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Short communication

Determination of aminopeptidase X activity in tissues of normo- and hypertensive rats by capillary electrophoresis

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Abstract

Aminopeptidase X, an enzyme that degrades angiotensin I to des-asp-angiotensin I, was determined in the lung, liver, kidney, plasma, endothelium and aortic smooth muscle of the spontaneously hypertensive rat (SHR) and its normotensive control, the Wistar Kyoto rat (WKY). The enzyme activity in the lung, kidney, plasma and endothelium of the SHR was elevated and this supports an earlier suggestion that in certain critical tissues of the SHR, the degradation of angiotensin I is shunted in favour of the des-asp-angiotensin I pathway. In these tissues, the formation of pressor angiotensin II would be curtailed and that of des-asp-angiotensin I enhanced. As des-asp-angiotensin I lacks direct vasopressor action, its preferential formation over that of angiotensin II could be a physiological response to the prevailing hypertension in the SHR. ©1997 Elsevier Science B.V.

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1. I. Introduction

Recent studies using capillary electrophoresis to separate the immediate metabolite of angiotensin I have shown that homogenates of the rat aorta and hypothalamus degraded angiotensin I to mainly des-asp-angiotensin I instead of angiotensin II [1,2]. The degradation was brought about by a specific aminopeptidase (named aminopeptidase X), which was not inhibited by amastatin, bestatin and EDTA [3]. Aminopeptidase X, together with angiotensin-converting enzyme and aminopeptidase N, may form a specific pathway for the degradation of angiotensin I that bypasses the formation of angiotensin II [3]. The likely importance of this pathway in blood pressure regulation is shown by the fact that the activity of aminopeptidase X is significantly elevated in the hypothalamus of hypertensive rats [2], and that des-asp-angiotensin I attenuated the central pressor actions of angiotensin II and angiotensin III in both the normo- and hypertensive rats [4]. In addition, des-asp-angiotensin I has also been shown to be a functional peptide, being formed from angiotensin I under physiological conditions [5]. This study investigated the distribution of aminopeptidase X in six tissues of normo- and hypertensive rats. The differential distribution of the enzyme between the tissues of the normo- and hypertensive rats will further decipher the likely role of aminopeptidase X in blood pressure regulation.

2. Experimental

2.1. Animals

The animals were 3–4 month old male rats. SHR

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and WKY were obtained from the Animal Resource Centre, Murdoch, Western Australia.

2.2. Preparation of tissue homogenates

Endothelial and smooth muscle homogenates were prepared as described previously [1]. Briefly, the aorta was trimmed free of fat, blood clot and connective tissue and its weight was recorded. The aorta was then everted over a pasteur pipette, cut into two sections and immersed in two volumes of 0.1 *M* phosphate buffer, pH 7.5, containing 0.1 *M* NaCl in a test-tube placed in a vial, on ice. The endothelium of pooled everted sections of aortas from three rats were then ruptured by ultrasound sonication for 30 min at maximum speed in a Vibra Cell sonicator, to obtain the endothelial homogenate. Selective rupture of the endothelium by this method was confirmed histologically [6]. The remainder of the sonicated aortas was minced with a pair of fine scissors and homogenised with a teflon Potter-Elvehjem tissue grinder in two volumes of the same phosphate buffer, to obtain the smooth muscle cum adventitia homogenate. The lung, liver and kidneys were removed and freed of fat and connective tissue and each organ was homogenised in five volumes of 0.1 *M* phosphate buffer using the teflon Potter-Elvehjem tissue grinder. The blood was collected in heparinised tubes and then centrifuged at 4000 *g* for 10 min at 4°C to obtain the plasma. A 300-μl volume of each homogenate was then introduced separately into dialysis tubing (10 000 molecular mass cut-off) and dialysed against 1000 ml of 0.1 *M* phosphate buffer, pH 7.5, for 2 h.

The protein in the homogenates and plasma was determined by the method of Lowry et al. [7].

