in one embodiment, isolated peptides having at least 50% sequence identity to a peptide selected from the group consisting of δ V1-1 (SEQ ID NO:3), δ V1-2 (SEQ ID NO:4), and δ V1-5 (SEQ ID NO:6). The peptide can also be selected from the group consisting of SEQ ID NO:4 (δ V1-1) and the peptides identified as SEQ ID NOS:33-47. The peptide can also be selected from the group consisting of δ V1-2 (SEQ ID NO:4) and the peptides identified as SEQ ID NOS:64-70.

Compounds having activity to activate PKC are in one embodiment a peptide having at least 50% sequence identity to $\psi\delta$ RACK (SEQ ID NO:5). Such peptides include peptides selected from the group consisting of $\psi\delta$ RACK (SEQ ID NO:5) and the peptides identified as SEQ ID NOS:10-18, 21-32.

The peptides can be conjugated with a moiety to facilitate transfer across a cell membrane. The peptides can be administered by any suitable route including but not limited to intravenous, parenteral, subcutaneous, inhalation, intranasal, sublingual, mucosal, and transdermal route. In one embodiment, the peptide is administered during a period of reperfusion; that is, after a period of initial perfusion.

In another aspect, the invention includes a method for inhibiting development of, slowing the progression of, or treating arteriosclerosis by administering a compound effective to inhibit the activity of δ PKC. The compound is preferably a peptide having at least 50% sequence identity to a peptide selected from the group consisting of δ V1-1 (SEQ ID NO:3), δ V1-2 (SEQ ID NO:4), and δ V1-5 (SEQ ID NO:6). The peptide can be conjugated to a moiety to assist transport across a cell membrane.

In another aspect, the invention includes method for treating a disorder characterized by or preceded by an increased production of reactive oxygen species in a cell or tissue by administering a compound effective to inhibit the

activity of δ PKC. In one embodiment, the compound is a peptide having at least 50% sequence identity to a peptide selected from the group consisting of δ V1-1 (SEQ ID NO:3), δ V1-2 (SEQ ID NO:4), and δ V1-5 (SEQ ID NO:6). The peptide can be conjugated to a cell membrane transport moiety, such as Tat. The method
5 finds use, for example, in inflammatory disorders, such as arthritis, and vascular disorders.

In another aspect, the invention includes a method for stimulating production of reactive oxygen species in a cell or tissue, by administering a compound effective to agonize the activity of δ PKC. In one embodiment, the
10 compound is a peptide having at least 50% sequence identity to $\psi\delta$ RACK (SEQ ID NO:5). In another embodiment, the peptide is administered to a tumor cell. The agonist peptide can be linked to a moiety effective to facilitate transport across a cell membrane.

These and other objects and features of the invention will be more fully
15 appreciated when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

Fig. 1 shows the alignment of the primary sequence of rat δ PKC and mouse
20 Θ PKC V1 domains. The bracketed areas designated as δ V1-1, δ V1-2, and $\psi\delta$ R indicate regions of difference between the two isozymes.

Fig. 2 shows the production of reactive oxygen species (ROS), in fmols/mg protein, in rat vascular smooth muscle cells *in vitro*, after incubation in the presence of tempoamine alone (control) or with $\psi\delta$ RACK, phorbol 12-myristate 13-
25 acetate (PMA), and δ V1-1 plus PMA;

Fig. 3A shows the production of reactive oxygen species (ROS), in fmols/mg protein, in Zucker Lean rats (Fa/fa), Zucker obese rats (fa/fa), and Zucker obese rats treated with insulin-sensitizing thiazolidinedione;

Figs. 3B-3C is a graph showing the stress, in kPa, as a function of strain, in
30 m/m, of aortas excised from Zucker lean rats (lower curve) and Zucker obese (upper curve) rats in the circumferential (Fig. 3B) and longitudinal (fig. 3C) directions;

Fig. 4A shows a Western blot autoradiogram of the cytosol and membrane cell fractions of rat vascular smooth muscle cells incubated with bovine insulin and probed with anti- δ PKC;

Fig. 4B is a bar graph showing the relative δ PKC activity, based on the Western blot of Fig. 4A, in cells incubated with bovine insulin, the δ PKC activity normalized against the δ PKC activity in the control cells;

Fig. 5A is a bar graph showing the normalized tumor growth factor-beta (TGF- β) expression *in vitro* in rat vascular smooth muscle cells left untreated (control) and incubated with insulin

Fig. 5B is a bar graph showing the normalized TGF- β expression *in vitro* in rat vascular smooth muscle cells left untreated (control) and incubated with Tat-conjugated δ V1-1 peptide;

Fig. 5C is a bar graph showing the normalized Cbfa-1 gene expression *in vitro* in rat vascular smooth muscle cells left untreated (control) and incubated with Tat-conjugated δ V1-1 peptide; and

Fig. 6 is a bar graph showing the increase in osteopontin expression (expressed as percent of control) *in vivo* in Zucker obese rats relative to Zucker lean rats.

Brief Description of the Sequences

SEQ ID NO:1 corresponds to amino acids 1-141 from the V1 domain of rat δ PKC (accession no. KIRTCD).

SEQ ID NO:2 corresponds to amino acids 1-124 of V1 domain of mouse theta-PKC (accession no. Q02111).

SEQ ID NO:3 is an amino acid sequence from the first variable region of δ PKC (amino acids 8-17), δ V1-1.

SEQ ID NO:4 is an amino acid sequence from the first variable region of δ PKC (amino acids 35-44), δ V1-2.

SEQ ID NO:5 is an amino acid sequence from δ PKC (amino acids 74-81), and is referred to herein as "pseudo-delta" RACK, or $\psi\delta$ RACK.

SEQ ID NO:6 is an amino acid sequence from a region of δ PKC (amino acids 619-676), referred to herein as δ V1-5.

SEQ ID NO:7 is the *Drosophila* Antennapedia homeodomain-derived carrier

peptide.

SEQ ID NO:8 is a Tat-derived carrier peptide (Tat 47-57).

SEQ ID NO:9 is a β PKC-selective activator peptide.

SEQ ID NO:10 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

5 SEQ ID NO:11 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:12 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:13 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:14 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:15 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

10 SEQ ID NO:16 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:17 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:18 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:19 is a fragment of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:20 is a fragment of SEQ ID NO:5 ($\psi\delta$ RACK).

15 SEQ ID NO:21 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:22 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:23 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:24 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:25 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

20 SEQ ID NO:26 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:27 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:28 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:29 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:30 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

25 SEQ ID NO:31 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:32 is a modification of SEQ ID NO:5 ($\psi\delta$ RACK).

SEQ ID NO:33 is a modification of SEQ ID NO:3 (δ V1-1).

SEQ ID NO:34 is a modification of SEQ ID NO:3 (δ V1-1).

SEQ ID NO:35 is a modification of SEQ ID NO:3 (δ V1-1).

30 SEQ ID NO:36 is a modification of SEQ ID NO:3 (δ V1-1).

SEQ ID NO:37 is a modification of SEQ ID NO:3 (δ V1-1).

SEQ ID NO:38 is a modification of SEQ ID NO:3 (δ V1-1).

SEQ ID NO:39 is a modification of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:40 is a modification of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:41 is a modification of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:42 is a modification of SEQ ID NO:3 ($\delta V1-1$).
5 SEQ ID NO:43 is a modification of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:44 is a modification of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:45 is a modification of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:46 is a modification of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:47 is a modification of SEQ ID NO:3 ($\delta V1-1$).
10 SEQ ID NO:48 is a fragment of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:49 is a modified fragment of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:50 is a modified fragment of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:51 is a modified fragment of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:52 is a modified fragment of SEQ ID NO:3 ($\delta V1-1$).
15 SEQ ID NO:53 is a modified fragment of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:54 is a modified fragment of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:55 is a modified fragment of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:56 is a modified fragment of SEQ ID NO:3 ($\delta V1-1$).
SEQ ID NO:57 is a fragment of SEQ ID NO:3 $\delta V1-1$.
20 SEQ ID NO:58 is a fragment of SEQ ID NO:3 $\delta V1-1$.
SEQ ID NO:59 is a fragment of SEQ ID NO:3 $\delta V1-1$.
SEQ ID NO:60 is a fragment of SEQ ID NO:3 $\delta V1-1$.
SEQ ID NO:61 is a fragment of SEQ ID NO:3 $\delta V1-1$.
SEQ ID NO:62 is a fragment of SEQ ID NO:3 $\delta V1-1$.
25 SEQ ID NO:63 is a fragment of SEQ ID NO:3 $\delta V1-1$.
SEQ ID NO:64 is a modification of SEQ ID NO:4 ($\delta V1-2$).
SEQ ID NO:65 is a modification of SEQ ID NO:4 ($\delta V1-2$).
SEQ ID NO:66 is a modification of SEQ ID NO:4 ($\delta V1-2$).
SEQ ID NO:67 is a modification of SEQ ID NO:4 ($\delta V1-2$).
30 SEQ ID NO:68 is a modification of SEQ ID NO:4 ($\delta V1-2$).
SEQ ID NO:69 is a modification of SEQ ID NO:4 ($\delta V1-2$).
SEQ ID NO:70 is a modification of SEQ ID NO:4 ($\delta V1-2$).

SEQ ID NO:71 is the sequence of Annexin V.

Detailed Description of the Invention

I. Definitions

5 Unless otherwise indicated, all terms herein have the same meaning as they would to one skilled in the art of the present invention. Practitioners are particularly directed to *Current Protocols in Molecular Biology* (Ausubel, F. M. et al., John Wiley and Sons, Inc., Media Pa.) for definitions and terms of the art.

