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(54) **SYNTHESIS, MODIFICATION AND REDUCTION OF PRIMARY STRUCTURE OF HYPOTENSIVE PEPTIDES PRESENT IN SCORPION VENOM FOR OPTIMIZING THEIR USE AS A HYPOTENSIVE MEDICAMENT**

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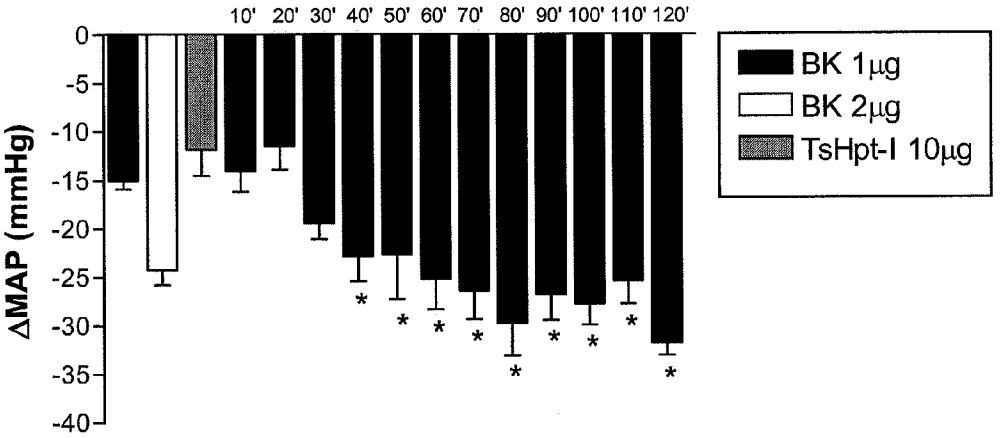
A61K 38/08	(2006.01)

(52) **U.S. Cl.** **514/15**; 514/16; 514/17; 514/18; 530/328; 530/329; 530/330; 530/331

(57) **ABSTRACT**

The present invention relates to synthetic and recombinant peptide primary structures including an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 12, wherein the primary structure includes an +aa-Pro-Pro amino acid in which "+aa" is Lys, Arg, His or a modified amino acid having a positive charge at physiological pH. The present invention also relates to a pharmaceutical composition including at least one peptide having a primary structure as defined above.

FIG. 1.1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0
-5
-10
-15
-20
-25
-30
-35
-40
ΔMAP (mmHg)
BK 1µg
BK 2µg
TsHpt-I 10µg

FIG. 1.2

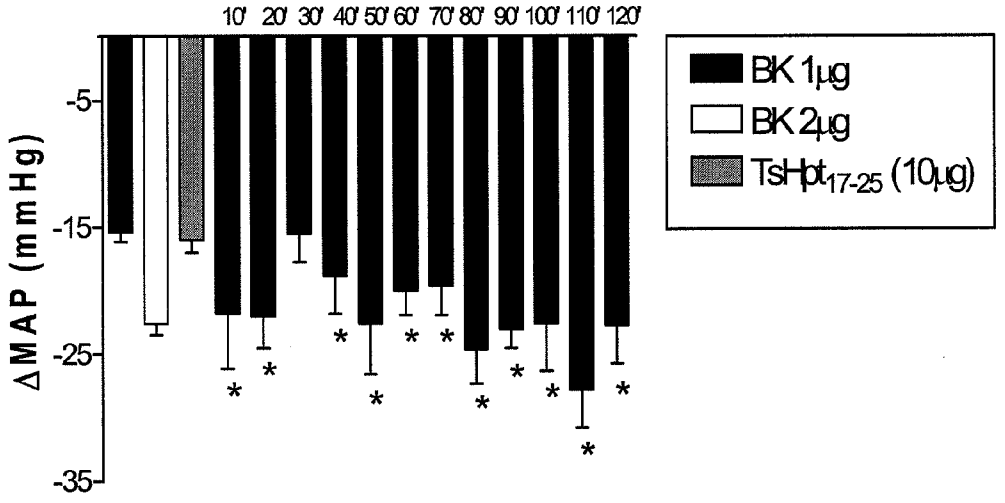

10' 20' 30' 40' 50' 60' 70' 80' 90' 100' 110' 120'
-5
-15
-25
-35
ΔMAP (mmHg)
BK 1µg
BK 2µg
TsHpt17-25 (10µg)

