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Short communication

Effects of des-Asp-angiotensin I on the contractile action of angiotensin II and angiotensin III

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Abstract

Nanomolar concentrations of des-Asp-angiotensin I potentiated the contractile action of angiotensin II on the rabbit aortic ring but attenuated the contractile action of angiotensin III in the same tissue. Indomethacin had no effect on the potentiation of angiotensin II but inhibited the attenuation of angiotensin III. The action of angiotensin II, angiotensin III and des-Asp-angiotensin I was not inhibited by (*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), an angiotensin AT_2 receptor antagonist. The data show that angiotensin II and angiotensin III act on different subclasses of angiotensin receptors and that their actions are differentially modulated by des-Asp-angiotensin I. The data also indicate the possibility that des-Asp-angiotensin I is a functional peptide that modulates the contractile action of the two angiotensins at sub-nanomolar concentrations.

Keywords: des-Asp-angiotensin I; Aortic ring; Captopril; Indomethacin

1. Introduction

Recently, we found 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 was a specific aminopeptidase that was not inhibited by amastatin, bestatin and EDTA (Sim, 1993; Sim and Qiu, 1994; Sim et al., 1994). The presence of this specific angiotensin pathway in tissues either directly or indirectly concerned with blood pressure regulation seems to indicate that des-Asp-angiotensin I is a likely 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 (SHR) and Wistar Kyoto (WKY) rats (Sim and Radhakrishnan, 1994a). This later finding may suggest that the nonapeptide exerts its vascular effect by modulating the central pressor actions of angiotensin II and angiotensin III. In the present study we showed that the contractile action of angiotensin II and angiotensin III on the rabbit aortic ring was also modulated by des-Asp-angiotensin I and that different angiotensin receptor subtypes were involved in the modulation.

2. Materials and methods

Male albino rabbits weighting 2.5–3 kg were obtained from the local University Animal Centre. The animals were paralysed by cervical dislocation, killed immediately by decapitation and the aortic rings with intact endothelium were prepared as reported previously (Sim and Singh, 1987). Briefly, each aorta was dissected free of adhering tissues and cut into 2 mm segments. Each segment was then suspended in a 10 ml organ bath of captopril-containing Krebs-Ringer bicarbonate solution (composition, Mm: captopril, 0.01; NaCl, 118; KCl, 5; $NaHCO_3$, 25; $CaCl_2$, 2.5; $MgSO_4$ $7H_2O$, 1.2; KH_2PO_4, 1.2; EDTA, 0.026) with one end connected via a silk thread to an isometric transducer (Ugo Basille) coupled to a MacLab/8 Virtual Instrument System via a MacLab Quad Bridge Amplifier and

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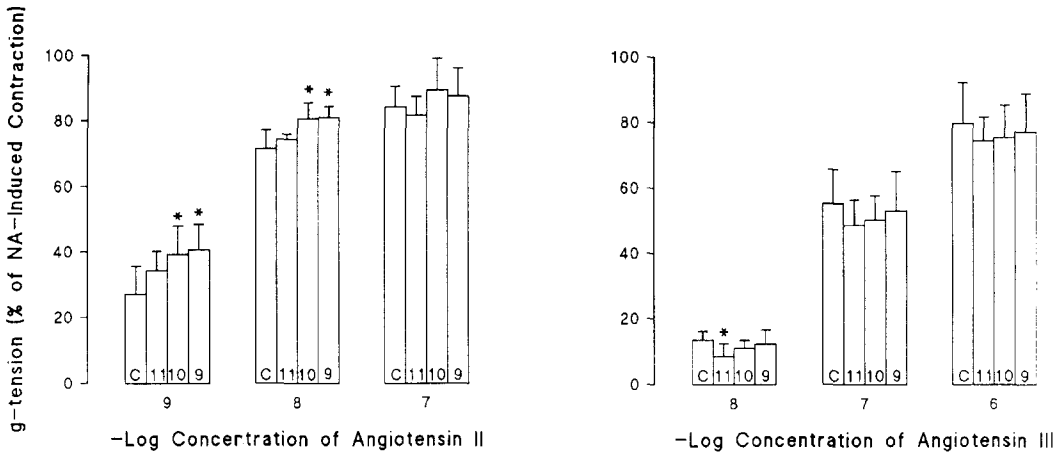