10 Abbreviations for amino acid residues are the standard 3-letter and/or 1-letter codes used in the art to refer to one of the 20 common L-amino acids.

A "conserved set" of amino acids refers to a contiguous sequence of amino acids that is conserved between members of a group of proteins. A conserved set may be anywhere from two to over 50 amino acid residues in length. Typically, a conserved set is between two and ten contiguous residues in length. For example,
15 for the two peptides MKAAEDPM (SEQ ID NO:10) and MRAPEDPM (SEQ ID NO:13), there are 4 identical positions (EDPM; SEQ ID NO:19) which form the conserved set of amino acids for these two sequences.

"Conservative amino acid substitutions" are substitutions which do not result in a significant change in the activity (e.g., δ V1-1 PKC activity) or tertiary
20 structure of a selected polypeptide or protein. Such substitutions typically involve replacing a selected amino acid residue with a different residue having similar physico-chemical properties. For example, substitution of Glu for Asp is considered a conservative substitution since both are similarly-sized negatively-charged amino acids. Groupings of amino acids by physico-chemical properties
25 are known to those of skill in the art.

"Peptide" and "polypeptide" are used interchangeably herein and refer to a compound made up of a chain of amino acid residues linked by peptide bonds. Unless otherwise indicated, the sequence for peptides is given in the order from the amino terminus to the carboxyl terminus.

30 Sequence "identity" is determined by comparing the amino acid sequences of polypeptides when aligned so as to maximize overlap and identity while minimizing sequence gaps. The percent identity of two amino acid or two nucleic acid sequences can be determined by visual inspection and/or mathematical calculation, commonly done for longer sequences by comparing sequence

information using a computer program. An exemplary, preferred computer program is the Genetics Computer Group (GCG; Madison, Wis.) Wisconsin package version 10.0 program, 'GAP' (Devereux *et al.*, *Nucl. Acids Res.*, 12: 387 (1984)). Other programs used by those skilled in the art of sequence comparison can also be used, such as, for example, the BLAST (BLASTP) and BLASTN programs, available for use via the National Library of Medicine website <http://www.ncbi.nlm.nih.gov/BLAST>. In preferred embodiments, sequences are considered homologous or identical to one another if their amino acid sequences are at least about 50% identical, more preferably if the sequences are 70% or 75% identical, still more preferably if the sequences are 80% or 85% identical, still more preferably if the sequences are 90% or 95% identical, when determined from a visual inspection or from one of the aforementioned computer programs.

A peptide or peptide fragment is "derived from" a parent peptide or polypeptide if it has an amino acid sequence that is identical or homologous to the amino acid sequence of the parent peptide or polypeptide.

"Reperfusion" refers to return of fluid flow into a tissue after a period of no-flow or reduced flow. For example, in reperfusion of the heart, fluid or blood returns to the heart through the coronary arteries after occlusion of these arteries has been removed.

The term "PKC" refers to protein kinase C, or C-kinase.

The term "RACK" refers to receptor for activated C-kinase.

An "agonist" refers to endogenous substance or a drug that can interact with a receptor and initiate a physiological or a pharmacological response characteristic of that receptor (contraction, relaxation, secretion, enzyme activation, etc.)

An "antagonist" refers to molecule that opposes the physiological effects of another. At the receptor level, it is a molecule that opposes the receptor-associated responses normally induced by another bioactive agent.

II. δ PKC Peptides Modulate Reactive Oxygen Species

In studies described herein, peptides effective to activate (agonize) delta protein kinase C (δ PKC) and peptides effective to inhibit (antagonize) δ PKC were administered to cells. Fig. 1 shows the sequences of the V1 domain of rat δ PKC

(SEQ ID NO:1; accession no. KIRTCO) and mouse theta-PKC (θ -PKC) V1 domain (SEQ ID NO:2, accession no. Q02111). Three regions in the V1 domain of δ PKC were identified with only ~10% identity to theta-PKC. These regions are indicated in Fig. 1 by the bars above the sequence of δ PKC and are referred to herein as

5 δ V1-1 having a sequence identified herein as SEQ ID NO:3 (SFNSYELGSL), δ V1-2 having a sequence identified herein as SEQ ID NO:4 (ALTDRGKLV), and $\psi\delta$ RACK having a sequence identified herein as SEQ ID NO:5 (MRAAEDPM). Not shown in Fig. 1 is another sequence from the δ PKC sequence identified as SEQ ID NO:6 and referred to herein as δ V1-5. δ V1-1, δ V1-2 and δ V1-5 are δ PKC

10 antagonists and $\psi\delta$ RACK is a δ PKC agonist.

In the studies described below, the δ PKC peptides were modified with a carrier peptide by cross-linking via an N-terminal Cys-Cys bond to Tat (SEQ ID NO:8). It will be appreciated that the δ PKC peptides can be conjugated to other moieties to facilitate transport of the peptide across a cell membrane, for example

15 *Drosophila* Antennapedia homeodomain (SEQ ID NO:7; Théodore, L. *et al. J. Neurosci.*, 15:7158 (1995); Johnson, J. A. *et al., Circ. Res.* 79:1086 (1996)) or polyarginine (Mitchell *et al., J. Peptide Res.*, 56:318-325 (2000); Rothbard *et al., Nature Med.*, 6:1253-1257 (2000)). It will also be appreciated that the transport peptide can be conjugated to a C-terminal amino acid residue.

20 Example 1 details a study where inhibitors and activators of δ PKC were administered to rat vascular smooth muscle cells *in vitro*. Cells were incubated in presence of (1) the agonist Tat-conjugated peptide $\psi\delta$ RACK (SEQ ID NO:5); (2) phorbol 12-myristate 13-acetate (PMA); or (3) the antagonist Tat-conjugated peptide δ V1-1 (SEQ ID NO:3) plus PMA. A control cell sample was left untreated.

25 Tempoamine ((2,2,6,6-tetramethyl-1-piperidin-4-yl)oxy-4-amine)) was added to each culture and the loss of tempoamine signal was measured by electron spin resonance to measure superoxide production. The results are shown in Fig. 2.

Fig. 2 shows the production of reactive oxygen species (ROS), in fmols/mg protein, in rat vascular smooth muscle cells *in vitro*, after incubation. The

30 presence of the δ PKC activator, $\psi\delta$ RACK, resulted in an increased production of ROS. PMA, a non-isozyme specific activator of PKC, stimulated a 3-4 fold increase in ROS in the cells. Addition of the delta isozyme specific inhibitor, δ V1-1 to cells co-incubated with PMA was effective to reverse or inhibit a significant part

of the ROS production stimulated by PMA. These results show that that selective activation of δ PKC caused a significant increase in production of ROS. Inhibition of this PKC isoform caused a decrease in PMA-stimulated ROS production.

Accordingly, a method for modulating (increasing or decreasing) production
5 of ROS in a cell or tissue is provided, by delivering a compound effective to activate δ PKC. More particularly, a compound that activates δ PKC will increase production of ROS. A compound that inhibits or antagonizes δ PKC will decrease ROS generation. The δ V1-1 inhibitor is merely exemplary of compounds that modulate ROS production.

10 The ability to modulate ROS production in cells is desirable since ROS have been implicated in the development of many disorders. ROS are involved in atherosclerotic lesions, in the evolution of various neurodegenerative diseases, and are also produced in association to epileptic episodes, in inflammation, in the mechanisms of action of various neurotoxicants, or as side effects of drugs. Thus,
15 further studies were conducted to examine the role of δ PKC in conditions involving ROS, such as arteriosclerosis.

Arteriosclerosis, or fibrotic hardening of the arteries, is a factor in the cardiovascular morbidity associated with several disease processes, including type 2 diabetes. Arterial stiffness, as measured by increased pulse-wave velocity, is an
20 independent predictor of stroke, and is significantly associated with cardiovascular mortality independent of age. Arteriosclerosis is thought to be due to increased deposition of extracellular matrix proteins, as well as modification of matrix structure towards a less compliant phenotype. Calcification of vascular tissue may be due to an active morphogenetic process that also contributes to
25 arteriosclerosis. Transforming Growth Factor-Beta-1, or TGF-Beta-1, is a cytokine known to induce fibrosis. TGF-beta signaling is transduced via intracellular Smad proteins. There may be some "cross-talk" between Smads and Core Binding Factor Alpha-1, or Cbfa-1, a transcription factor commonly associated with bone development, which itself can upregulate the expression of calcific proteins in the
30 vasculature.

Hyperglycemia may be an inciting factor for diabetes-associated arteriosclerosis, however, the arteriosclerotic process may begin during the insulin-resistant, euglycemic state. Studies were done to examine the effect of the factors

associated with insulin-resistance, with a focus on hyperinsulinemia, on the differential activation of PKC isoforms in vascular tissues. These studies also included an examination of whether changes in gene expression towards a pro-fibrotic phenotype were associated with activity of various PKC isoforms, and to what extent the effects seen could be via reactive oxygen species-dependent versus independent mechanisms. These studies will now be described.

Zucker obese rats (fa/fa), which bear a mutation (fa) in the leptin receptor gene, provide a model of obesity associated with type 2 diabetes and typically exhibit pronounced hyperinsulinemia and hyperlipidemia at 6 weeks of age and became diabetic after 14 weeks of age. Tissue homogenates from the aortas of Zucker obese rats and of Zucker lean (Fa/fa) rats were prepared and analyzed for superoxide production using electron spin resonance with a tempomaine probe. As seen in Fig. 3A, the obese rats had a significantly higher production of ROS than their lean counterparts. Obese rats treated with insulin-sensitizing thiazolidinedione (TZD1) brought the ROS production down to control levels.