FIG. 1.3

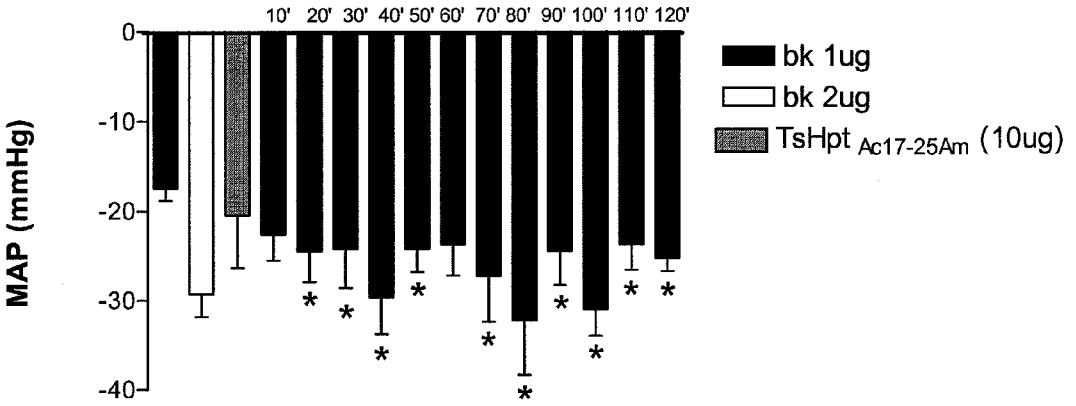
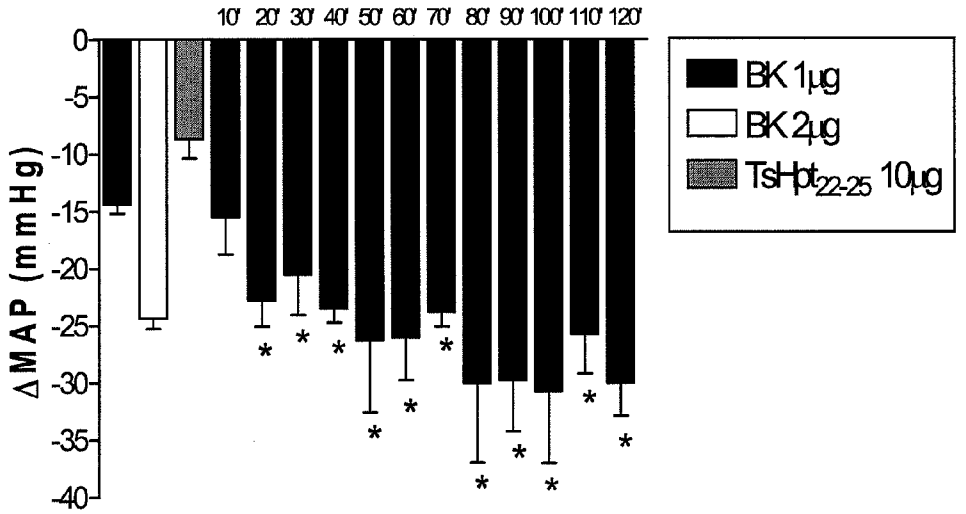

10' 20' 30' 40' 50' 60' 70' 80' 90' 100' 110' 120'
0
-10
-20
-30
-40
MAP (mmHg)
bk 1ug
bk 2ug
TsHpt Ac17-25Am (10ug)

FIG. 1.4


10' 20' 30' 40' 50' 60' 70' 80' 90' 100' 110' 120'
0
-5
-10
-15
-20
-25
-30
-35
-40
ΔMAP (mmHg)
BK 1μg
BK 2μg
TsHpt22-25 10μg

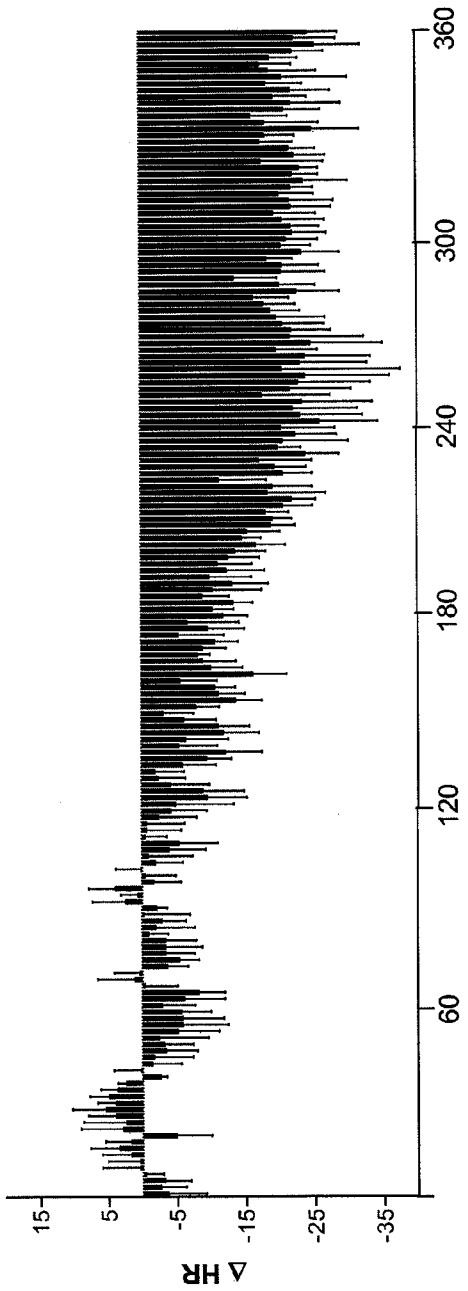

15
5
-5
-15
-25
-35
Δ HR
60
120
180
240
300
360
Time (min.)

