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Short communication

Effects of des-Asp-angiotensin I on the electrically stimulated contraction of the rabbit pulmonary artery

M.K. Sim [a,*], K.S. Soh [b]

[a] *Department of Pharmacology, Faculty of Medicine, National University of Singapore, Singapore 0511, Singapore*
[b] *Department of Pharmacology, Faculty of Medicine, University of Malaya, 591000 Kuala Lumpur, Malaysia*

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Abstract

In the presence of 3×10^{-6} M captopril, 5×10^{-7} M des-Asp-angiotensin I was found to inhibit the electrically (1 and 2 Hz) induced contraction of the rabbit pulmonary artery but had no significant effect on the noradrenaline-stimulated contraction. 2.8×10^{-6} M indomethacin and 10^{-6} M losartan but not 10^{-6} M (*S*) 1-{[4-(dimethylamino)-3-methylphenyl]methyl}-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo{4,5-*c*]pyridine-6-carboxylic acid, ditrifluoroacetate, dihydrate (PD123319) attenuated the inhibition. The inhibition of the electrically stimulated contraction by 5×10^{-7} M des-Asp-angiotensin I coincided with a significant drop in the accompanying evoked ^{3}H overflow from re-uptaken [^{3}H]noradrenaline. The results indicate that des-Asp-angiotensin I acts presynaptically on a subtype of angiotensin receptor that involves the release of prostaglandin(s). In addition, this receptor subtype is susceptible to blockade by angiotensin AT_1- but not AT_2-specific receptor antagonists. It was suggested that this receptor subtype is identifiable with the recently described angiotensin AT_{1B} receptor subtype found in the brain, pituitary and adrenal glomerulosa. These findings demonstrated a direct action of sub-micromolar concentrations of des-Asp-angiotensin I on a blood vessel and indicate that the nonapeptide is an active angiotensin per se.

Keywords: Des-Asp-angiotensin I; Losartan; PD123319; Pulmonary artery, rabbit; Transmural stimulation; (Superfusion)

1. Introduction

Angiotensin III can be formed from angiotensin I by a pathway that bypasses angiotensin II (Blair-West et al., 1971; Campbell et al., 1977). In this pathway, angiotensin I is degraded to des-Asp-angiotensin I by an aminopeptidase and the nonapeptide is then converted to angiotensin III by angiotensin converting enzyme. Whether this pathway is just an additional means for tissues to generate angiotensin III or whether it is an important pathway for the formation and degradation of a functional angiotensin, i.e. des-Asp-angiotensin I, remains unclear. Studies with des-Asp-angiotensin I seem to indicate that the nonapeptide has pressor and steroidogenic actions but that a major portion of these actions are dependent on its conversion to angiotensin III (Campbell et al., 1977; Sexton et al., 1979). However, recent studies from our laboratory show that homogenates of rat aorta and hypothalamus degrade exogenous angiotensin I to mainly des-Asp-angiotensin I instead of angiotensin II and the enzyme responsible for the degradation is a specific aminopeptidase that is not inhibited by amastatin, besatin and EDTA (Sim, 1993; Sim and Qiu, 1994; Sim et al., 1994). The presence of this specific angiotensin pathway in tissues that are either directly or indirectly concerned with blood pressure regulation seems to indicate that des-Asp-angiotensin I is likely a functional vascular angiotensin peptide. In fact, when prevented from degradation by prior administration of captopril, intracerebroventricularly administered des-Asp-angiotensin I attenuated dose dependently the central pressor actions of angiotensin II and angiotensin III in the spontaneously hypertensive rat and its normotensive control (Sim and Radhakrishnan, 1994). This latter finding may suggest that the nonapeptide exerts its vascular effect by modulating the central pressor actions of angiotensin II and angiotensin III. The present study describes the in-

* Corresponding author. Tel. (65) 7723268, fax (65) 7730579.

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hibitory action of des-Asp-angiotensin I on the electrically stimulated contraction of the rabbit pulmonary artery and discusses the likely angiotensin receptor subtype that is involved in the inhibition.

