PAP045

SPECIFICATION

THE USE OF DES-ASPARTATE-ANGIOTENSIN I (ARG-VAL-TYR-ILE-HIS-PRO-PHE-HIS-LEU) AS AN ANTI-CARDIAC HYPERTROPHIC AGENT

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THE USE OF DES-ASPARTATE-ANGIOTENSIN I (ARG-VAL-TYR-ILE-HIS-PRO-PHE-HIS-LEU) AS AN ANTI-CARDIAC HYPERTROPHIC AGENT

BACKGROUND OF THE INVENTION

Field of the Invention: This invention relates to the use of des-Aspartate-angiotensin I, a nine amino acid angiotensin peptide (Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), as an anti-cardiac hypertrophic agent.

Description of Prior Art: The interest in des-Aspartate-angiotensin I as a peptide of the renin-angiotensin system was first generated when Blair-West and colleagues (Blair-West et al., *J. Clin. Endocrinol. Metab.* (1971) 32:575-578) postulated a biosynthetic pathway for the production of angiotensin III by enzymatic NH_2-terminal degradation of angiotensin I to the nonapeptide and sequential action of angiotensin converting enzyme on this nonapeptide to produce the heptapeptide. Since then des-Aspartate-angiotensin I has been shown to be an excellent substrate of plasma and pulmonary angiotensin converting enzyme (Tsai et al., *J. Med. Chem.* (1975) 18:1180-1183) and that its pressor and steroidogenic actions are dependent on its conversion to angiotensin III (Campbell et al., *Endocrinology* (1977) 100:46-50). Recently, we found that homogenates of rat aorta and hypothalamus degrade exogenous angiotensin I to mainly des-Aspartate-angiotensin I instead of angiotensin II and the enzyme responsible for the degradation was a specific amnopeptidase that was not inhibited by amastatin, bestatin and EDTA (Sim, *Biochem. Pharmacol.* (1993) 45:1524-1527; Sim et al.,

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Blood Pressure (1994) 3:260-264; Sim et al., *Biochem. Pharmacol.* (1994) 48:1043-1046). Des-Aspartate-angiotensin I has also been shown to attenuate the pressor action of angiotensin II and angiotensin III in the brain (Sim and Radhakrishnan, *Eur. J. Pharmacol.* (1994) 257:R1-R3). Peripherally, it is able to potentiate the contractile action of angiotensin II on the rabbit aortic ring but to attenuate the contractile action of angiotensin III in the same tissue (Sim and Yuan, *Eur. J. Pharmacol.* (in press)). These recent findings of ours seem to indicate that des-Aspartate I is a functional peptide that may have undefined specific actions in ensuring the normal functioning of the cardiovascular system.

SUMMARY OF THE INVENTION

In the course of studying the cardiovascular pharmacology of des-Aspartate-angiotensin I, the nonapeptide has been found to attenuate significantly the experimentally-induced cardiac hypertrophy in rat. The novelty of this finding is the exceeding low dose at which des-Aspartate-angiotensin I has been found to be effective i.e. an i.v. dose of 180 ng (given over a period of 4 hours) per day for four days attenuates significantly the experimentally-induced cardiac hypertrophy in rats. Another significant finding is that, despite being a peptide, des-Aspartate-angiotensin I is equally effective in attenuating the cardiac hypertrophy when given orally at 285 μg per day for four days. These findings show that des-Aspartate-angiotensin I is a highly specific anti-cardiac hypertrophic agent and is effective at concentrations that produce minimum or no secondary effects.

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DETAILED DESCRIPTION OF THE INVENTION

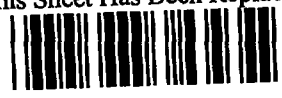
Materials and Methods

Sources of Materials: Des-Aspartate-angiotensin I was obtained from Bachem (Bubendorf, Switzerland). Adult Sprague Dawley rats (250 -300 g) were obtained from the Animal Centre, National University of Singapore.

Induction of Cardiac Hypertrophy: The abdominal aorta of each animal was co-arcted to the size of a 23-gauge hypodermic needle with a silk thread according to the method described by Everett et al (*Hypertension* (1994) 23:587-592). Briefly, each animal was anaesthetized with pentobarbital (5 mg/100 g, i.p.). An incision was made in the ventral abdominal wall to access the suprarenal portion of the abdominal aorta. This portion of the abdominal aorta was dissected free and a blunt 23-gauge needle was placed adjacent to the aorta. A ligature was placed around the blunt needle and the aorta. The blunt needle was then removed, leaving the aorta constricted to the size of the needle. The resulting co-arctation resisted the normal flow of blood from heart to the lower portion of the body and placed an extra load on the heart. This extra load is believed to cause the heart, especially the left ventricle, to hypertrophy.