Fig. 1. Concentration response of the rabbit aortic ring to angiotensin II (left histograms) and angiotensin III (right histograms) in the absence (C-labelled histograms) and presence of various concentrations of des-Asp-angiotensin I (11, 10, 9 in each histogram represent 10^{-11}, 10^{-10}, 10^{-9} M of des-Asp-angiotensin I, respectively). 10^{-12} M des-Asp-angiotensin I had no effects on the contraction and was not included in the figure. Each histogram is the mean of four rings obtained from four individual rabbits. * Significant difference when compared with the value obtained in the absence of the nonapeptide.

the other attached to the base of the gas inlet steel tubing. The bath was aerated with 95% O_2 and 5% CO_2 and maintained at 37° C.

After 60 min equilibration with a load of 2 g, each ring was exposed to increasing concentrations of either angiotensin II or angiotensin III (10^{-11}–10^{-6} M) to obtain a cumulative concentration-response curve. The interval between two consecutive concentrations of either angiotensin II or angiotensin III was 5 min. The experiment was repeated 10 min after the tissue had been exposed to various concentrations (10^{-12}–10^{-7} M) of des-Asp-angiotensin I; each concentration of the nonapeptide conducted using a separate aortic ring. In another set of experiments either indomethacin (10^{-6} M) or (*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) (10^{-6} M) was added to the incubation mixture at concentrations of des-Asp-angiotensin I which modified the actions of angiotensin II and angiotensin III. The two drugs were added 20 min before the addition of des-Asp-angiotensin I. At the end of the experiment each ring was contracted with 10^{-7} M noradrenaline and rings that did not relax to 10^{-6} M acetylcholine were excluded from the study.

Angiotensin II, angiotensin III, des-Asp-angiotensin

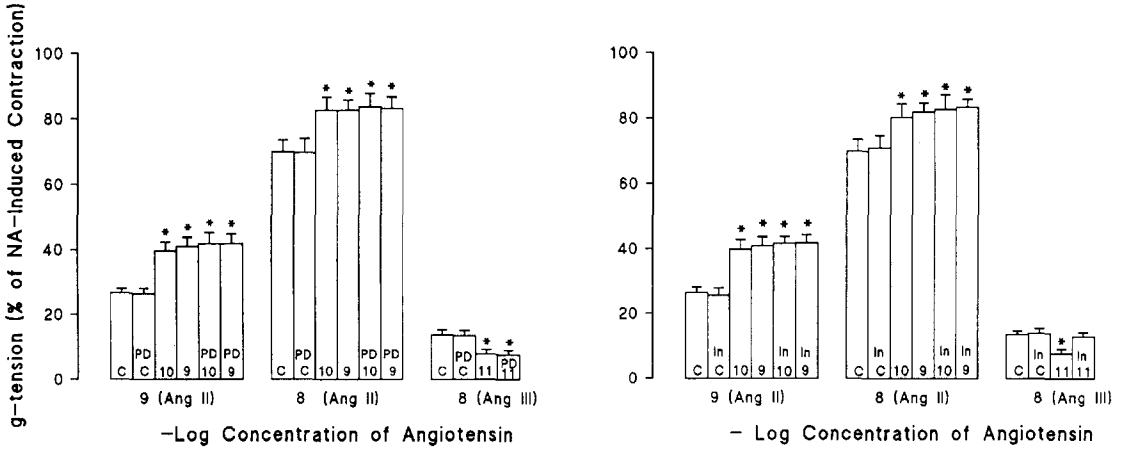

Fig. 2. Effect of 10^{-6} M indomethacin (In-labelled histograms) and 10^{-6} M PD123319 (PD-labelled histograms) on the des-Asp-angiotensin I-induced potentiation of angiotensin II action and the des-Asp-angiotensin I-induced attenuation of angiotensin III action. C, 11, 10, 9 in each histogram represent control experiments and experiments carried out in 10^{-11}, 10^{-10}, 10^{-9} M of des-Asp-angiotensin I, respectively. * Significant difference when compared with the corresponding value obtained in the absence of the nonapeptide.