FIG. 2

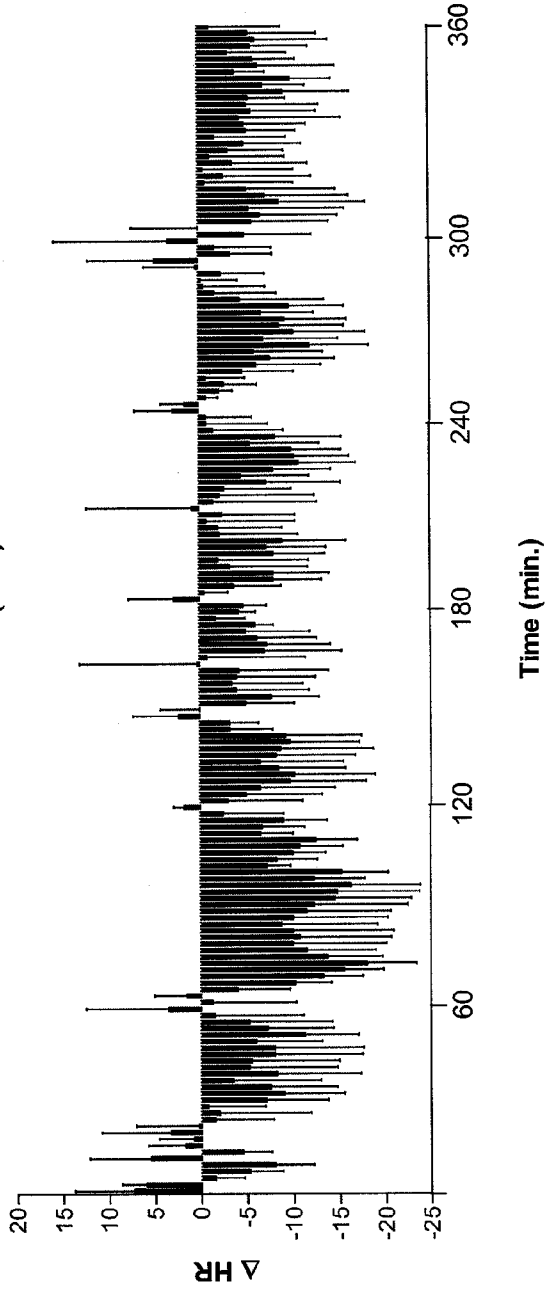

20
15
10
5
0
-5
-10
-15
-20
-25
Δ HR
60
120
180
240
300
360
Time (min.)

FIG. 3

FIG. 4.1

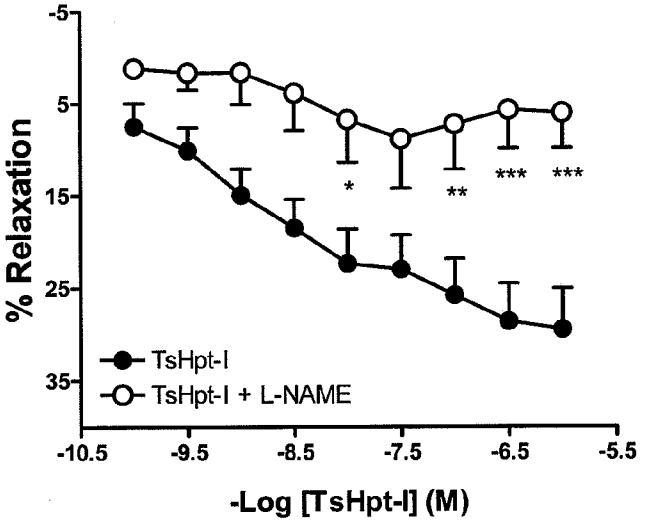


FIG. 4.2

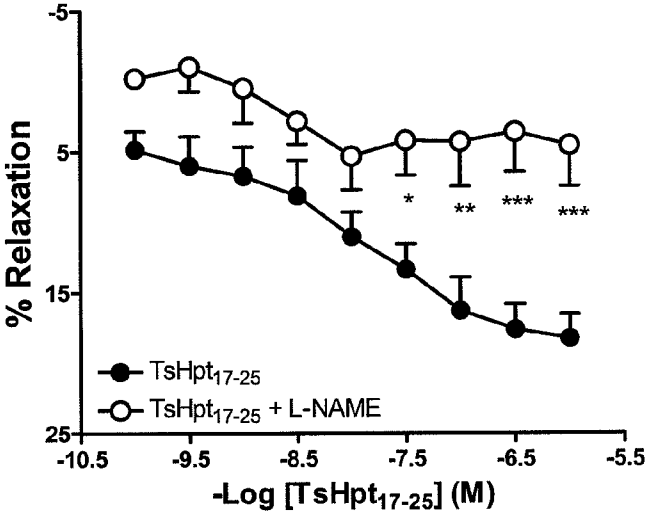
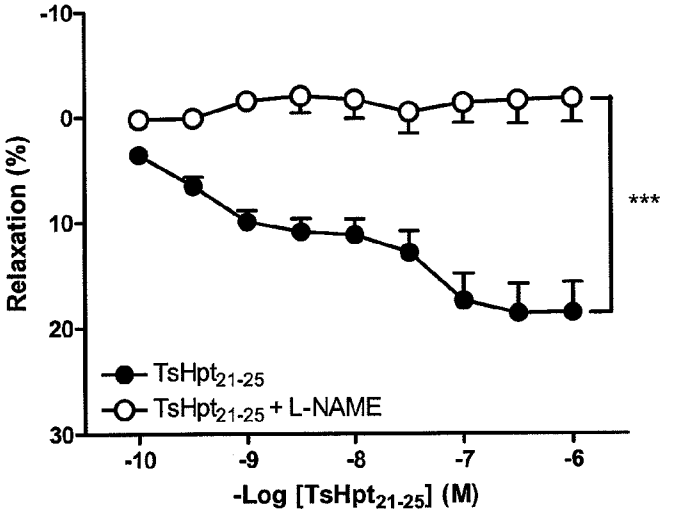


