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Subtypes of losartan-sensitive angiotensin receptor in the rabbit pulmonary artery

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1 The short rabbit pulmonary artery was denuded of endothelium and divided into three sections, the cardiac end (cardiac), middle and pulmonary end (pulmonary) sections, respectively. Des-Asp-angiotensin I attenuated the contractions of the cardiac and middle sections to transmural nerve stimulation but potentiated the contractions in the pulmonary section.

2 The actions of the nonapeptide were inhibited completely by 10^{-6} M losartan; however, a similar concentration of PD123319 had no effect. Indomethacin (10^{-6} M) also inhibited completely the attenuation in the cardiac and middle sections but had no effect on the potentiation seen in the pulmonary section.

3 The data suggest that the two differential responses of the pulmonary artery to des-Asp-angiotensin I are mediated by two separate subtypes of the losartan-sensitive angiotensin AT_1 receptor.

Keywords: des-Asp-angiotensin I; angiotensin AT_1 receptor subtypes; pulmonary artery; losartan; indomethacin

Introduction

Cloning and expression studies have shown that the AT_1 receptor exists as two subtypes classified by Kakar and co-workers as AT_{1A} and AT_{1B} (Kakar *et al.*, 1992a, b) and by Sandbert and co-workers as AT_1 and AT_3 (Sandberg *et al.*, 1992). Both these subtypes are susceptible to inhibition by losartan but not PD123319. In addition, the AT_{1B} subtype does not appear to mediate the contraction of vascular smooth muscle, it being found in the brain, pituitary and adrenal glomerulosa, sites in which AT_{1A} receptors are also found. However the distinct functions mediated by each subtype, especially when they co-exist in the same tissue, are not yet fully deciphered. In this study we described the probable existence of two losartan-sensitive angiotensin receptor subtypes in the rabbit pulmonary artery, each mediating a distinct response to the action of des-Asp-angiotensin I.

Methods

Male albino rabbits weighing 2–2.5 kg were supplied by the local University Animal Centre. Briefly, each rabbit was killed by cervical dislocation and the pulmonary artery was rapidly removed. The artery was then denuded of the endothelium by gently rubbing the luminal surface with a glass rod and cut into three sections each about 3 mm wide, named as the cardiac (cardiac end section) the middle (middle section) and the pulmonary (pulmonary end section) sections, respectively. Each section was then cut open and 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^{-1} with Krebs solution that was prewarmed to 37°C and saturated with O_2 (95%) and CO_2 (5%). The composition of the solution (in mM) was as follows: captopril 0.01, 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 transmural experiment was carried out as described previously (Sim & Soh, 1995). Briefly, after 60 min of superfusion, each tissue 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 Hz. The electrically-stimulated contraction was recorded isometrically with a Ugo Basile isometric transducer attached to the MacLab/8 Virtual Instrument System via a MacLab Quad Bridge Amplifier. After the contraction had reached a steady plateau (between 10 to 11 min), the superfusion solution was changed to one that contained 5×10^{-6} M des-Asp-angiotensin I and the effect of the nonapeptide on the electrically-stimulated contraction was observed for a further 10 min. The effect of 10^{-6} M losartan, indomethacin and PD123319 on the action of des-Asp-angiotensin I was studied by incorporating each drug in the perfusion solution and repeating the experiment after 30 min of superfusion.

The rationale of the experiment was based on preliminary observations that attenuation of the contractions of the rabbit pulmonary strip to transmural nerve stimulation by des-Asp-angiotensin I varied with the length of the strip. Shortening the length of the strip by fixed portions from the pulmonary end of the artery enhanced the attenuation. This led to the division of the pulmonary artery into three sections as described in Methods. With the whole pulmonary artery (cutting it into a strip), it was not possible to employ higher concentrations (higher than 5×10^{-7} M) of des-Asp-angiotensin I to produce greater attenuation of the contractions without evoking the direct contractile action of the nonapeptide (Sim & Soh, 1995).

Results

Figure 1 shows that des-Asp-angiotensin I exerted differential effects on the electrically-stimulated contraction of the three sections of the rabbit pulmonary artery. With the cardiac and middle sections, the nonapeptide caused a slight potentiation in the contraction followed by a marked inhibition. In the pulmonary section, the nonapeptide caused a marked potentiation in the contraction. The magnitude of the des-Asp-angiotensin I-induced inhibition and potentiation (obtained from six animals) ranges from 38 to 58% and 70 to 120% of the pre-drug treated contraction, respectively. The inset shows that the inhibition in the cardiac section was concentration-dependent. The inhibition in the middle section and the potentiation in the third section were also concentration-dependent (data not shown).

PD123319 had no effect on the action of des-Asp-angiotensin I, i.e. it did not affect the inhibition of the cardiac and middle sections or the potentiation of the third section whilst

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10^{-6} M losartan inhibited completely the inhibition and potentiation of cardiac and middle and the pulmonary sections, respectively, as shown in Figure 2 for the cardiac and pulmonary sections. Indomethacin (10^{-6} M) had no effect on the potentiation of the third ring but prevented completely the inhibition of the cardiac and middle sections (see Figure 3).

