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Short communication

Actions of D-amino acid-substituted analogues of des-Asp-angiotensin I on the central pressor action of angiotensin III

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Abstract

The ability of intracerebroventricularly (i.c.v.) administered D-amino acid-substituted analogues of des-Asp-angiotensin I to attenuate the central pressor action of angiotensin III in the rat was investigated. Of the 9 D-amino acid-substituted analogues, only D-tyrosine-des-Asp-angiotensin I was active. I.c.v. D-tyrosine-angiotensin I but not i.c.v. D-isoleucine-angiotensin I (when prevented from degradation by angiotensin converting enzyme with captopril) also attenuated the central pressor action of angiotensin III. In vitro incubation of angiotensin I, D-tyrosine-angiotensin I and D-isoleucine-angiotensin I with brain homogenate resulted in the formation of des-Asp-angiotensin I, D-tyrosine-des-Asp-angiotensin I and D-isoleucine-des-Asp-angiotensin I, respectively. This shows that i.c.v. angiotensin I and D-tyrosine-angiotensin I were converted by brain aminopeptidase to des-Asp-angiotensin I and D-tyrosine-des-Asp-angiotensin I, respectively, which then attenuated the pressor action of angiotensin III. When compared to the findings of similar D-substitution studies carried out with angiotensin II and [Sar^1,Ile^8]angiotensin II by other investigators, des-Asp-angiotensin I has a stringent structural-activity relationship. These findings suggest that, at the physiological level, des-Asp-angiotensin I is formed from angiotensin I and that the nonapeptide probably acts on a distinct subtype of angiotensin receptors.

Keywords: Des-Asp-angiotensin I; Angiotensin analog, D-amino acid substituted; Angiotensin III; Blood pressure

1. Introduction

Des-Asp-angiotensin I is a component peptide of the renin-angiotensin system (Garrison and Peach, 1991). In homogenates of the rat hypothalamus and aorta, des-Asp-angiotensin I is formed from angiotensin I by a specific aminopeptidase that is not inhibited by amastatin, bestatin and EDTA (Sim, 1993; Sim et al., 1994). The nonapeptide has also been shown to attenuate the pressor action of angiotensin II and angiotensin III in the brain (Sim and Radhakrishnan, 1994). Peripherally, it is able to potentiate the contractile action of angiotensin II on the rabbit aortic ring and to attenuate the contractile action of angiotensin III in the same tissue (Sim and Yuan, 1995). These findings seem to indicate that des-Asp-angiotensin I is a functional peptide that may have as yet to be defined specific actions in blood pressure regulation. This study is an attempt to determine which of the nine amino acids that make up its structure is essential for its central action, i.e. its ability to attenuate the central pressor action of angiotensin III. Findings from this study also indicate that, physiologically, des-Asp-angiotensin I is formed from angiotensin I.

2. Materials and methods

Twenty-week-old male Wistar Kyoto (WKY) rats (350–400 g) were obtained from the Resource Centre, Perth, Western Australia and the experiment was carried out as described previously (Sim and Radhakrishnan, 1994). Briefly, an i.c.v. cannula and a telemetry blood pressure transmitter (Data Sciences International, USA) were implanted into each rat under pentobarbital (50 mg/kg i.v.) anaesthesia. The catheter of the transmitter was inserted into the abdominal aorta 1 mm above the bifurcation. Four days after implantation, the blood pressure of conscious animals was recorded via a receiver and displayed on a per-