FIG. 4.3



SYNTHESIS, MODIFICATION AND REDUCTION OF PRIMARY STRUCTURE OF HYPOTENSIVE PEPTIDES PRESENT IN SCORPION VENOM FOR OPTIMIZING THEIR USE AS A HYPOTENSIVE MEDICAMENT

FIELD OF THE INVENTION

[0001] The present invention relates to the synthesis, modification and reduction of the primary structure of arterial hypotension inducing peptides (hypotensive peptides) named *Tityus serrulatus* Hypotensins I to IV found in yellow scorpion *Tityus serrulatus*.

BACKGROUND OF THE INVENTION

[0002] Hypertension is an important challenge for the international public health authorities due to its high occurrence in modern society, in addition to the cardiovascular and renal risks derived therefrom. It is estimated that approximately 26% of the world population, that is, 972 million people suffered from hypertension in year 2000.

[0003] Blood pressure, or arterial pressure, is measured as systolic (pressure of the blood in the arteries when the ventricular systoles occurs) and diastolic (when diastole occurs). According to World Health Organization (WHO) the optimal blood pressure is less than 120 systolic and 80 diastolic (120/80 mmHg). High blood pressure, or hypertension, is considered to be a pressure greater than or equal to 140/90 mmHg and "high normal" blood pressure is between 130/85 and 140/90 mmHg (Bulletin of the World Health Organization, 1999).

[0004] Hypertension may be classified as primary and secondary. Primary or essential hypertension does not have a specific cause, being the most common form of hypertension and being presented by 95% of the patients. Genetic factors seem to be one of the major causes of this kind of hypertension.

[0005] The causes of secondary hypertension are known and pregnancy, cirrhosis and renal disorders may contribute for a temporary hypertensive condition. Some medicaments such as cortisone and estrogen may also temporarily increase the blood pressure. Prolonged use of anti-inflammatory drugs (NSAIDs) such as aspirin may cause renal disorders and can also affect the treatment of individuals suffering from hypertension who make use of anti-hypertensive drugs such as β-blockers and diuretics. It is known that cocaine causes acute hypertension conditions although apparently it does not cause a chronic condition. A study shows that about 10% of the cases related to hypertension are caused by the abuse of alcohol ingestion. Caffeine causes a temporary increase of blood pressure. However, studies show that its regular consumption increases the risks of heart diseases in health individuals. The dangers associated with caffeine, however, cannot be compared to smoking, which may increase the risk of death by cardiomyopathy and hypertension. Blood pressure may also temporarily increase due to stress or physical activities.

[0006] At the present time, five classes of drugs are recognized as efficient for hypertensive treatment: diuretics, alpha-blockers, beta-blockers, angiotensin converting enzyme (ACE) inhibitors, calcium antagonists, and angiotensin II antagonists.

[0007] Diuretics cause the body to excrete water and salt, thus decreasing the blood plasma volume and, consequently, decreasing the blood pressure. ACE inhibitors reduce the production of angiotensin-II and reduce the degradation of bradykinin. Beta-blockers inhibit the increase of the heart rate caused by the exciting effect of noradrenaline. Vasodilators expand blood vessels and calcium channel blockers help decrease the contractions of the heart.

[0008] All drugs used for hypertension have side effects and, therefore, there is still a need for novel anti-hypertensive drug classes.

[0009] The use of peptides as active ingredients for medicaments is a promising application of biotechnology. The major advantages rely on the selectivity and affinity of those molecules, as well as on the possible decrease of side effects and toxicity that result from chemical intermediates and/or metabolites. There are, on the other hand, important drawbacks that must be overcome for using those molecules such as their size that has influence on their absorption and distribution, as well as the resistance thereof against enzymatic hydrolysis and degradation in vivo.

[0010] Presently known anti-hypertensive peptides are isolated from animal venoms and have a primary structure comprising from 5 to 13 amino acid residues. Such peptides have anti-hypertensive activity based on the inhibition of angiotensin converting enzyme (ACE) that prevents the hydrolysis of bradykinin (a hypotensive agent) and the synthesis of angiotensin-II (a hypertensive agent), thereby acting as anti-hypertensive peptides.