The isolated aortas were isolated from the animals and analyzed using a tissue mechanical tester, as described in Example 2, in both the circumferential direction and the longitudinal direction. Figs. 3B-3C shows the stress-strain relationship in the circumferential direction and the longitudinal direction, respectively. The averages under the curve for each data set indicates increased developed strain in Zucker obese rats (upper curve) compared to Zucker lean rats (lower curve) in both the circumferential and longitudinal directions. Modeling this data with a soft tissue strain energy equation further demonstrated decreased anisotropy (i.e., varying tissue properties based on the direction of strain) in the Zucker obese rat aorta as a consequence of the greater increase in the longitudinal component than the circumferential component.

Example 3 describes a study conducted to examine to role of PKC isozymes, and in particular δ PKC, in vascular fibrosis, which is accelerated during the insulin-resistant, pre-diabetic state in animals. In this study, rat vascular smooth muscle cells were incubated with bovine insulin. Translocation of PKC isozymes was assessed by Western blot analysis of the cytosolic and membrane (particulate) fractions of the cells. The subcellular localization of various PKC isozymes was assessed by immunofluorescence by probing the blot with isozyme

specific antibodies, such as anti- δ PKC, anti- α PKC, and anti- ϵ PKC, etc. The results for a blot probed with anti- δ PKC antibodies are shown in Figs. 4A-4B.

Fig. 4A shows the Western blot autoradiogram of the cytosol and membrane cell fractions of rat vascular smooth muscle cells incubated with bovine insulin and probed with anti- δ PKC. Visual inspection of the figure and comparison to the control cells which were not incubated with insulin shows that δ PKC is significantly stimulated by insulin. Fig. 4B is a bar graph showing the relative δ PKC activity, based on the Western blot of Fig. 4A, of cells incubated with bovine insulin. This semi-quantitative representation of the blot shows that δ PKC activity is stimulated by a factor of about 1.7 in the presence of insulin.

Accordingly, the methods described herein include a method for inhibiting δ PKC activity (e.g., cellular translocation) to treat or to slow progression of arteriosclerosis in a person, particularly in a diabetic person.

In another study, described in Example 4, the gene transcriptional effects of insulin and the selective inhibition of PKC isoforms was examined using real-time polymerase chain reaction (TaqMan[®] PCR). In vitro cells were incubated with insulin and in the presence or absence of a δ PKC inhibitor, δ V1-1 peptide. The expression of TGF- β and core binding factor α -1 (Cbf α -1) were monitored using TaqMan[®] real time PCR. The results are shown in Figs. 5A-5C.

Fig. 5A is a bar graph showing the normalized TGF- β 1 expression *in vitro* in rat vascular smooth muscle cells left untreated (control) and incubated with insulin. Insulin alone did not appear to stimulate the genes involved in vascular fibrosis, such as TGF- β 1.

Figs. 5B-5C show the effect of a δ PKC inhibitor compound on expression of TGF- β 1 and Cbfa-1. In Fig. 5B, the TGF- β 1 expression is inhibited when cells are incubated with Tat-conjugated δ V1-1 peptide. Similarly, as seen in Fig. 5C, Cbfa-1 gene expression *in vitro* is inhibited with cells are incubated with Tat-conjugated δ V1-1 peptide.

In summary, these studies show that inhibition of δ PKC was associated with a significant decrease in the expression of TGF- β 1 and Cbfa-1. δ PKC also decreased the mRNA expression of PAI-1, extracellular matrix proteins such as fibronectin and collagen 4 α 3, and pro-calcific proteins such as osteopontin (data not shown). Expression of TGF- β 1 is involved in sclerotic processes,

including myocardial fibrosis, diabetic nephropathy, and scleroderma. Overexpression leads to excessive matrix deposition and fibrosis of certain organs. Accordingly, it will be appreciated that administering a compound that inhibits δ PKC activity in a cell decreases expression of factors involved in, for example, arteriosclerosis, and other disorders associated with increased TGF- β 1 and Cbfa-1 expression, such as inflammation.

In another study, described in Example 5, aortas were isolated from obese and lean Zucker rats. RNA isolated from the aortas were analyzed for expression of osteopontin. Fig. 6 is a bar graph showing the increase in osteopontin expression *in vivo* in Zucker obese rats relative to Zucker lean rats. The isolated aortas were also tested for elasticity of the vessel, and as Fig. 3B shows, aortas excised from older Zucker obese rats demonstrated increased wall strain compared to aortas of age-matched lean Zucker rats. The data demonstrating increased vascular stiffness in the insulin-resistant phenotype, ex-vivo, confirms that the fibrotic processes seen at the mRNA and protein level has pathophysiological consequences.

III. Treatment Methods

As described above, the peptides δ V1-1, δ V1-2, δ V1-5, and $\psi\delta$ RACK act as translocation inhibitors or activators of δ PKC. $\psi\delta$ RACK is an agonist, inducing translocation of δ PKC to promote production of ROS. δ V1-1, δ V1-2, and δ V1-5 are antagonists, inhibiting δ PKC translocation and inhibiting production of ROS. Thus, methods of modulating the level of ROS in a cell or tissue by administering compounds that modulate appropriately the activity of δ PCK are contemplated.

It will be appreciated that administration of a peptide having inhibitory or stimulatory activity on δ PKC is merely exemplary of the general concept of administration of any compound having inhibitory or stimulatory activity on δ PKC. Those of skill in the art will understand that compounds having such activity are known or can be identified, and can include organic compounds that act as peptidomimetics.

The method of the invention is intended for use in treating any condition associated with oxidative stress, e.g., any disorder characterized by or preceded by an increased level of ROS. The method also finds use in cancer therapy,

where the level of ROS in tumor cells is increased by administration of a δ PKC compound that activates δ PKC to increase superoxide and other ROS intracellularly. Conditions associated with increased levels of ROS, where a method of decreasing the level of ROS by administering a compound effective to

5 inhibit δ PKC activity, include, but are not limited to vascular disorders, such as vascular fibrosis, arteriosclerotic lesions, etc., various neurodegenerative diseases, disorders characterized by inflammation, such as arthritis, inflammatory bowel disease, etc., kidney disease, cardiovascular disease, and general tissue damage.

10 In embodiments where a peptide is administered it will be appreciated that the peptides can be used in native form or modified by conjugation to a carrier, such as those described above. In one embodiment, the peptide is modified to include a terminal cysteine residue for attachment to a cell-permeable carrier peptide. It will also be appreciated that one or two amino acids from the

15 sequences can be substituted or deleted while still retaining the desired peptide activity. Exemplary modifications for some of the peptides described above are given below.

For the $\psi\delta$ RACK peptide, identified as SEQ ID NO:5, potential modifications include the following changes shown in lower case: MkAAEDPM (SEQ ID NO:10),

20 MRgAEDPM (SEQ ID NO:11), MRAgEDPM (SEQ ID NO:12), MRApEDPM (SEQ ID NO:13), MRAnEDPM (SEQ ID NO:14), MRAAdDPM (SEQ ID NO:15), MRAAEDPv (SEQ ID NO:16), MRAAEDPi (SEQ ID NO:17), MRAAEDPI (SEQ ID NO:18), and MRAAEDmp (SEQ ID NO:21), MeAAEDPM (SEQ ID NO:22), MdAAEDPM (SEQ ID NO:23), MRAAEePI (SEQ ID NO:24), MRAAEDPI (SEQ ID

25 NO:25), MRAAEePi (SEQ ID NO:26), MRAAEePv (SEQ ID NO:27), MRAAEDPv (SEQ ID NO:28), and any combination of the above. The following modifications to $\psi\delta$ RACK are also contemplated and are expected to convert the peptide from agonist to an antagonist: MRAAnDPM (SEQ ID NO:29), and MRAAqDPM (SEQ ID NO:30), MRAAEqPM (SEQ ID NO:31), MRAAEnPM (SEQ ID NO:32). Suitable

30 fragments of $\psi\delta$ RACK are also contemplated, and SEQ ID NOS: 19, 20 are exemplary.

Accordingly, the term "a δ PKC agonist" as used herein intends a $\psi\delta$ RACK peptide, which refers to SEQ ID NO:5 and to peptides having a sequence

homologous to SEQ ID NO:5 and to peptides identified herein, but not limited to, as SEQ ID NO:10-18 and SEQ ID NO:20-28. The term a δ PKC agonist further refers to fragments of these $\psi\delta$ RACK peptides, as exemplified by SEQ ID NOS:19-20.

5 For δ V1-1, potential modifications include the following changes shown in lower case: tFNSYELGSL (SEQ ID NO:33), aFNSYELGSL (SEQ ID NO:34), SFNSYELGtL (SEQ ID NO:35), including any combination of these three substitutions, such as tFNSYELGtL (SEQ ID NO: 36). Other potential modifications include SyNSYELGSL (SEQ ID NO:37), SFNSfELGSL (SEQ ID NO:38), SNSYdLGSL (SEQ ID NO:39), SFNSYELpSL (SEQ ID NO:40). Other
10 potential modifications include changes of one or two L to I or V, such as SFNSYEiGSv (SEQ ID NO:41), SFNSYEvGSi, (SEQ ID NO:42) SFNSYELGSv (SEQ ID NO:43), SFNSYELGSi (SEQ ID NO:44), SFNSYEiGSL (SEQ ID NO:45), SFNSYEvGSL (SEQ ID NO:46), aFNSYELGSL (SEQ ID NO:47), and any
15 combination of the above-described modifications. Fragments and modification of fragments of δ V1-1 are also contemplated, such as YELGSL (SEQ ID NO:48), YdLGSL (SEQ ID NO:49), fdLGSL (SEQ ID NO:50), YdiGSL (SEQ ID NO:51), YdvGSL (SEQ ID NO:52), YdLpSL (SEQ ID NO:53), YdLgIL (SEQ ID NO:54), YdLGSi (SEQ ID NO:55), YdLGSv (SEQ ID NO:56), LGSL (SEQ ID NO:57), iGSL
20 (SEQ ID NO:58), vGSL (SEQ ID NO:59), LpSL (SEQ ID NO:60), LGIL (SEQ ID NO:61), LGSi (SEQ ID NO:62), LGSv (SEQ ID NO:63).