2.3. Assay of aminopeptidase

A 105-μl volume of each homogenate was added to 210 μl of ANG I in 0.1 *M* potassium phosphate buffer, pH 7.5, containing 300 μ*M* ANG I, 0.1 m*M* EDTA, 0.1 m*M* amastatin, O.1 m*M* bestatin and 0.05 *M* NaCl, in a final volume of 315 μl. Incubation was carried out at 37°C and three sequential aliquots of 90 μl of this incubation solution were inactivated by 10 μl of 5 *M* perchloric acid at 10, 15 and 20 min. The solutions were then centrifuged at 100 000 *g* and 2°C (Beckman TL100) for 60 min. The angiotensins in each supernatant were then separated and quantitated by capillary electrophoresis (Waters Quanta 4000 capillary electrophoresis system), as described previously [8].

2.4. Quantitation by capillary electrophoresis

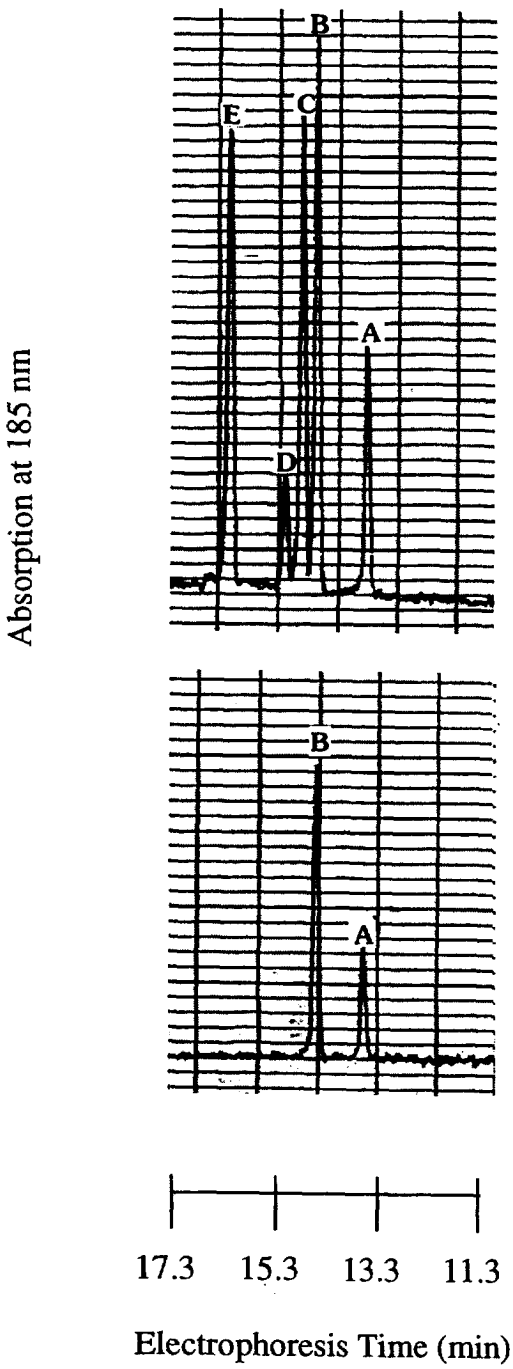
For each series of capillary electrophoresis analysis, the peak of interest, i.e. that of des-asp-angiotensin I, was verified and quantitated with a standard solution of the nonapeptide (5–40 μ*M*). Tubes containing the standard solutions were interspersed among the tubes containing the sample solutions. This is to randomise the error of analysis by the capillary electrophoresis that may arise as a result of changes in capillary conditions as the run progressed. The des-asp-angiotensin I in five aliquots of a sample was quantitated by the same protocol on the same day. From this, the intra-day coefficient of variation was found to be 5%. Five aliquots of a des-asp-angiotensin I solution were quantitated (one aliquot each day) by the same protocol on five successive days. From this, the inter-day coefficient of variation was found to be 5.5%.

2.5. Drugs

Amastatin and bestatin were purchased from Sigma. Angiotensin I and des-asp-angiotensin I were purchased from Bachem Feinchemikalien.

