Location of vascular α_2 -adrenoceptors in man

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To determine whether vascular postsynaptic α_2 -adrenoceptors are innervated by sympathetic nerves in man, normal volunteers were infused for 90 min with angiotensin II (A II) and the α_2 -adrenoceptor agonist α -methylnoradrenaline (MNA) in a double-blind cross-over study. Whilst systolic blood pressure returned to baseline within 5 min of terminating the A II infusion it remained elevated 40 min after stopping MNA. The prolongation of the pressor response to MNA, a substrate for neuronal uptake, was probably due to activation of α_2 -adrenoceptors by MNA re-released from contiguous sympathetic nerve endings. The proximity of α_2 -adrenoceptors to sympathetic nerve terminals suggests that they could contribute to blood pressure regulation in man.

Keywords α_2 -adrenoceptors blood pressure angiotensin II
 α -methylnoradrenaline

Introduction

There is increasing evidence that postsynaptic α -adrenoceptors are not homogenous and that the pressor response to catecholamines is mediated by both α_1 - and α_2 -adrenoceptors on vascular smooth muscle in many species (Timmermans *et al.*, 1979; Madjar *et al.*, 1980; Langer *et al.*, 1980). Whether vascular α_2 -adrenoceptors are important in blood pressure regulation in man is unclear. Elliot & Reid (1983) have demonstrated a significant pressor response to infusion of the selective α_2 -adrenoceptor agonist α -methylnoradrenaline (α MNA) in normal volunteers whilst van Brumellen *et al.* (1983) have demonstrated an increase in forearm vascular resistance following infusion of the α_2 -adrenoceptor agonist BHT920 and a decrease after yohimbine administration. On the other hand, we have been unable to demonstrate any inhibition of the pressor response to infused adrenaline after pre-treatment with the selective α_2 -adrenoceptor antagonist, RX781094, in normal man (Brown & Dollery, 1984).

A major factor determining the functional role of postsynaptic α_2 -adrenoceptors might be

their anatomical location in relation to adrenergic nerve endings. Under most conditions, the circulating adrenaline and noradrenaline concentrations in man are low relative to noradrenaline concentrations in the synaptic cleft and are unlikely to contribute to blood pressure regulation by stimulation of vascular extrasynaptic α_2 -adrenoceptors. However, α_2 -adrenoceptors could be important if stimulated by noradrenaline released from nerve endings directly into the synaptic cleft.

We have shown previously that a 90 min infusion of adrenaline in man is followed by prolonged tachycardia and a small increase in blood pressure (Brown & Macquin, 1981). These effects were attenuated by prior treatment with desmethyylimipramine, suggesting that the mechanism involved uptake of adrenaline during the infusion, and its re-release into the synapse when the infusion had stopped. α MNA is also a substrate for neuronal uptake (Iversen, 1967), is not subject to metabolism by monoamine oxidase and elevates blood pressure in man. It seemed likely, therefore,

that intravenous infusion might similarly load sympathetic nerve endings with α MNA which would be re-released intrasynaptically later. If the pressor response seen during the infusion persisted after circulating α MNA had been cleared, this would provide evidence that α_2 -adrenoceptor stimulation by noradrenaline released directly into the synaptic cleft can contribute to regulation of blood pressure.

Methods

The study involved six normal male volunteers (mean weight 72 ± 8 kg, mean age 29 ± 2 years). The protocol of the study was approved by the Research Ethics Committee of the Royal Postgraduate Medical School and Hammersmith Hospital. All subjects were drug free and avoided caffeinated drinks and smoking for 12 h prior to infusions.

A cannula was inserted in an antecubital vein for drug infusion and a 19 gauge 'butterfly' needle in the opposite forearm for blood sampling. Blood pressure was measured by Roche Arteriosonde, the mean of duplicate readings being recorded. Heart rate was counted for 1 min, at intervals, from a Grass Polygraph recording.

After 20 min rest, a 10 min infusion of 0.9% saline was administered. This was followed by 10 min infusions of α MNA (Corbasil), the rates ranging from 0.03 to $0.2 \mu\text{g kg}^{-1} \text{min}^{-1}$, or angiotensin II (Hypertensin) at rates of 4 to $32 \text{ ng kg}^{-1} \text{min}^{-1}$. The infusion rate resulting in an increase of 20–30 mm Hg in systolic pressure was then administered for a further 90 min. Blood pressure and heart rate were monitored for a further 40 min after the termination of agonist administration. At least 1 week separated the two infusions. The order of infusions was randomised and the study was double-blind.