Accordingly, the term "a δ V1-1 peptide" as used herein refers to an isolated peptide identified by SEQ ID NO:3 and to peptides having at least about 50% sequence identity, more preferably 70% or 75% sequence identity, still more
25 preferably 80% sequence identity, even still more preferably 90% or 95% sequence identity to SEQ ID NO:3, including but not limited to the peptides set forth in SEQ ID NOS:33-47, as well as fragments of any of these peptides that retain activity, as exemplified by but not limited to SEQ ID NOS:48-63.

For δ V1-2, potential modifications include the following changes shown in lower case: ALsTDRGKTLV (SEQ ID NO:64), ALTsDRGKTLV (SEQ ID NO:65),
30 ALTTDRGKsLV (SEQ ID NO:66), and any combination of these three substitutions, ALTTDRpKTLV (SEQ ID NO:67), ALTTDRGrTLV (SEQ ID NO:68), ALTTDkGKTLV (SEQ ID NO:69), ALTTDkGkTLV (SEQ ID NO:70), changes of

one or two L to I, or V and changes of V to I, or L and any combination of the above. In particular, L and V can be changed to V, L, I R and D, E can change to N or Q.

Accordingly, the term "a δ V1-2 peptide" as used herein refers to an isolated peptide identified by SEQ ID NO:4 and to peptides having at least about 50% sequence identity, more preferably 70% or 75% sequence identity, still more preferably 80% or 85% sequence identity, even still more preferably 90% or 95% sequence identity to SEQ ID NO:4, including but not limited to the peptides set forth in SEQ ID NOS:64-70, as well as fragments of any of these peptides that retain activity.

For δ V1-5 (SEQ ID NO: 6), potential modifications include those similar to the modifications described for δ V1-2. The term "a δ V1-5 peptide" as used herein refers to isolated peptides that retain the desired activity and have at least about 50% sequence identity, more preferably 70% or 75% sequence identity, still more preferably 80% or 85% sequence identity, even still more preferably 90% or 95% sequence identity to SEQ ID NO:6.

Accordingly, the term "a δ PKC peptide antagonist" as used herein intends an isolated δ PKC peptide, exemplified herein by a δ V1-1 peptide, a δ V1-2 peptide and a δ V1-5 peptide.

In still other embodiments, the peptide can be part of a fusion protein or a transport protein conjugate. Typically, to form a fusion protein, the peptide is bound to another peptide by a bond other than a Cys-Cys bond. An amide bond from the C-terminal of one peptide to the N-terminal of the other is exemplary of a bond in a fusion protein. The second peptide to which the δ PKC agonist/antagonist peptide is bound can be virtually any peptide selected for therapeutic purposes or for transport purposes. For example, it may be desirable to link the δ V1-1 peptide to a cytokine or other peptide that elicits a biological response.

Where the peptide is part of a conjugate, the peptide is typically conjugated to a carrier peptide, such as Tat-derived transport polypeptide (Vives *et al.* *J. Biol. Chem.*, 272:16010-16017 (1997)), polyarginine (Mitchell *et al.*, *J. Peptide Res.*, 56:318-325 (2000); Rothbard *et al.*, *Nature Med.*, 6:1253-1257 (2000)) or Antennapedia peptide by a Cys-Cys bond. See U.S. Patent No. 5,804,604. As

noted above, the transport peptide can be attached to the C-terminus or the N-terminus of the peptide. In another general embodiment, the peptides can be introduced to a cell, tissue or whole organ using a carrier or encapsulant, such as a liposome in liposome-mediated delivery.

5 The peptide may be (i) chemically synthesized or (ii) recombinantly produced in a host cell using, e.g., an expression vector containing a polynucleotide fragment encoding said peptide, where the polynucleotide fragment is operably linked to a promoter capable of expressing mRNA from the fragment in the host cell.

10 It will be appreciated that the dose of peptide administered will vary depending on the condition of the subject, the timing of administration (that is, whether the peptide is administered to prevent an increase in ROS, to treat an existing increase in ROS). Those of skill in the art are able to determine appropriate dosages, using, for example, the dosages used in *in vitro* and animal
15 studies.

 The peptides can be administered to the cell, tissue or whole organ *in vitro*, *in vivo*, or *ex vivo*. All modes of administration are contemplated, including intravenous, parenteral, subcutaneous, inhalation, intranasal, sublingual, mucosal, and transdermal. A preferred mode of administration is by infusion or
20 reperfusion through arteries to a target site. It will also be appreciated that a suitable pharmaceutical preparation can be prepared based on the selected route of administration. For example, the peptide can be delivered in a saline-based formulation. Other pharmaceutically-acceptable carriers, vehicles, and excipients are well known in the art and literature sources describing preparations for *in vivo*
25 administration are readily available.

 It will also be appreciated that any of the embodiments described here, preferred to non-preferred, can be combined with any other embodiment, preferred to non-preferred.

30 V. Examples

 The following examples further illustrate the invention described herein and are in no way intended to limit the scope of the invention.

Example 1

Production of Reactive Oxygen Species *In Vitro*

A. Peptide Preparation

5 δ V1-1 (SEQ ID NO:3) and $\psi\delta$ RACK (SEQ ID NO:5) were synthesized and purified (>95%) at the Stanford Protein and Nucleic Acid Facility. The peptides were modified with a carrier peptide by cross-linking via an N-terminal Cys-Cys bond to Tat (SEQ ID NO:8).

B. Peptide Delivery Into Cells

10 Rat vascular smooth muscle cells were harvested from Zucker lean aortas by enzymatic digestion and cultured in Dulbecco's modified eagle's medium with 10% fetal bovine serum and penicillin/streptomycin under standard tissue culture conditions. The cells were incubated in the presence tempoamine ((2,2,6,6-tetramethyl-1-piperidi-nyloxy-4-amine)) (100 μ M) and in the presence of (1) the
15 agonist Tat-conjugated peptide $\psi\delta$ RACK (10 μ M); (2) phorbol 12-myristate 13-acetate (PMA, 500ng/mL); or (3) the antagonist Tat-conjugated peptide δ V1-1 (10 μ M) plus PMA (500ng/mL). A control cell sample was left untreated. The loss of tempoamine signal was measured by electron spin resonance to measure superoxide production. The results are shown in Fig. 2.

20

Example 2

Mechanical Testing of Tissue

Seven male Zucker lean and eight Zicker obese (fa/fa, Harlan) rats were maintained on a normal chow diet and housed in a room with a 12-hour light/12-
25 hour dark cycle. Aortas were harvested and snap-frozen in liquid nitrogen for subsequent RNA isolation while a subset were fixed in a 10% formalin for histological evaluation.

Aortic tissue mechanics were analyzed using a custom-made tissue mechanical tester. Thoracic aorta segments were chosen that had minimal
30 branching vessels, and any branding vessels were ligated prior to explant. Within 4 hours of explant, 15 mm-long cylindrical thoracic aorta specimens were cannulated to barbed-end fittings with suture. Under constant longitudinal strain, held at *in vivo* length, pressure was gradually increased from 0-330 mmHg. Then, strain, held at mean physiological pressure, length was extended from relaxed to

150% of *in vivo* length. Constant pressure was maintained by using a large reservoir held approximately 6.5 feet above the specimen as a pressure source, making any leakage out of the explanted vessel negligible. Intraluminal pressure and axial load were recorded through a data acquisition system and correlated to regional tissue strain via video images. From this information, stress-strain relationships were computed. The isotropy index was computed using Fung's soft tissue strain energy equation (Fung, Y.C. *et al.*, *Am. J. Physiol.*, 237(5):H620-H631 (1979)). Results are shown in Figs. 3B-3C.

Example 3

Translocation of δ PKC in Cells Incubated with Insulin

Rat vascular smooth muscle cells passages three through seven were prepared as described in Example 1B. The cells were incubated with bovine insulin (100 nM) for time periods of up to 24 hours. Activation of various PKC isozymes was analyzed by harvesting the cells and separating the protein into cytosolic and membrane (particulate) fractions and examining both fractions by Western blot using anti- δ PKC, anti- α PKC, and anti- ϵ PKC for the presence of different PKC isoforms. The activity of the isoform was assessed as amount of PKC in the membrane fraction divided by the total amount of protein in both fractions (cytosol and membrane), normalized to control. The results are shown in Figs. 4A-4B.

Example 4

Gene Transcriptional Effects of Insulin *in vitro*

Rat vascular smooth muscle cells passages three through seven were prepared as describe in Example 1B. The cells were incubated with insulin (100 nm, SourceLife Technologies) or with the δ PKC inhibitor peptide δ V1-1, prepared as described in Example 1A. RNA isolation and purification were performed as described in Example 2 and TaqMan[®] real time PCR was used to analyze the relative quantity TGF- β 1 and core binding factor α -1 (Cbf α -1). PCR primers and probes were obtained commercially from Applied Biosystems, Inc. and the PCR was performed on an Applied Biosystems ABI Prism 7700. The results are shown in Figs. 5A-5C.