2. Materials and methods

Male albino rabbits weighing 2–2.5 kg were supplied by the local University Animal Centre. Each rabbit was killed by cervical dislocation and the pulmonary artery was rapidly removed. The artery was then denuded of the endothelium by gentle rubbing of the luminal surface with a glass rod and cut spirally into a strip of about 3 mm wide and 20 mm long. The strip was then mounted vertically under 2 g tension between two platinum electrodes in a water-jacket chamber maintained at 37°C. It was superfused at a rate of 3 ml/min with Krebs solution that was prewarmed to 37°C and saturated with oxygen (95%) and CO_2 (5%). The composition of the solution (in mM) was as follows: NaCl 118, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 10 and ascorbic acid 0.3.

The strip was equilibrated by superfusion with the Krebs solution containing only one of the following: 3×10^{-6} M captopril, 3×10^{-6} M captopril plus 3×10^{-6} M indomethacin, 3×10^{-6} captopril plus 10^{-6} M losartan, or 3×10^{-6} M captopril plus 10^{-6} M PD123319 for 90 min. Following this, the strip was electrically stimulated by monophasic square waves (Harvard Dual Impedance Research Stimulator) of supramaximal voltage of 25 V and 0.3 ms duration at 1, 2, 4 and 8 Hz. Contractions were recorded isometrically with a Ugo Basile isometric transducer attached to a MacLab/8 Virtual Instrument System via a MacLab Quad Bridge Amplifier. Frequencies were increased when the contraction for a particular frequency had become constant, usually within 2.5 min. The tissue was stimulated for about 12–15 min in each frequency-response experiment. Three control frequency-response curves were obtained with an interval of 30 min between each curve. Following this, the effect of des-Asp-angiotensin I on the electrically stimulated contraction was studied after 30 and 60 min of superfusing the tissue with a solution containing either 5×10^{-8} or 5×10^{-7} M of the nonapeptide in addition to each of the above drugs. At the end of the experiment the strip was contracted by superfusion with Krebs solution containing 10^{-7} M noradrenaline. This was then followed by Krebs solution containing 10^{-7} M noradrenaline and 10^{-7} M acetylcholine to test the extent of endothelial denudation. Most strips did not respond to acetylcholine. The few that did were not included in the study. In a preliminary parallel experiment the neurogenic nature of the electrically stimulated contraction was confirmed by repeating the second frequency response in the presence of 2 μM tetrodotoxin. The toxin inhibited completely the contractions elicited by all frequencies.

The effect of 5×10^{-7} M des-Asp-angiotensin I on the noradrenaline (10^{-7} M)-induced contraction of the strip was also studied. The protocol used was as described previously (Sim and Singh, 1987). Briefly, the strip was exposed to a dose of 10^{-7} M noradrenaline in the presence of 3×10^{-6} M captopril (included in the Krebs solution). The drugs were washed off and the experiment was repeated after the strip had been exposed to 5×10^{-7} M des-Asp-angiotensin I for a period of 30 min.

The protocol to study the effect of des-Asp-angiotensin I on adrenergic neurotransmision of the pulmonary arterial strip was as follows. The mounted strip was preincubated in 20 ml Krebs solution containing 68 nM [^{3}H]noradrenaline, specific activity 1.5 Ci/mmol for 60 min. It was then superfused with Krebs solution (containing 3×10^{-6} M captopril) at a rate of 3 ml/min for 100 min. The strip was then stimulated electrically at 1 Hz. Three minutes after the contraction had plateaued, two 3 ml aliquots of superfusate were collected. The superfusion Krebs solution was then changed to one that contained 5×10^{-7} M [des-Asp^1]angiotensin I in addition to 3×10^{-6} M captopril. Collection of superfusate was begun at the onset of attenuation of the electrically stimulated contraction by the nonapeptide (see legend of Fig. 2 for details). The radioactivity in 3 ml aliquots of each collected superfusate was then counted in 10 ml of scintillation cocktail (Beckman Ready Solv-HP) using a Beckman LS 1801 with a counting efficiency of 60%.