Discussion

The present data demonstrated, for the first time, the differential responses of adjacent sections of the rabbit pulmonary artery to the same concentration of des-Asp-angiotensin I. Both the inhibitory action and the potentiation produced by

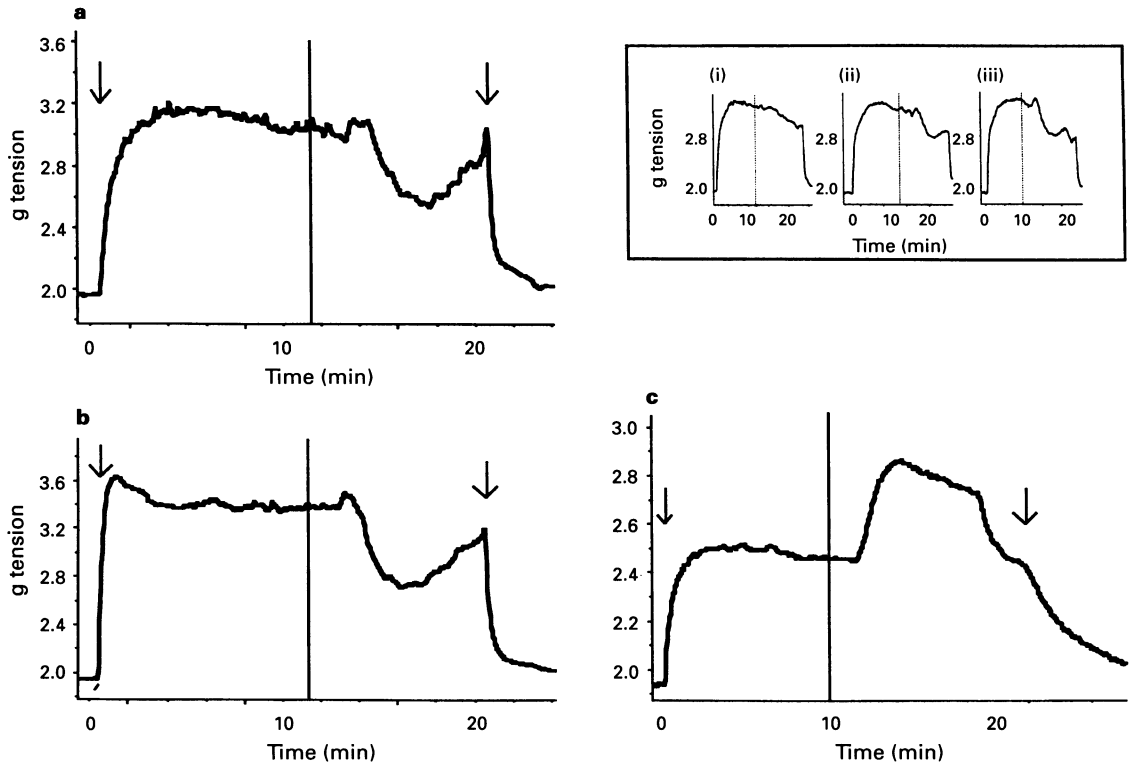

Figure 1 Differential actions of des-Asp-angiotensin I on the electrically-stimulated contraction of three sequential sections of the rabbit pulmonary artery: (a) cardiac section, (b) middle section; (c) pulmonary section of the artery. The vertical line indicates the change of the perfusing Krebs solution to one that contained 5×10^{-6} M des-Asp-angiotensin I (it took about 2 min for the latter solution to reach the tissue and produce its effect). The period between the two arrows was the period of electrical stimulation. The inset shows the response of the cardiac section to increasing concentrations ((i) 10^{-7}, (ii) 10^{-6}, (iii) 10^{-5} M) of des-Asp-angiotensin I.

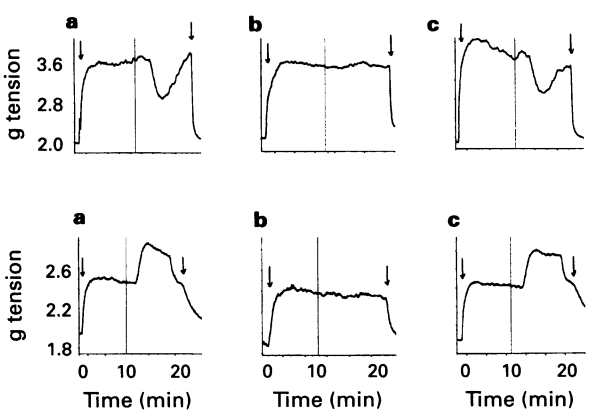

Figure 2 Effects of 10^{-6} M losartan on the action of des-Asp-angiotensin I in the cardiac section (upper three tracings) and pulmonary section (lower three tracings) of the rabbit pulmonary artery: (a) carried out in Krebs solution that did not contain losartan, (b) carried out in Krebs solution that contained 10^{-6} M losartan and after the tissue had been perfused for 30 min in the losartan-containing Krebs solution, (c) carried out in losartan-free Krebs solution after the losartan-treated tissue had been perfused with losartan-free Krebs for 30 min. The vertical line indicates the change of the perfusing Krebs solution to one that contained 5×10^{-6} M des-Asp-angiotensin I. The period between the two arrows was the period of electrical stimulation.