Example 5**Expression of Factors Associated with Vascular Fibrosis in
Zucker Obese and Lean Rats**

5 Aortas from Zucker obese rats and Zucker lean (Fa/fa) rats, 10-16 weeks of age, were isolated. RNA from the aortas was analyzed for expression of osteopontin according to the methods described in Examples 2 and 4 above. The aortas were also tested for elasticity via mechanical testing of stress-strain, as described in Example 2 above. The results are shown in Fig. 6 and Fig. 3B.

10 Although the invention has been described with respect to particular embodiments, it will be apparent to those skilled in the art that various changes and modifications can be made without departing from the invention.

IT IS CLAIMED:

1. A composition for preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue, comprising, a compound
5 effective to modulate the activity of δ PKC.
2. The composition of claim 1, wherein said compound has activity to inhibit δ PKC activity to reduce production of reactive oxygen species in said cell or tissue.
- 10 3. The composition of claim 1, wherein said compound has activity to activate δ PKC activity to stimulate production of reactive oxygen species in said cell or tissue.
4. The composition of claim 2, wherein said compound is a peptide having at
15 least 50% sequence identity to a peptide selected from the group consisting of δ V1-1 (SEQ ID NO:3), δ V1-2 (SEQ ID NO:4), and δ V1-5 (SEQ ID NO:6).
5. The composition of claim 4, wherein said peptide is selected from the group consisting of SEQ ID NO:3 (δ V1-1) and the peptides identified as SEQ ID
20 NOS:33-47.
6. The composition of claim 4, wherein said peptide is selected from the group consisting of δ V1-2 (SEQ ID NO:4) and the peptides identified as SEQ ID
25 NOS:64-70.
7. The composition of claim 3, wherein said compound is a peptide having at least 50% sequence identity to $\psi\delta$ RACK (SEQ ID NO:5).
8. The composition of claim 7, wherein said peptide is selected from the group
30 consisting of $\psi\delta$ RACK (SEQ ID NO:5) and the peptides identified as SEQ ID NOS:10-18, 21-32.

9. The composition according to any preceding claim wherein the peptide is linked to a moiety effective to facilitate transport across a cell membrane.

10. The composition according to claim 9, wherein said moiety is a Tat-derived peptide.

11. The composition according to any preceding claim for use in treating or slowing the progression of arteriosclerosis.

10 12. The composition according to any one of claims 1-10 for use in treating a disorder preceded by an increased production of reactive oxygen species in a cell or tissue.

13. The composition according to claim 12, wherein said disorder is an inflammatory disorder.

14. The composition according to claim 13, wherein said inflammatory disorder is associated with the vasculature.

20 15. The composition according to claim 12, wherein said inflammatory disorder is arthritis.

16. The composition according to claim 1, for use in stimulating production of reactive oxygen species in a cell or tissue, wherein said compound is effective to agonize the activity of δ PKC.

17. The composition according to claim 16, wherein said compound is a peptide having at least 50% sequence identity to $\psi\delta$ RACK (SEQ ID NO:5).

30 18. The composition according to claim 16 or claim 17, wherein said peptide is linked to a moiety effective to facilitate transport across a cell membrane.

19. The composition according to any one of claims 16-18, wherein said cell is a tumor cell.

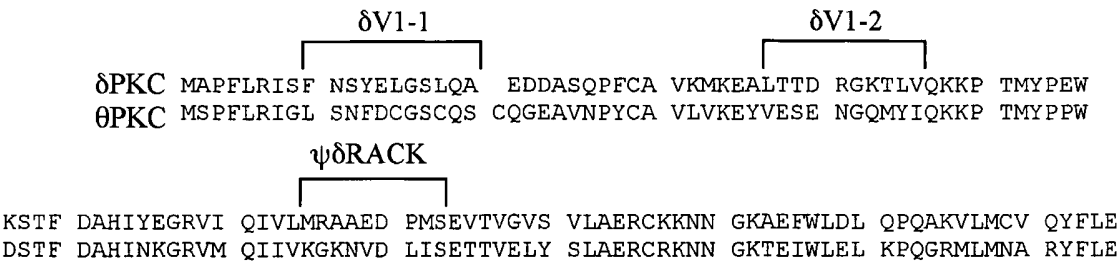


Fig. 1

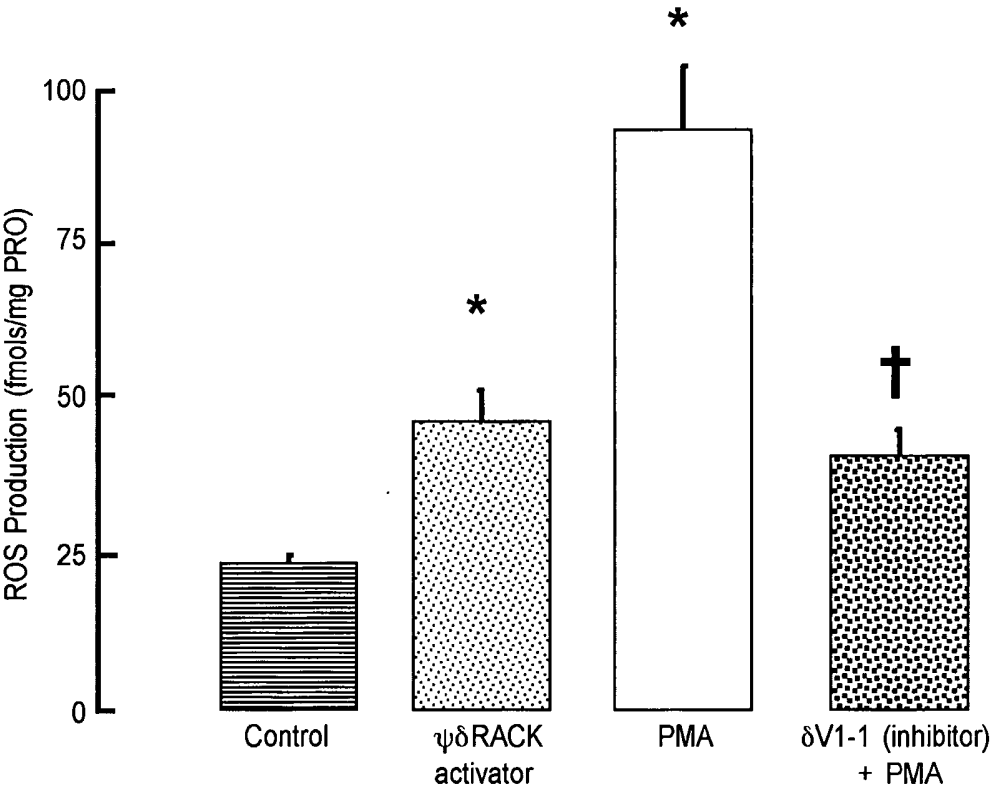
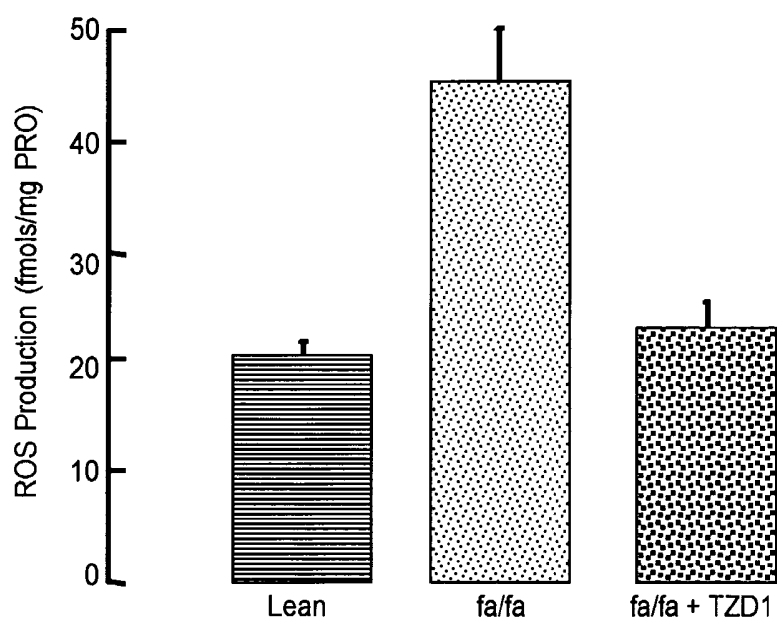
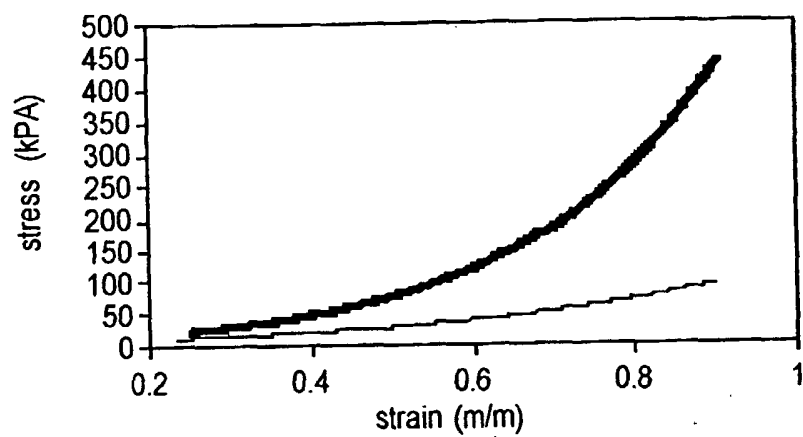
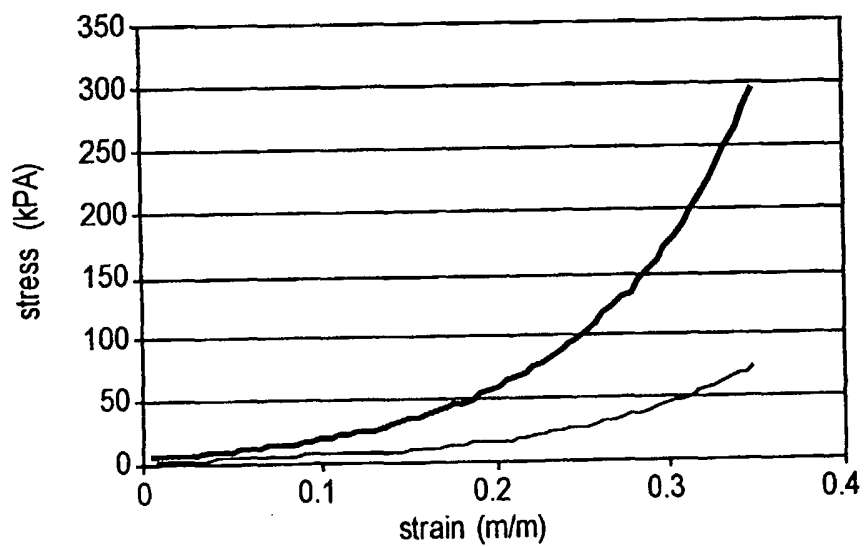