I were purchased from Bachem Feinchemikalien. Captopril and indomethacin were purchased from Sigma. PD123319 was a generous gift from Dr Carol L. Germain, Parke-Davis Pharmaceutical Research.

The data were analyzed by analysis of variance followed by the post-hoc Fisher test. A P value of less than 0.05 indicates a significant difference.

3. Results

The threshold concentrations for angiotensin II and angiotensin III for the majority of the aortic rings were 10^{-9} and 10^{-8} M, respectively. des-Asp-angiotensin I tended to potentiate the contractile action of angiotensin II but to attenuate that of angiotensin III (see Fig. 1). The effect was more marked at the lower concentrations of the two angiotensin peptides. However, only the contractile action of 10^{-9} and 10^{-8} M angiotensin II was significantly potentiated by 10^{-10} and 10^{-9} M des-Asp-angiotensin, and only the action of 10^{-8} M angiotensin III was significantly attenuated by 10^{-11} M des-Asp-angiotensin I.

Fig. 2 shows that the potentiation of angiotensin II by des-Asp-angiotensin I was not affected by indomethacin. However, the attenuation of angiotensin III by des-Asp-angiotensin I was significantly inhibited by indomethacin, i.e. in the presence of 10^{-6} M indomethacin, the attenuation of angiotensin III action by des-Asp-angiotensin I was no more significant. PD12319 had no effect on either the potentiation of angiotensin II or the attenuation of angiotensin III action by des-Asp-angiotensin I.

Parallel experiments also showed that indomethacin (10^{-6} M) and PD123319 (10^{-6} M) had no effect on the contractile action of angiotensin II and angiotensin III (see Fig. 2).

4. Discussion

The data seem to indicate that angiotensin II and angiotensin III act on different sub-classes of vascular angiotensin receptors and that these receptors are differentially modulated by des-Asp-angiotensin I. Evidence for the existence of a different sub-class of angiotensin receptors that mediate the vascular actions of angiotensin II and angiotensin III, respectively, has also been reported by Tabrizchi and Pang (1987) in their study using intact rat. Two other studies on the central actions of angiotensin II and angiotensin III also indicate that the heptapeptide acts on a subtype of angiotensin receptor where its pressor action is attenuated to a greater extent by des-Asp-angiotensin I (Sim and Radhakrishnan, 1994a) and pentobarbital and chlordiazepoxide (Sim and Radhakrishnan, 1994b).

Although des-Asp-angiotensin I has been shown to attenuate the pressor action of i.c.v. angiotensin II in the rat (Sim and Radhakrishnan, 1994a), it potentiated the contractile action of angiotensin II on the rabbit aortic ring. The difference could be due to the fact that the angiotensin receptors in the brain are of a different subtype. Neither the direct contractile action of angiotensin II nor its potentiation by des-Asp-angiotensin II was affected by indomethacin indicating that the vascular angiotensin receptors involved are not modulated by prostanoid (Lin and Nasjletti, 1991). As the direct contractile action of angiotensin II and its potentiation by des-Asp-angiotensin I were also not affected by PD123319, the receptors involved are also not of the angiotensin AT_2 subtype. Because losartan was found to block the direct contractile action of angiotensin II (Chiu et al., 1991) and angiotensin III (unpublished data), the effect of the blocker on the potentiation by des-Asp-angiotensin I could not be studied. A higher concentration (10^{-6} M) of des-Asp-angiotensin I has, nevertheless, been shown by us (data not shown) and others (Ackerly et al., 1977) to cause contraction of the rabbit aorta. This contractile action has also been found by us to be blocked by losartan (unpublished data). It is, thus, likely that the potentiation by des-Asp-angiotensin I could also be mediated by the AT_1 receptors.

Nanomolar concentrations of des-Asp-angiotensin I potentiate the contractile effect of angiotensin II but attenuate that of angiotensin III. The potentiation and attenuation occur at the threshold concentrations of the two angiotensins. These data may suggest that des-Asp-angiotensin I is a functional peptide that modulates the contractile action of angiotensin II and angiotensin III.

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