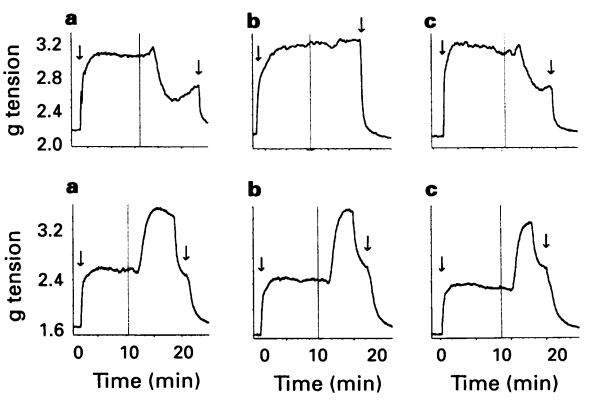

Figure 3 Effects of 10^{-6} M indomethacin on the action of des-Asp-angiotensin I in the cardiac section (upper three tracings) and the pulmonary section (lower three tracings) of the rabbit pulmonary artery: (a) carried out in Krebs solution that did not contain indomethacin; (b) carried out in Krebs solution that contained 10^{-6} M indomethacin and after the tissue had been perfused for 30 min in the indomethacin-containing Krebs solution, (c) carried out in indomethacin-free Krebs solution after the indomethacin-treated tissue had been perfused with indomethacin-free Krebs for 30 min. The vertical line indicates the change of the perfusing Krebs solution to one that contained 5×10^{-6} M des-Asp-angiotensin I. The period between the two arrows was the period of electrical stimulation.

des-Asp-angiotensin I were not sustained. As the experiment was carried out in 10 μM captopril, the transient actions were not due to the conversion of des-Asp-angiotensin I to angiotensin III by angiotensin converting enzyme. Rapid angiotensin-receptor internalization has been shown to be responsible for the transient contractile action of angiotensin II (Auguet *et al.*, 1991) and it is possible the same phenomenon contributes to the transient actions of des-Asp-angiotensin I. As both responses were inhibited by losartan, the angiotensin receptors mediating them are of the AT_1 type (Timmermans *et al.*, 1993).

The present findings also support an earlier suggestion that the indomethacin-sensitive subtype of angiotensin AT_1 receptor is identifiable with the angiotensin AT_{1B} receptor (Sim & Soh, 1995) as this receptor subtype does not mediate the contraction of vascular tissues (Kakar *et al.*, 1992a, b). Thus, it is likely that the other subtype which is indomethacin-resistant and mediates the contractile action of des-Asp-angiotensin I is identifiable with the angiotensin AT_{1A} receptor. The physiological significance and the differential distribution of the two receptor subtypes in such a short blood vessel remains to be studied further. However, the response of the cardiac and middle sections to the nonapeptide with an initial slight potentiation in the contraction may indicate that the distribution of the receptors, although differential, is not absolute.

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References

AUGUET, M., DELAFLOTTE, S., GUILLON, J-M., CHABRIER, P-E. & BRAQUET, P. (1991). Different regulation of vascular tone by angiotensin II and endothelin-1 in rat aorta. *Eur. J. Pharmacol.*, **196**, 21–27.

KAKAR, S.S., RIEL, K.K. & NEILL, J.D. (1992a). Differential expression of angiotensin II receptor subtype mRNAs (AT-1A and AT-1B) in the brain. *Biochem. Biophys. Res. Commun.*, **185**, 688–692.

KAKAR, S.S., SELLERS, J.C., DEVOR, D.C., MUSGROVE, L.C. & NEILL, J.D. (1992b). Angiotensin II subtype cDNAs: Differential tissue expression and hormonal regulation. *Biochem. Biophys. Res. Commun.*, **183**, 1090–1096.

SANDBERG, K., HONG, J., CLARK, A.J.L., SHAPIRA, H. & CATT, K.J. (1992). Cloning and expression of a novel angiotensin II receptor subtype. *J. Biol. Chem.*, **267**, 9455–9458.

TIMMERMANS, P.B.M.W.M., WONG, P.C., CHIU, A.T., HERBLIN, W.F., BENFIELD, P., CARINI, D.J., LEE, R.J., WEXLER, R.R., SAYE, J.A.M. & SMITH, R.D. (1993). Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol. Rev.*, **45**, 205–251.

SIM, M.K. & SOH, K.S. (1995). Effects of des-Asp-angiotensin I on the electrically-stimulated contraction of the rabbit pulmonary artery. *Eur. J. Pharmacol.*, **284**, 215–219.

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