Fig. 2

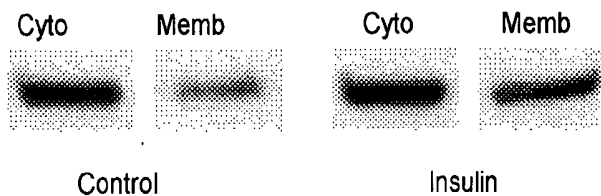
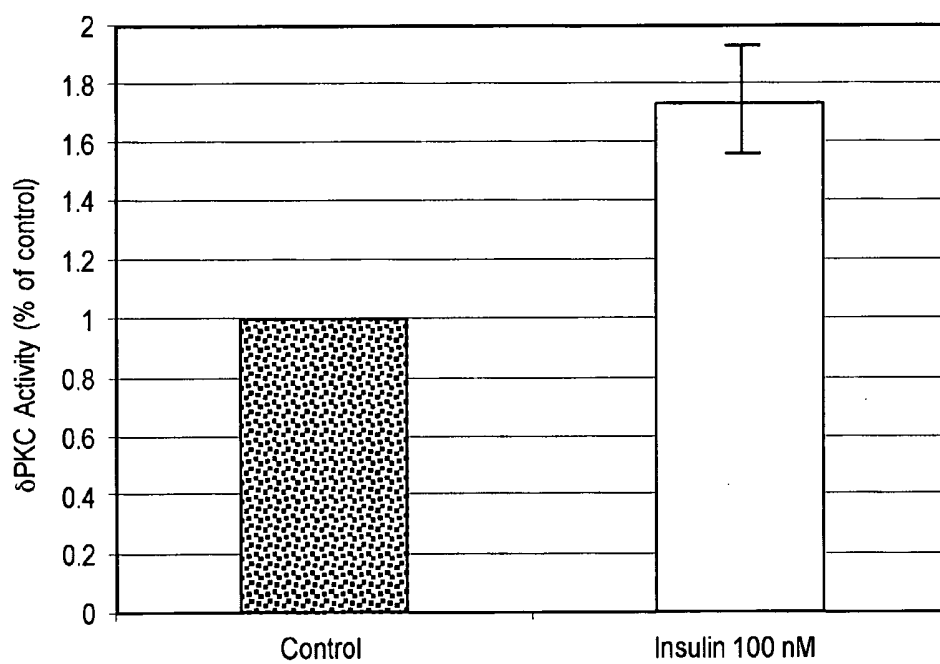
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**Fig. 3A**

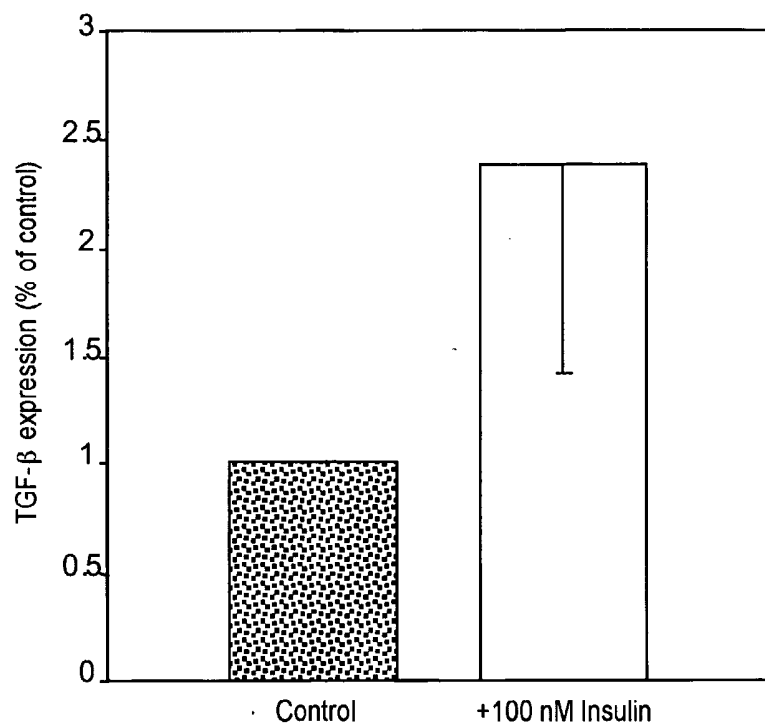
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**Fig. 3B****Fig. 3C**

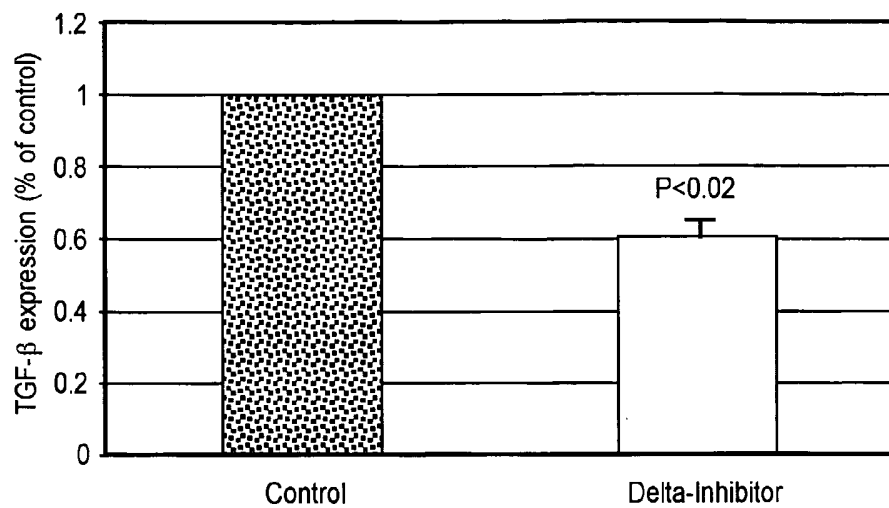
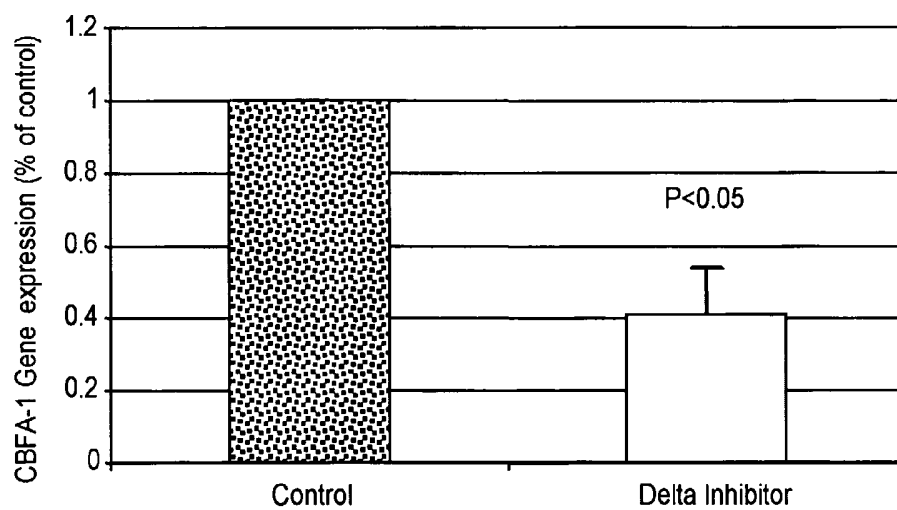
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**Fig. 4A****Fig. 4B**