[0011] Scorpion Hypotensive Peptides (SHptP) named TsHpt-I, TsHpt-II, TsHpt-III and TsHpt-IV, which are disclosed in patent application U.S. Ser. No. 10/517,097 refer to novel anti-hypertensive peptides found in animal origin venom. They i) consist of 24 to 25 amino acid residues, not crosslinked by disulfide bridges (absence of cysteine) and having molecular weights in the range of 2500 to 3000 Da; ii) have a molecular signature with amino acid residues Pro-Pro or Pro-Pro-Ala in their carboxy-terminal ends; and iii) induce a potent, long and sustained arterial hypotension, with one of the action mechanisms thought to be the potentialization of bradykinin occurring independently from the inhibition of the angiotensin converting enzyme.

SUMMARY OF THE INVENTION

[0012] The present invention relates to synthetic peptide primary structures comprising an amino acid sequence selected from the group of SEQ ID NOs:1 to 12, wherein said primary structure comprises an +aa-Pro-Pro amino acid in which "+aa" is Lys, Arg, His or any other modified amino acid that has a positive charge at physiological pH.

[0013] In another embodiment, the present invention also relates to a recombinant peptide primary structure as defined above prepared by recombinant techniques in heterologous expression systems such as viral systems, bacterial systems, fungal systems or in any other expression systems in eukaryotic or prokaryotic cells, or combinations thereof.

[0014] Still in accordance with another embodiment the present invention relates to a pharmaceutical composition comprising at least one peptide having a primary structure as defined above.

[0015] The present invention also relates to a method for treating hypertension, comprising administering to an animal at least one peptide of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The invention will be described in conjunction with the following drawings wherein:

[0017] FIG. **1.1** is a graph showing the effect on blood pressure of the injection in bolus (i.v.) of TsHpt-I (35 μg/Kg) (grey bar), the injection in bolus (i.v.) of 1 μg of bradykinin (BK) (black bars), and 2 μg of BK (white bar) (n=5; *P<0.05);

[0018] FIG. **1.2** is a graph showing the effect on blood pressure of the injection in bolus (i.v.) of $TsHpt_{17-25}$ (37 μg/Kg) (grey bar), the injection in bolus (i.v.) of 1 μg of BK (black bars) and 2 μg of BK (white bar) (n=5; *P<0.05);

[0019] FIG. **1.3** is a graph showing the effect on blood pressure of the injection in bolus (i.v.) of $TsHpt_{ac17-25am}$ peptide with a N-terminal acetylation and C-terminal amidation (43 μg/Kg) (grey bar), the injection in bolus (i.v.) of 1 μg of BK (black bars) and 2 μg of BK (white bar) (n=4; *P<0.05);

[0020] FIG. **1.4** is a graph showing the effect on blood pressure of the injection in bolus (i.v.) of $TsHpt_{22-25}$ (37 μg/Kg) (grey bar), the injection in bolus (i.v.) of 1 μg of BK (black bars) and 2 μg of BK (white bar) (n=4; *P<0.05);

[0021] FIG. **2** is a graph showing the effect on mean arterial pressure (MAP) in hypertensive rats SHR (n=4) of the injection (i.v.) of 100 μg of peptide $TsHpt_{17-25}$ (333 μg/Kg);.

[0022] FIG. **3** is a graph showing the effect on mean arterial pressure in hypertensive rats TGR (n=4) of the injection (i.v.) of 100 μg of peptide $TsHpt_{17-25}$ (333 μg/Kg);

[0023] FIG. **4.1** is a graph showing the vasodilation effect of peptide TsHpt-I on rings isolated from aorta in absence (black circles) and presence (white circles) of L-NAME ((n=4) *P<0.05; **P<0.01; ***P<0.001);

[0024] FIG. **4.2** is a graph showing the vasodilation effect of peptide $TsHpt_{17-25}$ on rings isolated from aorta in absence (black circles) and presence (white circles) of L-NAME ((n=4) *P<0.05; **P<0.01; ***P<0.001); and

[0025] FIG. **4.3** is a graph showing the vasodilation effect of peptide $TsHpt_{21-25}$ on rings isolated from aorta in absence (black circles) and presence (white circles) of L-NAME ((n=4) ***P<0.001).

DETAILED DESCRIPTION OF THE INVENTION

[0026] In order to reduce the native structure of the hypotensive peptides TsHpt-I, TsHpt-II, TsHpt-III and TsHpt-IV, the inventors developed a synthesis based on the Fmoc solid phase technique (see, e.g., CHAN, W. C & WHITE, P. D. "Fmoc solid-phase peptide synthesis. A practical approach." Oxford University Press; 2000) to synthesize peptides analogous to TsHpt, the structures of which were reduced to a minimum functional structure. Such minimization aims also to decrease production costs, to optimize their absorption and distribution of the active ingredient in the individual organism and to optimize their protection against enzymatic hydrolysis and degradation in vivo.