Administration of Des-Aspartate-Angotensin I: Following the operation, each animal was administered one of the various doses of des-Aspartate-angiotensin I (dissolved in saline) per day for four days (see Table 1 for details). The nonapeptide was administered either intravenously via a femoral vein catheter which was implanted during the co-arctation

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operation or orally via a 1 ml syringe with a blunt needle. The intravenous administration was carried out using a microinjector which delivered 10 µl of the peptide solution per hour for four hours. For oral administration, the peptide was dissolved in 0.5 ml saline. Control animals with co-arcted abdominal aorta were administered saline instead of the peptide solution. Sham animals were animals that underwent the same surgical operation but their aortae were not the co-arcted.

Determination of Cardiac Hypertrophy: On the fourth day following the co-arctation of the abdominal aorta, the animal was again anaesthetized with pentobarbital and the carotid and femoral blood pressure were determined via a carotid artery catheter and a femoral artery catheter, respectively. Each catheter was connected to a Gould Statham (P23 ID) pressure transducer. The transducers were in turn connected to a MacLab Quad Bridge Amplifier coupled to a MacLab/8 Virtual Instrument System which displayed the mean arterial blood pressure in mm Hg. The difference in the two readings indicated the extent of co-arctation. The heart of each animal was then excised and the weight of the ventricles was determined. The index of the ventricle weight (in mg) over the body weight of the animal (in g) was taken as the index of hypertrophy. For sham-operated animal the index was around 2.5, for aorta-co-arcted animal the index was above 3.7.

Results

The results of the study are summarised in Table 1. The findings are displayed in Figs. 1 and 2. Des-Aspartate-angiotensin I has been found to be an effective agent in preventing the development of experimentally-induced cardiac hypertrophy. The anti-

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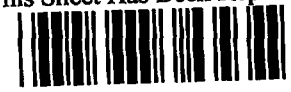
hypertrophic action is dose-dependent and its maximum action is brought about by an i.v. dose of 180 ng/day for four days or an oral dose of 285 μg/day for four days.

Table 1 Effects of des-Aspartate-angiotensin I on cardiac hypertrophy in rats

Dose	Hypertrophy Index*	CBP (mm Hg)	FBP (mm Hg)	BP (mm Hg)
No Administration				
Sham animals	2.53 ± 0.06	129 ± 13	129 ± 13	0
Intravenous Administration				
Control animals	3.77 ± 0.06	151 ± 19	107 ± 22	44
23 ng (18 pmol)	3.72 ± 0.18	158 ± 17	106 ± 13	52
45 ng (38 pmol)	3.51 ± 0.09	159 ± 25	119 ± 18	40
90 ng (76 pmol)	3.47 ± 0.13	156 ± 15	124 ± 14	32
180 ng (180 pmol)	3.17 ± 0.14	142 ± 22	111 ± 25	31
Oral Administration				
Control animals	3.75 ± 0.06	159 ± 18	110 ± 20	49
64 μg (63.5 nmol)	3.40 ± 0.10	153 ± 10	89 ± 27	64
128 μg (125 nmol)	3.23 ± 0.12	163 ± 27	121 ± 30	42
285 μg (250 nmol)	2.93 ± 0.09	171 ± 24	119 ± 33	52
590 μg (500 nmol)	3.13 ± 0.22	153 ± 22	110 ± 19	43
1180 μg (1000 nmol)	3.34 ± 0.16	154 ± 29	107 ± 25	46
1770 μg (1500 nmol)	3.57 ± 0.17	165 ± 14	123 ± 28	42

Each value is a means ± SEM obtained from 6 individual animals. Sham animals were animals that underwent the surgical operation but not the co-arctation of the abdominal aorta. Control animals were animals that underwent co-arctation of the abdominal aorta but were given saline instead of the peptide solution. *Hypertrophy Index = ventricle weight in mg/body weight in g. CBP = mean arterial blood pressure obtained from the carotid artery catheter, FBP = mean arterial blood pressure obtained from the femoral artery catheter, BP = CBP - FBP.

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CLAIM

What is claimed is:

The use of des-aspartate-angiotensin I (Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) as an anti-cardiac hypertrophic agent in both the prevention and treatment of cardiac hypertrophy.

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