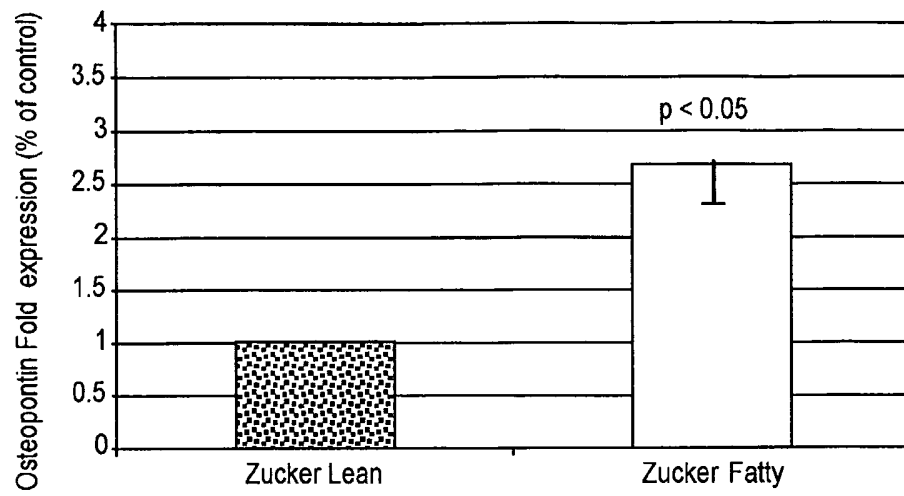
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**Fig. 5A**

6/7

**Fig. 5B****Fig. 5C**

7/7

**Fig. 6**

SEQUENCE LISTING

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Junior University
Tsao, Philip S.
Mochly-Rosen, Daria

<120> Use of DeltaPKC Peptides for Modulation of Reactive Oxygen Species

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 Asn Pro Lys Phe Glu His Leu Leu Glu Asp
 50 55

<210> 7
 <211> 17
 <212> PRT
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<220>
 <223> Drosophila Antennapedia homeodomain-derived
 carrier peptide

<400> 7

Cys Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys
1 5 10 15
Lys

<210> 8
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Tat-derived carrier peptide

<400> 8
Tyr Gly Lys Lys Arg Arg Gln Arg Arg Arg
1 5 10

<210> 9
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> beta-PKC-selective activator peptide

<400> 9
Ser Val Glu Ile Trp Asp
1 5

<210> 10
<211> 8
<212> PRT
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<220>
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<400> 10
Met Lys Ala Ala Glu Asp Pro Met
1 5

<210> 11
<211> 8
<212> PRT
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<220>
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<400> 11
Met Arg Gly Ala Glu Asp Pro Met
1 5

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<211> 8
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<220>
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<400> 12
Met Arg Ala Gly Glu Asp Pro Met
1 5

<210> 13
<211> 8
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<400> 13
Met Arg Ala Pro Glu Asp Pro Met
1 5

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<400> 14
Met Arg Ala Asn Glu Asp Pro Met
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<220>
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<400> 16
Met Arg Ala Ala Glu Asp Pro Val
1 5

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<220>
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<400> 17

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1 5

<210> 18

<211> 8

<212> PRT

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<220>

<223> modified pseudo-delta RACK peptide

<400> 18

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1 5

<210> 19

<211> 4

<212> PRT

<213> Rattus norvegicus

<400> 19

Glu Asp Pro Met
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<210> 20

<211> 5

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<213> Rattus norvegicus

<400> 20

Ala Glu Asp Pro Met
1 5

<210> 21

<211> 8

<212> PRT

<213> Artificial Sequence

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<400> 21

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<210> 22

<211> 8

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<400> 22

Met Glu Ala Ala Glu Asp Pro Met
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<210> 23

<211> 8

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<400> 23
Met Asp Ala Ala Glu Asp Pro Met
1 5

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<400> 24
Met Arg Ala Ala Glu Glu Pro Leu
1 5

<210> 25
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<220>
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<400> 25
Met Arg Ala Ala Glu Asp Pro Leu
1 5

<210> 26
<211> 8
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<220>
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<400> 26
Met Arg Ala Ala Glu Glu Pro Ile
1 5

<210> 27
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<220>
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<400> 27
Met Arg Ala Ala Glu Glu Pro Val
1 5

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<211> 8
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<220>
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<400> 28
Met Arg Ala Ala Glu Asp Pro Val
1 5

<210> 29
<211> 8
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<220>
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<400> 29
Met Arg Ala Ala Asn Asp Pro Met
1 5

<210> 30
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<400> 30
Met Arg Ala Ala Gln Asp Pro Met
1 5

<210> 31
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<400> 31
Met Arg Ala Ala Glu Gln Pro Met
1 5

<210> 32
<211> 8
<212> PRT
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<220>
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<400> 32
Met Arg Ala Ala Glu Asn Pro Met
1 5

<210> 33
<211> 10
<212> PRT
<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 33

Thr	Phe	Asn	Ser	Tyr	Glu	Leu	Gly	Ser	Leu
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<210> 34

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 34

Ala	Phe	Asn	Ser	Tyr	Glu	Leu	Gly	Ser	Leu
1			5						10

<210> 35

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 35

Ser	Phe	Asn	Ser	Tyr	Glu	Leu	Gly	Thr	Leu
1			5						10

<210> 36

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 36

Thr	Phe	Asn	Ser	Tyr	Glu	Leu	Gly	Thr	Leu
1			5						10

<210> 37

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 37

Ser	Tyr	Asn	Ser	Tyr	Glu	Leu	Gly	Ser	Leu
1			5						10

<210> 38

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 38
Ser Phe Asn Ser Phe Glu Leu Gly Ser Leu
1 5 10

<210> 39
<211> 9
<212> PRT
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<220>
<223> modified delta V1-1 peptide

<400> 39
Ser Asn Ser Tyr Asp Leu Gly Ser Leu
1 5

<210> 40
<211> 10
<212> PRT
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<220>
<223> modified delta V1-1 peptide

<400> 40
Ser Phe Asn Ser Tyr Glu Leu Pro Ser Leu
1 5 10

<210> 41
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> modified delta V1-1 peptide

<400> 41
Ser Phe Asn Ser Tyr Glu Ile Gly Ser Val
1 5 10

<210> 42
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> modified delta V1-1 peptide

<400> 42
Ser Phe Asn Ser Tyr Glu Val Gly Ser Ile
1 5 10

<210> 43
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> modified delta V1-1 peptide

<400> 43

Ser	Phe	Asn	Ser	Tyr	Glu	Leu	Gly	Ser	Val
1				5					10

<210> 44

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 44

Ser	Phe	Asn	Ser	Tyr	Glu	Leu	Gly	Ser	Ile
1				5					10

<210> 45

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 45

Ser	Phe	Asn	Ser	Tyr	Glu	Ile	Gly	Ser	Leu
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<210> 46

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 46

Ser	Phe	Asn	Ser	Tyr	Glu	Val	Gly	Ser	Leu
1				5					10

<210> 47

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-1 peptide

<400> 47

Ala	Phe	Asn	Ser	Tyr	Glu	Leu	Gly	Ser	Leu
1				5					10

<210> 48

<211> 6

<212> PRT

<213> Rattus norvegicus

<400> 48

Tyr	Glu	Leu	Gly	Ser	Leu
1				5	

<210> 49
<211> 6
<212> PRT
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<220>
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<400> 49
Tyr Asp Leu Gly Ser Leu
1 5

<210> 50
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> modified fragment of delta V1-1 peptide

<400> 50
Phe Asp Leu Gly Ser Leu
1 5

<210> 51
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> modified fragment of delta V1-1 peptide

<400> 51
Tyr Asp Ile Gly Ser Leu
1 5

<210> 52
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> modified fragment of delta V1-1 peptide

<400> 52
Tyr Asp Val Gly Ser Leu
1 5

<210> 53
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> modified fragment of delta V1-1 peptide

<400> 53
Tyr Asp Leu Pro Ser Leu
1 5

<210> 54

<211> 6
<212> PRT
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<220>
<223> modified fragment of delta V1-1 peptide

<400> 54
Tyr Asp Leu Gly Leu Leu
1 5

<210> 55
<211> 6
<212> PRT
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<220>
<223> modified fragment of delta V1-1 peptide

<400> 55
Tyr Asp Leu Gly Ser Ile
1 5

<210> 56
<211> 6
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<220>
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<400> 56
Tyr Asp Leu Gly Ser Val
1 5

<210> 57
<211> 4
<212> PRT
<213> Rattus norvegicus

<400> 57
Leu Gly Ser Leu
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<210> 58
<211> 4
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<220>
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<400> 58
Ile Gly Ser Leu
1

<210> 59
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<212> PRT
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<220>
<223> modified fragment of delta V1-1 peptide

<400> 59
Val Gly Ser Leu
1

<210> 60
<211> 4
<212> PRT
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<220>
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<400> 60
Leu Pro Ser Leu
1

<210> 61
<211> 4
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<220>
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<400> 61
Leu Gly Leu Leu
1

<210> 62
<211> 4
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<220>
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<400> 62
Leu Gly Ser Ile
1

<210> 63
<211> 4
<212> PRT
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<220>
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<400> 63
Leu Gly Ser Val
1

<210> 64
<211> 11
<212> PRT
<213> Artificial Sequence

<220>

<223> modified delta V1-2 peptide

<400> 64

Ala	Leu	Ser	Thr	Asp	Arg	Gly	Lys	Thr	Leu	Val
1				5					10	

<210> 65

<211> 11

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<213> Artificial Sequence

<220>

<223> modified delta V1-2 peptide

<400> 65

Ala	Leu	Thr	Ser	Asp	Arg	Gly	Lys	Thr	Leu	Val
1				5					10	

<210> 66

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-2 peptide

<400> 66

Ala	Leu	Thr	Thr	Asp	Arg	Gly	Lys	Ser	Leu	Val
1				5					10	

<210> 67

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-2 peptide

<400> 67

Ala	Leu	Thr	Thr	Asp	Arg	Pro	Lys	Thr	Leu	Val
1				5					10	

<210> 68

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-2 peptide

<400> 68

Ala	Leu	Thr	Thr	Asp	Arg	Gly	Arg	Thr	Leu	Val
1				5					10	

<210> 69

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-2 peptide

<400> 69

Ala Leu Thr Thr Asp Lys Gly Lys Thr Leu Val
 1 5 10

<210> 70

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> modified delta V1-2 peptide