[0027] As the native TsHpts have a structure with 24 or 25 amino acid residues the primary sequence of which has 2 proline residues in its carboxy-terminal portion, the inventors developed a synthesis of peptides analogous to the native molecules based on their carboxy-terminal portion and obtained the following synthetic amino acid sequences 1 to 12:

SEQ ID NO:1 ($TsHpt_{17-25}$):
1 5 9
Lys-Glu-Thr-Asn-Ala-Lys-Pro-Pro-Ala

SEQ ID NO:2 ($TsHpt_{18-25}$):
1 5 8
Glu-Thr-Asn-Ala-Lys-Pro-Pro-Ala

SEQ ID NO:3 ($TsHpt_{19-25}$):
1 5 7
Thr-Asn-Ala-Lys-Pro-Pro-Ala

SEQ ID NO:4 ($TsHpt_{20-25}$):
1 5 6
Asn-Ala-Lys-Pro-Pro-Ala

SEQ ID NO:5 ($TsHpt_{21-25}$):
1 5
Ala-Lys-Pro-Pro-Ala

SEQ ID NO:6 ($TsHpt_{22-25}$):
1 4
Lys-Pro-Pro-Ala

SEQ ID NO:7 ($TsHpt_{17-24}$):
1 5 8
Lys-Glu-Thr-Asn-Ala-Lys-Pro-Pro

SEQ ID NO:8 ($TsHpt_{18-24}$):
1 5 7
Glu-Thr-Asn-Ala-Lys-Pro-Pro

SEQ ID NO:9 ($TsHpt_{19-24}$):
1 5 6
Thr-Asn-Ala-Lys-Pro-Pro

SEQ ID NO:10 ($TsHpt_{20-24}$):
1 5
Asn-Ala-Lys-Pro-Pro

SEQ ID NO:11 ($TsHpt_{21-24}$):
1 4
Ala-Lys-Pro-Pro

SEQ ID NO:12 ($TsHpt_{22-24}$):
1 3
Lys-Pro-Pro

[0028] The above peptide primary structures of the invention comprise +aa-Pro-Pro amino acids wherein "+aa" is Lys, Arg, His or any other modified amino acid that has a positive charge in physiological pH.

[0029] The insertion of chemical modifications such as acetylation, cyclization, amidation, amongst others, in the amine- and carboxy-terminal ends and/or in the side chains have the aim of providing a higher level of protection of the primary structure against enzymatic hydrolysis and degradation that may increase the active ingredient plasmatic half-life leading to a longer pharmacological effect.

[0030] The hypotensive effect of the synthetic analogues represented by SEQ ID NOs:1 to 12 as well as native peptide

TsHpt-I demonstrate by in vivo assays a capacity for inducing hypotension independently from the addition of bradykinin and from the inhibition of angiotensin converting enzyme. Those effects could be observed through, for example, the intravenous injection of those compounds in normotensive Wistar rats, that is, rats having blood pressure at acceptable levels, at doses varying in a range of 35 to 43 μg/kg as shown in FIGS. **1.1** to **1.5**. The experiment was also carried out with groups formed by rats with higher blood pressure from the strains SHR (Spontaneous Hypertensive Rats) and TGR(mRENs)27 (Transgenic Hypertensive Rats). The results are shown in FIGS. **2** and **3**, respectively.

[**0031**] In the normotensive rats the intravenous injection of both native and synthetic peptides led to an immediate decrease of the mean arterial pressure of 5 to 20 mmHg independently from the addition of bradykinin. The decrease of the mean arterial pressure due to administration of bradykinin (bradykinin potentializing effect) in those cases remains for more than 120 minutes.

[**0032**] In the rats of hypertensive strains (STR and TGR(mRENs)27) the mean arterial pressure decrease induced by intravenous injection of synthetic peptides, independently from administration of bradykinin, was measured for 6 hours with values varying from 5 to 35 mmHg. In vitro assays using preparations with aortic vessels isolated from male Wistar rats showed that synthetic peptides are capable of inducing vasodilatation dependent from production of nitric oxide, which explains its hypertensive effect (see FIGS. **4.1** to **4.4**).

[**0033**] Hypotensive effects independent from bradykinin and from inhibition of ACE as those observed for native peptide TsHpt-I for its synthetic analogues, confirm a enhanced pharmacological action in relation to other bradykinin potentializing peptides identified, isolated and studied up to this moment and place those new peptides in a new class of hypotension inducing peptides present in animal venoms.

[**0034**] It is known that one of the major problems for developing proteic drugs is their low stability in the gastrointestinal tract and their poor absorption by the intestinal cells. However, the synthetic peptide of SEQ ID NO:5, for example, was able to induce a prolonged reduction of blood pressure after oral administration at a dose of 2.5 mg/Kg of rat body weight), demonstrating that the molecule is resistant to the action of proteolytic enzymes present in the gastrointestinal tract, in addition to being properly absorbed in the mucosa thereof.

[**0035**] The peptide primary structures of the present invention may be prepared by recombinant techniques in heterologous expression systems such as viral systems, bacterial systems, fungal systems or in any other expression systems in eukaryotic or prokaryotic cells, or combinations thereof.

[**0036**] The peptide primary structures of the present invention are useful as active ingredients in pharmaceutical compositions.

EXAMPLES

[**0037**] Peptide Synthesis (Fmoc Route) and Chemical Modification of Peptides

[**0038**] Peptides were synthesized using the Fmoc/t-butyl route of synthesis on a solid support (CHAN, W. C & WHITE, P. D. "Fmoc solid-phase peptide synthesis. A practical approch." Oxford University Press; 2000).