3. Results and discussion

Fig. 1 shows the capillary electropherogram of a standard solution containing five angiotensin peptides and a solution of enzymatically formed des-asp-angiotensin I. The enzymatic formation of des-asp-angiotensin I from angiotensin I was linearly related to time (up to 20 min) under the conditions of incubation (data not shown). Table 1 shows that aminopeptidase X activity is present in the lung, kidney, plasma, liver, aortic endothelium and smooth muscle of the normo- and hypertensive rats. These organs are known to contain an active renin–angiotensin system where angiotensin I is converted to



angiotensin II [9]. The presence of aminopeptidase X in these tissues suggests that angiotensin I is also converted to des-asp-angiotensin I. As angiotensin II is a potent pressor and des-asp-angiotensin I lacks pressor action [10], ACE (angiotensin converting enzyme) and aminopeptidase X are important critical enzymes of the renin-angiotensin system that enable the system to either form, or bypass the formation of, pressor angiotensin II. The mechanism of how the system is regulated to shunt the degradation of angiotensin I into either pathway is not known.

The data (Table 1) also show an increase in the activity of aminopeptidase X in the lung, kidney, plasma and endothelium of the SHR and they support an earlier suggestion that, in certain critical tissues of the hypertensive rat, the degradation of angiotensin I is shunted in favour of the des-asp-angiotensin I pathway [3]. In these tissues, the formation of the pressor angiotensin II would be curtailed and that of des-asp-angiotensin I enhanced. Besides lacking direct vasopressor action, des-asp-angiotensin I has been shown to reduce the electrically stimulated contraction of the rabbit pulmonary artery by acting on pre-synaptic receptors to attenuate the release of norepinephrine [11]. These events will result in vascular conditions favouring a drop in blood pressure. It is possible that the degradation of angiotensin I to either of the metabolites is blood-pressure dependent. A rise in blood pressure favours the formation of des-asp-angiotensin I over angiotensin II, and vice versa for a drop in blood pressure. For some unexplained reasons, the blood pressure of the SHR remains high despite the fact that the elevated aminopeptidase X activity in these animals favours the formation of des-asp-angiotensin I. One likely scenario is that the increase in aminopeptidase X activity in response to an elevation of blood pressure in the SHR is compromised.

The angiotensin-converting enzyme and aminopeptidase X could also form an intra-renin–angioten-

Fig. 1. Capillary electropherogram of angiotensin peptides. Top panel: a standard solution containing 80 μ*M* des-Asp-angiotensin I, 100 μ*M* angiotensin I, 100 μ*M* angiotensin III, 40 μ*M* angiotensin (1–7) and 120 μ*M* angiotensin II. Bottom panel: centrifuged endothelial homogenate that had been incubated with 300 μ*M* angiotensin I for 10 min. Electrophoresis was carried out at 10 kV for a period of 20 min in 0.1 *M* phosphoric acid, pH 1.95. The angiotensins were detected at 185 nm. A=angiotensin I, B=des-asp-angiotensin I, C=angiotensin III, D=angiotensin (1–7) and E=angiotensin II.

Table 1
Aminopeptidase X activity in various tissues of the normo- and hypertensive rat

Rat	Concentration (pmol/mg protein/min)					
	Lung	Liver	Kidney	Plasma	Endothelium	Smooth muscle
WKY	9.9±1.4	4.7±0.7	4.4±0.1	0.7±0.1	12.3±0.4	4.5±0.5
SHR	12.4±0.7	4.2±0.4	6.5±1.1	1.2±0.1[a]	16.2±0.7[a]	4.6±0.5

Each value is the mean±S.E.M. of 5–7 separate determinations.
[a] Significantly different from the corresponding values of the WKY ($p<0.05$, Student's t test).

sin system for the regulation of local or regional blood pressure and flow. This supports the paracrine role of angiotensin II [12,13] and possibly also that of des-asp-angiotensin I.

Acknowledgments

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