Des-Asp-angiotensin I was purchased from Bachem Feinchemikalein, [^{3}H]noradrenaline (levo-[ring-2,5,6-^{3}H]) from New England Nuclear and indomethacin, captopril and tetrodotoxin from Sigma, USA. Losartan and PD123319 were gifts from DuPont Merck Pharmaceutical Company, Wilmington, USA and Parke-Davis Pharmaceutical Research, Michigan, USA, respectively.

2.1. Rationale for experimental design

The rationale of the overall experimental design was based on data obtained from preliminary experiments. In the absence of captopril, 5×10^{-8} and 5×10^{-7} M des-Asp-angiotensin I potentiated the electrically induced contractions. This potentiation was brought about by angiotensin III which was formed by the action of angiotensin converting enzyme on the nonapeptide. 3×10^{-6} M captopril was found to completely inhibit the potentiation produced by 5×10^{-8} M des-Asp-angiotensin I, and to reverse the potentiation to inhibition in the case of 5×10^{-7} M des-Asp-angiotensin I. Hence 3×10^{-6} M captopril was incorporated

in all superfusion solutions to inhibit angiotensin converting enzyme. 10^{-6} M des-Asp-angiotensin I in the presence of 3×10^{-6} M of captopril exerted a contractile action (increase in basal tone) on the strip. The response was not abolished by higher concentrations (up to 5×10^{-5} M) of captopril, indicating that it was a direct action. 10^{-6} M des-Asp-angiotensin I has also been found by us to produce contraction in rat aortic rings in the presence of similar concentrations of captopril (unpublished data). Hence the highest concentration of des-Asp-angiotensin I used in this study was 5×10^{-7} M. 2.8×10^{-6} M indomethacin was used to inhibit the formation of prostaglandin(s) because in an earlier study, this concentration was found to produce an enhancement of the electrically stimulated contraction by inhibiting the formation of prostaglandin(s) in the same tissue (Soh, 1986). The concentration of 10^{-6} M losartan was chosen to maximize the blockade of the angiotensin AT_1 receptors. This concentration is approximately 50 times the IC_{50} of losartan for inhibition of angiotensin II binding in nervous tissues and vascular smooth muscle (Timmermans et al., 1993). In order to make comparisons with losartan, a similar concentration (10^{-6} M) of PD123319 was used. Both 10^{-6} M losartan and PD123319 had no direct effect on the electrically induced contraction of the preparation.

3. Results

Preliminary parallel experiments showed that, except for the first frequency-response curve (which was in most cases smaller in magnitude than the rest), the other four frequency-response curves obtained sequentially from each strip did not vary significantly from one another. With the sixth frequency-response curve, some strips produced diminished responses. Based on this, only the second and third frequency-response curves were averaged to give the control curve, and the fourth and fifth were used to assess the extent of des-Asp-angiotensin I action. As des-Asp-angiotensin I did not affect the 8 Hz-induced contraction, the magnitude of each contraction was expressed as a percentage of the 8 Hz-induced contraction. A stimulation frequency of 1 Hz was used in the study of des-Asp-angiotensin I action on adrenergic neurotransmission because in parallel experiments using 2 Hz stimulation the evoked ^3H overflow declined progressively over the 18–20 min of stimulation.

Fig. 1A shows that in the presence of 3×10^{-6} M captopril, 5×10^{-7} M des-Asp-angiotensin I inhibited significantly ($P < 0.05$, Student's t-test) the contractile responses of the superfused rabbit pulmonary strip to 1 and 2 Hz stimulation. The contractions induced by 4 and 8 Hz were not significantly affected (the 8 Hz-induced contraction is not shown as it was used as a denominator to express the contractions induced by the lower frequencies). 5×10^{-8} M des-Asp-angiotensin I had no significant effect on the 1- to 8 Hz-induced contractions (data not shown). Fig. 1B shows that 2.8×10^{-6} M indomethacin attenuated significantly the inhi-