[**0039**] In the end of the synthesis process the peptides were cleaved from resins by using the following cleavage solution: TFA:TES:H_2O (95:2.5:2.5 v:v:v). After the cleavage reactions were completed, TFA was removed by bubbling N_2 into the reaction tubes. Then, the peptides were washed six times with cold diisopropylic ether which is responsible for the removal of exceeding protecting groups and carbo-cations sequestrants, in addition to causing the precipitation of the peptides. The peptides were then extracted from the resins by means of washings with water, followed by filtration through a porous plate. This procedure was immediately followed by lyophilization of the resulting material.

[**0040**] After being lyophilized the peptides were purified by high performance liquid chromatography (HPLC) in a Äkta Explorer system, in a reverse phase column (C-18 or other). The peptides thus obtained were analyzed by mass spectrometry for quality control.

[**0041**] As example of a chemical modification of the synthetic peptides, the amino-terminal and carboxi-terminal of $TsHpt_{17-25}$ (SEQ ID NO:1) were modified. The amino-terminal was acetylated using an acetic anhydride: diclorometane solution (1:1, v/v). The carboxi-terminal of $TsHpt_{17-25}$ (SEQ ID NO:1) was amidated using the Rink Amide resin to synthesized this peptide, since after the cleavage of the peptide-resin bond, this resin causes the amidation of the carboxi-terminal of the peptide.

[**0042**] Surgical Procedures

[**0043**] Rats were subjected to a surgery for implanting polyethylene cannules into femoral vessels 24 hours before the experiments. The cannules were prepared from polyethylene with two different diameters (PE50 and PE10), fused by heating and filled with a solution of NaCl 0.9% w/v. The cannule of the femoral artery was intended to register the mean arterial pressure (MAP) and the one introduced in the femoral vein was used for administration of the peptides to be tested. The cannules were exteriorized in the interscapular of the animals.

[**0044**] Experiments for Potentialization of Hypotensive Effect of BK (FIGS. **1.1** to **1.4**)

[**0045**] The peptides were diluted from a stock solution (1 mg/mL) with a solution of NaCl 0.9% w/v for injection at 10 μg/animal body (approximately 38 μg/Kg of animal body) resulting in a final volume of 200 μL of solution.

[**0046**] Firstly 1 μg of bradykinin (BK) was injected in a final volume of 100 μL. Later, 2 μg of bradykinin were injected with the same final volume, and the ΔMAP was calculated again. The peptides were, then, administrated intravenously and the MAP variation was measured.

[**0047**] MAP variation (ΔMAP) was calculated by means of the following equation:

$$\Delta MAP = MAP_{final} - MAP_{initial}$$

[**0048**] After administration of the peptide, 1 μg of BK was injected again in intervals of 10 minutes for 120 minutes and the ΔMAP was calculated to evaluate whether that variation is near a BK control (1 or 2 μg).

[0049] As an example, the scorpion's native peptide TsHpt-I was injected in bolus (35 μg/Kg), single dose, in normotensive male Wistar rats—weighting 250 to 300 g. After 40 minutes of the intravenous administration (i.v.), the peptide was able to double the effect of a single dose of BK (1 μg). This effect was observed up to 2 hours after the administration. A rapid and strong hypotensive response was observed after the injection of TsHpt-I (FIG. **1.1**). The synthetic peptides comprising all claimed sequences (SEQ ID NO:1 to SEQ ID NO:12) were also submitted to BK potentiation in vivo tests. As examples, FIGS. **1.2** to **1.4** show TsHpt-I analogs (SEQ ID NO:1 and SEQ ID NO:6) when injected, in bolus, in normotensive male Wistar rats—weighting 245 to 300 g. Those analogs were also able to induce a hypotensive response independent of BK in the same way as TsHpt-I. Although these synthetic analogs were able to potentiate the BK hypotension, these effects could be observed earlier. While TsHpt-I started to potentiate BK within 40 minutes (FIG. **1.1**), the analog $TsHpt_{17-25}$ (SEQ ID NO:1) started its action within 10 minutes (FIG. **1.2**). As an example of peptide structure modification, an analog to $TsHpt_{17-25}$ (SEQ ID NO:1) was synthesized with an acetylation of its N-terminal residue and an amidation of its C-terminal residue. As seen from FIG. **1.3**, this analog is also able to induce both BK potentiation and BK independent hypotension.

[0050] Experiments for Evaluation of Hypotensive Effect of Synthetic Peptides in Hypertensive Rats (SHR and TGR) (FIGS. **2** and **3**)

[0051] As a control condition MAP and heart rate of animals were observed and noted at intervals of 2 minutes during 1 hour. In the event MAP figures were lower than 150 mmHg, the animal was not considered as hypertensive and the experiment was interrupted. If MAP values were equal of higher than 150 mmHg, the peptides were administrated intravenously (100 μg/rat) and the MAP profile and heart rate (HR) of the animals were monitored for 6 hours with measurements of MAP and HR at each 2 minutes.