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sonal computer. After 1 h of acclimatization, the following protocol of drug administration was carried out. A bolus i.c.v. dose of 100 pmol of angiotensin III in 10 μl of saline was administered to initiate a blood pressure rise which lasted for about 6 min. Sixty minutes later, a bolus i.c.v. dose of 0.5 μmol captopril, 1000 pmol of a D-amino acid-substituted analogue of des-Asp-angiotensin I (namely: D-arginine-des-Asp-angiotensin I, D-valine-des-Asp-angiotensin I, D-tyrosine-angiotensin I, D-isoleucine-des-Asp-angiotensin I, D-histidine(6)-des-Asp-angiotensin I, D-proline-des-Asp-angiotensin I, D-phenylalanine-des-Asp-angiotensin I, D-histidine(9)-des-Asp-angiotensin I) and 100 pmol of angiotensin III were sequentially administered in 10 μl saline at an interval of 15 min. The dose of 0.5 μmol captopril has been shown, in an earlier study, not to affect the pressor action to angiotensin III (Sim and Radhakrishnan, 1994). The experiment was repeated in the same animal 2 days later followed by a control experiment after another 2 days where the dose of the D-substituted des-Asp-angiotensin I was replaced with saline. Angiotensin III and not angiotensin II was used to induce the pressor response in this study because it had been shown earlier that 1000 pmol (but not 100 pmol) of des-Asp-angiotensin I attenuated completely the pressor response of the haptapeptide and not that of the octapeptide in the WKY (Sim and Radhakrishnan, 1994). Similarly, from preliminary experiments using 10, 100 and 1000 pmol of angiotensin III, it was found that 100 pmol angiotensin III produced a sizable response (comparable to that obtained by Wright et al., 1990) that could be completely attenuated by 1000 pmol des-Asp-angiotensin I. The response induced by 1000 pmol angiotensin III was only partially attenuated by the nonapeptide.

The above experiment was then repeated using angiotensin I, D-tyrosine-angiotensin I, D-isoleucine-angiotensin I, angiotensin-(3–10), angiotensin-(2–9) and angiotensin-(1–9) instead of the D-amino acid-substituted des-Asp-angiotensin I. The purpose of this experiment was to show that i.c.v. angiotensin I and its D-amino acid-substituted derivatives were converted to des-Asp-angiotensins which could then affect the pressor action of angiotensin III. That the same aminopeptidase was responsible for the conversion of the three forms of angiotensin I to the des-Asp-angiotensins was confirmed by incubating them with brain homogenate and monitoring the formation of des-Asp-angiotensins using capillary electrophoresis as described previously (Sim et al., 1994). Briefly, homogenates of whole brain (minus the cerebellum) of WKY rats were prepared in 10 volumes (w/v) of 0.1 M Tris-HCl buffer pH 7.5 by ultrasonication. 105 μl of homogenate was added to 210 μl of solution containing one of the three forms of angiotensin I in 0.1 M Tris-HCl buffer, pH 7.5, containing 300 μM of the substrate and 10^{-4} M of amastatin, bestatin, and EDTA in a final volume of 315 μl. Incubation was carried out at 37°C and three sequence aliquots of 100 μl of this incubation solution were pipetted into three separate vials containing 100 μl of 0.5 M perchloric acid at 5, 10, and 15 min, respectively. These three solutions were then centrifuged at 100000 × *g* and 4°C for 2 h. The angiotensin in each supernatant was then separated and identified by capillary electrophoresis as described previously (Lim and Sim, 1994).

Angiotensin III, des-Asp-angiotensin I, angiotensin-(3–10), angiotensin-(2–9) and angiotensin-(1–9) were purchased from Bachem Feinchemikalien. Captopril was purchased from Sigma. The D-amino acid analogues of des-Asp-angiotensin I and angiotensin I were custom made by Chiron Mimotopes, Australia.

3. Results

Of the nine D-amino acid-substituted forms of des-Asp-angiotensin I, only D-tyrosine-des-Asp-angiotensin I retained the ability to attenuate the pressor action of angiotensin III. Similarly, only D-tyrosine-angiotensin I but not D-isoleucine-angiotensin I attenuated the pressor action of angiotensin III. These data are graphically displayed in Fig. 1. Angiotensin-(3–10), angiotensin-(2–9) and angiotensin-(1–9) were also with-