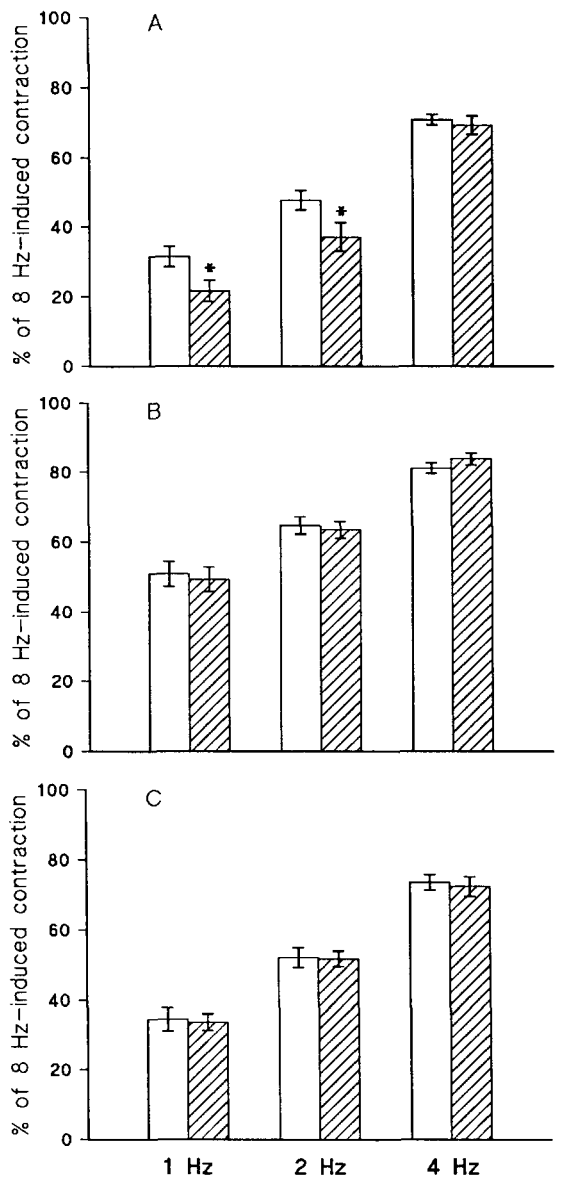

Fig. 1. Effect of 5×10^{-7} M des-Asp-angiotensin I on the electrically stimulated contractions of the rabbit pulmonary aortic strip in the absence (A) and presence of 2.8×10^{-6} M indomethacin (B) and 10^{-6} M losartan (C). The experiment was carried out in the presence of 3×10^{-6} M captopril to prevent the degradation of des-Asp-angiotensin I to angiotensin III (please see text for explanation). Empty and hatched histograms represent values obtained before (control) and after the administration of des-Asp-angiotensin I, respectively. Each value is the mean of six determinations obtained from six separate strips. The vertical bars represent the SEM. * Significantly different ($P < 0.05$, Student's t-test) from the corresponding control value.