[0052] As example, the peptide $TsHpt_{17-25}$ (SEQ ID NO:1) (303 μg/Kg) was intravenous injected in SHR hypertensive rats. FIG. **2** shows that this peptide could quickly reduce the MAP, followed by a rise of this parameter. Approximately 40 minutes after the peptide administration, a strong and long-lasting hypotension could be observed. Differently from the results obtained in SHR rat strain, when the synthetic analog $TsHpt_{17-25}$ (SEQ ID NO:1) (223 μg/Kg) was administered (i.v.) into TGR rats, a brief raise in the MAP was observed, followed by a rapid and temporary decrease in the MAP. About 24 minutes after the peptide injection, a strong and long-lasting decrease in MAP was observed, with few sudden raises in blood pressure (FIG. **3**).

[0053] Evaluation of Vasodilating Effect in Rats Aorta Rings (FIGS. **4.1** to **4.4**)

[0054] After being sacrificed, male Wistar rats had their thoracic aorta carefully removed, isolated from its fat and conjunctive tissue, and incubated with Krebs-Henseleit solution (mmol/L): NaCl 110.8; KCl 5.9; $NaHCo_3$ 25.0; $MgSO_4$ 1.07; $CaCl_2$ 2.49; KH_2PO_4 2.33; glucose 11.51. The aorta was, then, cut into rings (3-4 mm) that were connected to metallic rod coupled with isometric transducers. This preparation was kept in Krebs-Henseleit solution aired with carbogen (95% O_2 and 5% CO_2). Tension (1 g) was regulated during the first hour for stabilizing the preparation, the solution being changed at intervals of 15 minutes to avoid the accumulation of metabolism products. After the stabilization period, the vessels were pre-contracted with phenylephrine (0.1 μM) and, during the tonic contraction phase, cumulative concentration-answer curves with the synthetic peptides (10^{-10} at 3×10^{-7}) were built.

[0055] As an example, endothelium-containing aortic rings pre-contracted with phenylephrine, the synthetic TsHpt-I (FIG. **4.1**) and its analogs $TsHpt_{17-25}$ (SEQ ID NO:1—FIG. **4.2**) and $TsHpt_{21-25}$ (SEQ ID NO:5—FIG. **4.3**) produced a concentration-dependent vasodilator effect. To study the participation of nitric oxide (NO) on the relaxation induced by TsHpt-I (FIG. **4.1**) and its analogs $TsHpt_{17-25}$ (SEQ ID NO:1—FIG. **4.2**) and $TsHpt_{21-25}$ (SEQ ID NO:5—FIG. **4.3**), experiments were performed in the presence of L-NAME (100 μM), an inhibitor of NO synthase. After the blockade of NO synthase, the endothelium-dependent relaxation induced by TsHpt-I and $TsHpt_{17-25}$ were partially inhibited (FIGS. **4.1** and **4.2**), while the relaxation induced by analog $TsHpt_{21-25}$ was completely inhibited (FIG. **4.3**).

[0056] Telemetry and Gavage in SHR Rats

[0057] A radio transducer was implanted in the stomach of hypertensive rats (SHR strain) for measuring, amongst other variables, the heart rate (HR) and mean arterial pressure (MAP).

[0058] The synthetic peptides were resuspended in saline solution (0.9% NaCl w/v) and orally administrated as a single dose (2.5 mg/KG; 0.1 mL per 100 g rat body weight). MAP and HR were measure by telemetry after the synthetic analog $TsHpt_{21-25}$ (SEQ ID NO:5) was administrated by gavage. This analog was able to induce a reduction on MAP that was maintained regular up to 72 hours.

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What is claimed is:

1. A peptide comprising at least one member selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11 SEQ ID NO:12, and analogs thereof, wherein each of the analogs has a primary structure identical to one of SEQ ID NOs: 1-12 with a substitute amino acid substituted for a lysine closest to a carboxy-terminus of the peptide, said substitute amino acid having a positive charge at physiological pH.

2. The peptide of claim 1, wherein the peptide possesses anti-hypertensive activity.

3. The peptide of claim 1, containing less than 24 amino acids.

4. The peptide of claim 1, consisting of one member of the group.

5. The peptide of claim 1, wherein the peptide is synthetic.

6. The peptide of claim 1, wherein the peptide is recombinant.

7. A pharmaceutical composition comprising at least one peptide of claim 1 and a pharmaceutically acceptable carrier.

8. The pharmaceutical composition of claim 7, wherein the at least one peptide is present in the composition in an anti-hypertensive amount.

9. The pharmaceutical composition of claim 7, wherein the at least one peptide contains less than 24 amino acids.

10. The pharmaceutical composition of claim 7, wherein the at least one peptide consists of one member of the group.

11. The pharmaceutical composition of claim 7, wherein the at least one peptide is synthetic.

12. The pharmaceutical composition of claim 7, wherein the at least one peptide is recombinant.

13. The pharmaceutical composition of claim 7, wherein the at least one peptide comprises SEQ ID NO: 1.

14. The pharmaceutical composition of claim 7, wherein the at least one peptide comprises SEQ ID NO: 5.

15. The pharmaceutical composition of claim 7, wherein the at least one peptide comprises SEQ ID NO: 6.

16. A method for treating hypertension, comprising administering to an animal an anti-hypertensive amount of at least one peptide of claim 1.

* * * * *