The effect of angiotensin II on haemodynamic and plasma noradrenaline responses to tyramine infusion in man

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Summary. Six normal volunteers were studied on four separate occasions. On each occasion they received two concomitant infusions which were either placebo/placebo, placebo/tyramine, angiotensin II/placebo or angiotensin II/tyramine. Angiotensin II infusion was given at a constant rate of 2 ng/kg/min whereas the tyramine infusion consisted of 10 min increments at 1.25, 2.5, 3.75, 5, 7.5 and 10 μg·kg⁻¹·min⁻¹.

Tyramine infusion caused a dose dependent increase in systolic blood pressure with increases in diastolic blood pressure and plasma noradrenaline only at the highest doses. These changes were not affected by concomitant angiotensin infusion.

We have therefore found no evidence to support the enhancement of haemodynamic or plasma noradrenaline responses to tyramine infusion by low dose infusion of angiotensin II in man.

Key words: Angiotensin II, Noradrenaline release; tyramine infusion, arterial pressure

The renin-angiotensin system (RAS) and the sympathetic nervous system (SNS) are essential for blood pressure homeostasis in man and their independent roles in this respect are well established. The RAS influences arterial pressure in a number of ways through a combination of the direct and indirect actions of angiotensin II (ANG-II). In addition to established mechanisms facilitation of adrenergic neurotransmission has been recognised as a further indirect mechanism whereby ANG-II can influence cardiovascular control in animals [Vanhoutte et al. 1981; Zimmerman et al. 1984]. In a review of this work de Jonge et al. (1984) identify four proposed mechanisms, three of which occur presynaptically and one postsynaptically. Firstly that ANG-II has a Tyramine-like activity whereby ANG-II caused a release of noradrenaline (NA) from the stores at sympathetic nerve terminals. Secondly blockade of the neuronal reuptake of NA has been found in some studies. Thirdly ANG-II causes prejunctional facilitation of sympathetic neurotransmission in vascular smooth muscle by enhancing the amount of NA released per nerve impulse. Finally, a postsynaptic sensitization of vascular alpha-adrenoceptors might occur.

Despite this compelling animal evidence to suggest that ANG-II facilitates sympathetic transmission, it has been difficult to convincingly establish a similar mechanism in man. We have previously found that a subpressor infusion of ANG-II does not alter the haemodynamic or plasma NA response to a cold pressor test, bicycle exercise or forearm isometric exercise [Seidelin et al. 1987]. In case the kind of SNS stimulation is a crucial factor, we have now sought evidence for an ANG-II/NA interaction by examining whether exogenous ANG-II is able to augment the presynaptic release of endogenous NA in response to infused tyramine (TYR) in normal man.

Subjects and methods

Six normotensive volunteers (4 m and 2 f) were studied. Their mean age was 24 y (range 18-33 y) and mean weight 70 kg (range 54-87.5 kg). Clinical examination, routine haematology, blood biochemistry, urinalysis and electrocardiography (ECG) were normal in all subjects. Each subject gave written informed consent to the study which had been approved by the Medical and Dental research ethical committee of the University of Dundee.

No subject was on any regular medication and all were instructed not to take any medication for 7 days prior to each study day. They were also instructed to abstain from alcohol and smoking for 24 h prior to each study day and to take no caffeine containing drinks from 22.00 h the evening before each study day. They were studied on four separate occasions at least 5 days apart. Sodium intake was not strictly controlled but each subject was asked to maintain the same approximate sodium intake for 3 days before each study day which was assessed by baseline plasma ANG-II levels.

The subjects attended the clinical laboratory at 09.30 h after a light breakfast or at 13.30 h after a light luncheon. Each individual was investigated at the same time of day on all four study days. They were positioned supine and intravenous cannulae were placed in veins in both forearms. After 20 min of supine rest an infusion of either ANG-II (2 ng·kg⁻¹·min⁻¹) or placebo (5% dextrose) was commenced and infused at a constant rate in a volume of 0.5 ml·min⁻¹ throughout the rest of the study. After a further 20 min
supine rest an incremental infusion of placebo (5% dextrose) or TYR was commenced at 1.25 μg·kg⁻¹·min⁻¹ and increased at 10 min intervals to 2.50, 3.75, 5.00, 7.50 and 10.0 μg·kg⁻¹·min⁻¹. The infusions were given in randomised single blind fashion. Throughout each experiment blood pressure (BP) was recorded semi-automatically (Dinamap vital signs monitor 1846, Critikon USA) and heart rate was monitored continuously on an ECG oscilloscope (Hewlett Packard, USA).

Blood samples were taken and divided into two aliquots for later analysis of plasma ANG-II and plasma NA. The first aliquot was taken into chilled lithium heparin tubes for measurement of NA. The second aliquot was taken into chilled glass tubes containing a solution of 0.05 M O-phenanthroline, 2 g·l⁻¹ neomycin, 0.125 M EDTA disodium salt and 2% ethanol for measurement of ANG-II. The samples were immediately centrifuged at 4°C, separated and stored at −70°C until assayed. Plasma NA was measured by our double isotope radioenzymatic assay [Brown & Jenner, 1981]. The intra-assay coefficient of variation for this method in our laboratory was 8% and the interassay coefficient of variation was 11.1%. Plasma ANG-II was measured, after plasma extraction, by a commercially available radioimmunoassay kit (Immuno-diagnostics Ltd., U.K.). The intra-assay coefficient of variation for this method in our laboratory was 5.8% and the interassay coefficient of variation was 12.1%.

Results were analysed by repeated measures analysis of variance (MANOVA, SPSS/PC+ ) to examine the effect of time, treatments and for an interaction between treatments. A P-value of < 0.05 was considered significant. Results are given as mean (SEM) in the figure and tables.

**Results**

Resting systolic BP, diastolic BP and heart rate were not significantly different on all four study days (Fig. 1 and Table 1). There were no significant changes in any of these observations in response to ANG-II infusion. Fig. 1 (a) shows the effect of TYR infusion on systolic and diastolic BP with concomitant placebo infusion, and Fig. 1 (b) shows the effect of TYR infusion with concomitant ANG-II infusion. ANG-II alone produced no significant change in systolic or diastolic BP TYR alone (Fig. 1) caused a large dose-dependent increase in systolic BP (118 (3) to 138 (5) mmHg, P < 0.02 MANOVA) and a smaller but significant increase in diastolic BP at the two highest doses of TYR (P < 0.03). When infused together, ANG-II/TYR produced a similar dose-dependent increase in systolic BP (115 (2) to 143 (6) mmHg, P < 0.02) and a small rise in

<table>
<thead>
<tr>
<th>Table 1. Changes in heart rate (beats per min)</th>
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<tr>
<td>Dose of tyramine (μg/kg/min)</td>
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PL = placebo infusion (5% dextrose), ANG = angiotensin II infusion (2 ng·kg⁻¹·min⁻¹), TYR = tyramine infusion (1.25–10.0 μg·kg⁻¹·min⁻¹ as indicated). Resting = baseline measurements taken after a 20 min infusion of either ANG-II or PL as indicated. This was followed by an incremental infusion of TYR in the doses indicated. Results are given as mean with (SEM)