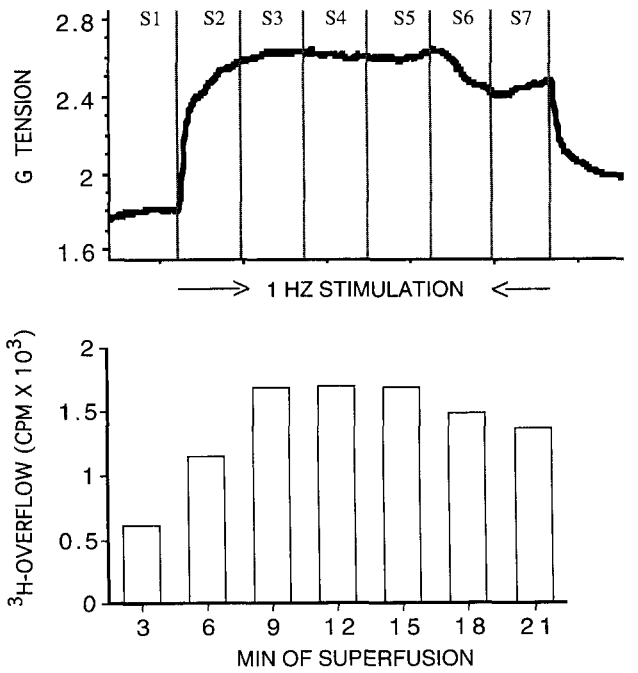

Fig. 2. Effect of 5×10^{-7} M des-Asp-angiotensin I on the 1-Hz-induced contraction and the corresponding evoked ^{3}H overflow in the rabbit pulmonary aortic strip. The strip was electrically stimulated for 18 min. Seven 3-min superfusate aliquots were collected as indicated by S1, S2, S3, S4, S5, S6, S7. Perfusion with Krebs solution containing 5×10^{-7} M des-Asp-angiotensin I in addition to 3×10^{-6} M captopril was initiated immediately after S4. The perfusion solution took approximately 3 min to reach the tissue and hence the action of des-Asp-angiotensin I was seen only in S6 and S7. The basal (non-stimulated) ^{3}H overflow (S1) for this particular experiment was 610 counts/min and the radioactivity in the S2, S3, S4, S5, S6 and S7 aliquots collected during the 1-Hz stimulation was 1147, 1688, 1703, 1690, 1491 and 1311 counts/min respectively. The normalized values for S5, S6 and S7 (normalized against the average of S3 and S4) derived from three separate experiments were 99 ± 8, 79 ± 6 and 75 ± 6%, respectively, while those of the control were 97 ± 7, 95 ± 7 and 96 ± 8, respectively. The latter two values of the des-Asp-angiotensin data were significantly different ($P < 0.05$, Student's t-test) from that of S5, i.e. attenuation of the contraction by des-Asp-angiotensin I was accompanied by a signifcant drop in evoked ^{3}H overflow.

bition by 5×10^{-7} M des-Asp-angiotensin I of the 1- and 2 Hz-induced contractions, indicating that the non-apeptide action was probably mediated via the release of prostaglandin(s). Fig. 1C show that the inhibition of the 1- and 2 Hz-induced contractions by 5×10^{-7} M des-Asp-angiotensin I was significantly attenuated by losartan (i.e. the inhibition of the electrically stimulated contraction by des-Asp-angiotensin I was no more significant, $P > 0.05$, Student's t-test). However, PD123319 had no effect on the inhibition (data not shown).

5×10^{-7} M des-Asp-angiotensin I had no significant effect on the noradrenaline-induced contraction of the pulmonary aortic strips (data not shown), indicating that it probably did not interfere with the action of noradrenaline on the postsynaptic α_1-adrenoceptor.

Fig. 2 shows that attenuation of the electrically stimulated contraction by 5×10^{-7} M des-Asp-angiotensin I coincided with a drop in the accompanying evoked ^{3}H overflow.

4. Discussion

Des-Asp-angiotensin I appears to act presynaptically on a subtype of angiotensin receptor that has been shown by Jaiswal et al. (1991) and Trachte et al. (1990) to mediate the release of prostaglandin E_2. The data obtained with losartan and PD123319 tend to indicate that this receptor subtype is susceptible to blockade by angiotensin AT_1 (losartan) but not by AT_2 (PD123319) receptor antagonists. This is in line with current findings of other investigators that vascular angiotensin receptors are of the angiotensin AT_1 receptor subtype or that the functional activity of the vascular system is mediated through angiotensin AT_1 receptors (Timmermans et al., 1993). Of related interest is the finding showing that angiotensin II could concurrently increase adrenergic neurotransmission and prostaglandin E_2 release in the rabbit vas deferens (Trachte et al., 1990). These two responses were also inhibited by losartan. The latter two findings are supportive of our assumption that the indomethacin-sensitive inhibition of neurogenic contraction by des-Asp-angiotensin I (which is also blocked by losartan) is probably mediated by vasorelaxant prostaglandins including prostaglandin E_2.

Recent cloning and expression studies have shown that the angiotensin AT_1 receptor exists as two subtypes classified by Kakar and co-workers as angiotensin AT_{1A} and AT_{1B} (Kakar et al., 1992a,b) and by Sandberg and co-workers as angiotensin AT_1 and AT_3 receptor subtypes (Sandberg et al., 1992). Both these subtypes are susceptible to inhibition by losartan but not PD123319. It is likely the presynaptic angiotensin AT_1 receptor described in the present study is identifiable with the angiotensin AT_{1B} or AT_3 subtype found in the brain, pituitary and adrenal glomerulosa as this receptor subtype does not mediate the contraction of vascular tissues.

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