<400> 70

Ala Leu Thr Thr Asp Lys Gly Lys Thr Leu Val
 1 5 10

<210> 71

<211> 320

<212> PRT

<213> Homo sapiens

<400> 71

Met Ala Gln Val Leu Arg Gly Thr Val Thr Asp Phe Pro Gly Phe Asp
 1 5 10 15
 Glu Arg Ala Asp Ala Glu Thr Leu Arg Lys Ala Met Lys Gly Leu Gly
 20 25 30
 Thr Asp Glu Glu Ser Ile Leu Thr Leu Thr Ser Arg Ser Asn Ala
 35 40 45
 Gln Arg Gln Glu Ile Ser Ala Ala Phe Lys Thr Leu Phe Gly Arg Asp
 50 55 60
 Leu Leu Asp Asp Leu Lys Ser Glu Leu Thr Gly Lys Phe Glu Lys Leu
 65 70 75 80
 Ile Val Ala Leu Met Lys Pro Ser Arg Leu Tyr Asp Ala Tyr Glu Leu
 85 90 95
 Lys His Ala Leu Lys Gly Ala Gly Thr Asn Glu Lys Val Leu Thr Glu
 100 105 110
 Ile Ile Ala Ser Arg Thr Pro Glu Leu Arg Ala Ile Lys Gln Val
 115 120 125
 Tyr Glu Glu Glu Tyr Gly Ser Ser Leu Glu Asp Asp Val Val Gly Asp
 130 135 140
 Thr Ser Gly Tyr Tyr Gln Arg Met Leu Val Val Leu Leu Gln Ala Asn
 145 150 155 160
 Arg Asp Pro Asp Ala Gly Ile Asp Glu Ala Gln Val Glu Gln Asp Ala
 165 170 175
 Gln Ala Leu Phe Gln Ala Gly Glu Leu Lys Trp Gly Thr Asp Glu Glu
 180 185 190
 Lys Phe Ile Thr Ile Phe Gly Thr Arg Ser Val Ser His Leu Arg Lys
 195 200 205
 Val Phe Asp Lys Tyr Met Thr Ile Ser Gly Phe Gln Ile Glu Glu Thr
 210 215 220
 Ile Asp Arg Glu Thr Ser Gly Asn Leu Glu Gln Leu Leu Ala Val
 225 230 235 240
 Val Lys Ser Ile Arg Ser Ile Pro Ala Tyr Leu Ala Glu Thr Leu Tyr
 245 250 255
 Tyr Ala Met Lys Gly Ala Gly Thr Asp Asp His Thr Leu Ile Arg Val
 260 265 270
 Met Val Ser Arg Ser Glu Ile Asp Leu Phe Asn Ile Arg Lys Glu Phe
 275 280 285
 Arg Lys Asn Phe Ala Thr Ser Leu Tyr Ser Met Ile Lys Gly Asp Thr
 290 295 300

Ser	Gly	Asp	Tyr	Lys	Lys	Ala	Leu	Leu	Leu	Leu	Cys	Gly	Glu	Asp	Asp
305					310					315					320

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/014886

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K38/08 A61K38/17 C07K7/06 C07K14/47 A61P29/00 A61P39/06 A61P19/02 A61P9/10		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K C07K A61P		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, CHEM ABS Data, WPI Data, PAJ, MEDLINE, EMBASE, BIOSIS, COMPENDEX		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	INAGAKI KOICHI ET AL: "Inhibition of delta-protein kinase C protects against reperfusion injury of the ischemic heart in vivo" CIRCULATION, AMERICAN HEART ASSOCIATION, DALLAS, TX, US, vol. 108, no. 19, 11 November 2003 (2003-11-11), pages 2304-2307, XP002307971 ISSN: 0009-7322 abstract discussion	1-5, 9-16,18, 19
X	WO 02/057413 A (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 25 July 2002 (2002-07-25) page 5, line 30 - page 7, line 14 ----- -/--	1-5, 9-16,18, 19
☒ Further documents are listed in the continuation of box C. ☒ Patent family members are listed in annex.		
* Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
Date of the actual completion of the international search	Date of mailing of the international search report	
26 July 2005	13. 10. 2005	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Pilling, S	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/014886

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEN LEON ET AL: "Opposing cardioprotective actions and parallel hypertrophic effects of deltaPKC and epsilonPKC" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 98, no. 20, 25 September 2001 (2001-09-25), pages 11114-11119, XP002307969 ISSN: 0027-8424 "peptide synthesis" on page 11114 Discussion -----	1-5, 9-16,18, 19
X	INAGAKI KOICHI ET AL: "Additive protection of the ischemic heart ex vivo by combined treatment with delta-protein kinase C inhibitor and epsilon-protein kinase C activator" CIRCULATION, AMERICAN HEART ASSOCIATION, DALLAS, TX, US, vol. 108, no. 7, 19 August 2003 (2003-08-19), pages 869-875, XP002307970 ISSN: 0009-7322 abstract -----	1-5, 9-16,18, 19
P,X	US 2004/259816 A1 (PANDOL STEPHEN J ET AL) 23 December 2004 (2004-12-23) paragraphs '0021!, '0022!, '0025! -----	1-5, 9-16,18, 19
A	WO 01/28543 A (MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH; GLEICH, GERALD, J) 26 April 2001 (2001-04-26) claims 1,10; example 2 -----	1-5, 9-16,18, 19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/014886

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5, 9-16, 18, 19 part

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-5, 9-16, 18,19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:3 or sequences with at least 50% identity

2. claims: 1-4, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:4 or sequences with at least 50% identity

3. claims: 1-4, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:6 or sequences with at least 50% identity

4. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:33 or sequences with at least 50% identity

5. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:34 or sequences with at least 50% identity

6. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:35 or sequences with at least 50% identity

7. claims: 1-3, 5, 9-16, 18, 19 part

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:36 or sequences with at least 50% identity

8. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:37 or sequences with at least 50% identity

9. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:38 or sequences with at least 50% identity

10. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:39 or sequences with at least 50% identity

11. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:40 or sequences with at least 50% identity

12. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:41 or sequences with at least 50% identity

13. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:42 or sequences with at least 50% identity

14. claims: 1-3, 5, 9-16, 18, 19 part

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:43 or sequences with at least 50% identity

15. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:44 or sequences with at least 50% identity

16. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:45 or sequences with at least 50% identity

17. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:46 or sequences with at least 50% identity

18. claims: 1-3, 5, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:47 or sequences with at least 50% identity

19. claims: 1-3, 6, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:64 or sequences with at least 50% identity

20. claims: 1-3, 6, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:65 or sequences with at least 50% identity

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

21. claims: 1-3, 6, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:66 or sequences with at least 50% identity

22. claims: 1-3, 6, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:67 or sequences with at least 50% identity

23. claims: 1-3, 6, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:68 or sequences with at least 50% identity

24. claims: 1-3, 6, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:69 or sequences with at least 50% identity

25. claims: 1-3, 6, 9-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:70 or sequences with at least 50% identity

26. claims: 1-3, 7-19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:5 or sequences with at least 50% identity

27. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:10 or sequences with at least 50% identity

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

28. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:11 or sequences with at least 50% identity

29. claims: 1-3, 8-16, 18, 19 partt

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:12 or sequences with at least 50% identity

30. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:13 or sequences with at least 50% identity

31. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:14 or sequences with at least 50% identity

32. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:15 or sequences with at least 50% identity

33. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:16 or sequences with at least 50% identity

34. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:17 or sequences with at least 50% identity

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

35. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in
modulating production of reactive oxygen species in a cell
or tissue comprising the peptide of SEQ ID NO:18 or
sequences with at least 50% identity

36. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in
modulating production of reactive oxygen species in a cell
or tissue comprising the peptide of SEQ ID NO:21 or
sequences with at least 50% identity

37. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in
modulating production of reactive oxygen species in a cell
or tissue comprising the peptide of SEQ ID NO:22 or
sequences with at least 50% identity

38. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in
modulating production of reactive oxygen species in a cell
or tissue comprising the peptide of SEQ ID NO:23 or
sequences with at least 50% identity

39. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in
modulating production of reactive oxygen species in a cell
or tissue comprising the peptide of SEQ ID NO:24 or
sequences with at least 50% identity

40. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in
modulating production of reactive oxygen species in a cell
or tissue comprising the peptide of SEQ ID NO:25 or
sequences with at least 50% identity

41. claims: 1-3, 8-16, 18, 19 part

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:26 or sequences with at least 50% identity

42. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:27 or sequences with at least 50% identity

43. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:28 or sequences with at least 50% identity

44. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:29 or sequences with at least 50% identity

45. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:30 or sequences with at least 50% identity

46. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:31 or sequences with at least 50% identity

47. claims: 1-3, 8-16, 18, 19 part

a composition for the preparation of a medicament for use in modulating production of reactive oxygen species in a cell or tissue comprising the peptide of SEQ ID NO:32 or sequences with at least 50% identity

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Information on patent family members

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