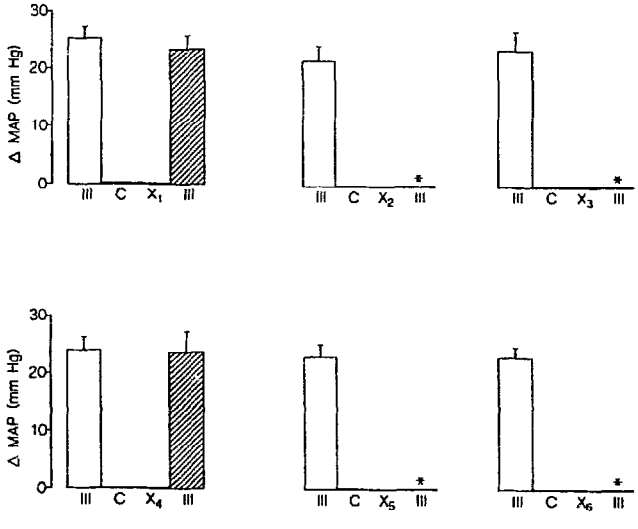

Fig. 1. Attenuation of the central pressor action of angiotensin III by D-amino acid-substituted analogues of angiotensin I (upper histograms) and des-Asp-angiotensin I (lower histograms) in the WKY rat. III = 100 pmol angiotensin III, C = 0.5 μmol captopril, X_1 = 1000 pmol D-isoleucine-angiotensin I, X_2 = 1000 pmol angiotensin I, X_3 = 1000 pmol D-tyrosine-angiotensin I, X_4 = 1000 pmol D-isoleucine-des-Asp-angiotensin I, X_5 = 1000 pmol des-Asp-angiotensin I, X_6 = 1000 pmol D-tyrosine-des-Asp-angiotensin I. * Indicates complete attenuation of the pressor action of angiotensin III. Each histogram represents the mean value ± S.E.M. (error bar) obtained from 5–6 rats. The basal blood pressure and heart rate were 112 ± 2 mm Hg and 293 ± 19 beats/min, respectively.

out effect on the pressor action of angiotensin III (data not shown).

The brain homogenates converted D-tyrosine-angiotensin I and D-isoleucine-angiotensin I to the corresponding des-Asp-angiotensin I at the same rate as angiotensin I was converted to des-Asp-angiotensin I.

4. Discussion

The data tend to indicate that des-Asp-angiotensin I has a very stringent structural-activity relationship. The original configurations of all but one amino acid were required for its activity. Each of these eight essential configurations determines an all or none relationship, i.e. the L-configuration possesses 100% activity and the D-configuration possesses no activity. This is quite unlike angiotensin II or [Sar^1,Ile^8]angiotensin II, where the respective agonistic or antagonistic activity is still present when each of the 1–7 amino acids is replaced by the D-isomer (Samanen et al., 1988). In these two compounds the configuration of the eighth amino acid, phenylalanine, appears to be the critical in determining activity (Samanen et al., 1988).

The requirement for the original stereo-configurations of eight amino acids for evoking the activity of des-Asp-angiotensin I seems to indicate that the nonapeptide may act on a distinct subtype of the angiotensin receptor. The occupation of this receptor in the brain leads to differential attenuation of the central pressor action of angiotensin II and angiotensin III (Sim and Radhakrishnan, 1994) and to potentiation and attenuation of the contractile action of angiotensin II and angiotensin III, respectively, in the rabbit aorta (Sim and Yuan, 1995). In addition, our recent studies on transmural nerve stimulation in the rabbit pulmonary artery indicate that des-Asp-angiotensin acts presynaptically on a subtype of angiotensin AT_1 receptor that involves the release of prostaglandin/s (Sim and Soh, 1995).

The data also show that when the action of angiotensin converting enzyme was inhibited by captopril, i.c.v. angiotensin I, D-tyrosine-angiotensin I and D-isoleucine-angiotensin I were probably converted by an aminopeptidase to the corresponding form of the des-Asp-angiotensins. This aminopeptidase is probably the specific enzyme that has been described previously. Unlike the known aminopeptidases, it is not inhibited by amastatin, bestatin and EDTA (Sim et al., 1994). As only des-Asp-angiotensin I and D-tyrosine-des-Asp-angiotensin I were active in attenuating the pressor action of angiotensin III, des-isoleucine-angiotensin I would have no effect on the pressor action of angiotensin III even if it were converted to D-isoleucine-des-Asp-angiotensin I. The lack of activity of angiotensin-(3–10), angiotensin-(2–9) and angiotensin-(1–9) also indicates that the activity of angiotensin I and D-tyrosine-angiotensin I could not have been due to their degradation to these products. In this study, 1000 pmol of both angiotensin I and D-tyrosine-angiotensin I were probably converted to their respective active des-Asp-angiotensins in situ in conscious moving rats. The conversion was under physiological and rate-limiting conditions, leading to the production of physiological levels of the des-Asp-angiotensins during the 15-min interval. These data provide further support for the suggestion that des-Asp-angiotensin I is a physiological peptide that is formed from angiotensin I by the action of a specific aminopeptidase.

Acknowledgements

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