Blood samples for plasma noradrenaline (NA) and α MNA estimation were taken during dose ranging, during the 90th minute of the long infusion and at intervals during the offset. Plasma NA and α MNA were measured by radioenzymatic assay (Brown & Jenner, 1981).

Results are expressed as mean $\pm$ s.d. Differences between the pre- and post-infusion blood pressure levels after both drugs were analysed by two way analysis of variance. In addition, to determine the duration of a significant difference, the differences in blood pressure from baseline at each time point were also analysed by unpaired *t*-tests.

Results

The mean systolic blood pressure prior to α MNA infusion (110 ± 10 mm Hg) was slightly lower than that prior to A II (118 ± 8 mm Hg). The infusion rates required to produce an increase of 20–30 mm Hg during dose ranging were $0.15 \mu\text{g kg}^{-1} \text{min}^{-1}$ for α MNA and $16 \text{ ng kg}^{-1} \text{min}^{-1}$ for A II. Both agonists produced comparable increases in systolic blood pressure, $+21 \pm 7$ mm Hg after 90 min of α MNA infusion and $+21 \pm 6$ mm Hg at the end of A II administration. However, Figure 1 illustrates the difference in the response of diastolic pressure, α MNA produced a small decrease (-6 ± 6 mm Hg) whilst A II increased diastolic pressure ($+28 \pm 6$ mm Hg). Heart rate changes are also depicted in Figure 1.

Whilst plasma noradrenaline did not change appreciably during α MNA infusion, there was a dose-dependent increase in plasma MNA during the incremental infusions and this fell to baseline within 15 min of cessation of the long infusion.

On stopping the A II infusion, systolic blood

Figure 1 Changes in blood pressure (---) and heart rate (HR) (---) in normal volunteers in response to increasing doses (DR) of i.v. methylnoradrenaline (a) and angiotensin II (b) during 90 min infusions (Long infusion) and after infusions have been stopped (Offset). Systolic pressure remained elevated ($*P < 0.005$) compared with baseline after MNA infusion. ($n = 6$, mean $\pm$ s.e.)

Figure 2 Differences in blood pressure from baseline during 40 min following the infusion of methylnoradrenaline (MNA) (●) and angiotensin II (AII) (■) in normal volunteers ($n = 6$, mean $\pm$ s.e.). Blood pressure after MNA was significantly elevated ($P < 0.005$ by analysis of variance).

pressure fell to pre-infusion levels within 5 min. Although termination of the α MNA infusion resulted in a small initial reduction in pressure, it remained significantly elevated above baseline throughout the post-infusion observation period, $P < 0.005$ by analysis of variance. 40 min after cessation of the α MNA infusion, systolic blood pressure was 12.3 ± 2.7 mm Hg above control compared with 3.2 ± 5.7 mm Hg after A II infusion ($P < 0.05$) (Figure 2).

Discussion

The prolonged increase in systolic blood pressure following the cessation of α MNA infusion (and reduction in plasma α MNA to baseline) supports the existence of a significant number of α_2 -adrenoceptors located within, or contiguous to, the sympathetic synapses in man. During dose ranging, the pressor response was proportional to the circulating agonist concentration. At the end of the infusion, however, α MNA was rapidly cleared from the plasma and although there was a small reduction in systolic blood pressure, it remained significantly elevated compared to control levels. Since α MNA, unlike A II, is subject to uptake into sympathetic nerve endings we propose that the continuing pressor response was due to stimulation of peri- or intrasynaptic α_2 -adrenoceptors by re-released α MNA.

Are there alternative explanations? Certainly

the persistent elevation in systolic pressure after α MNA was not due to baroreflex resetting during the long infusion, since the same resetting should have accompanied A II infusion. On the other hand, the reduction in diastolic pressure during its infusion shows that α MNA is not a pure α -adrenoceptor agonist. Wilffert *et al.* (1983) have suggested that it is also a β_2 -adrenoceptor agonist because its ability to raise blood pressure in the pithed rat is enhanced by prior β_2 -adrenoceptor blockade. Thus its haemodynamic effects are probably the resultant of α_2 -adrenoceptor-mediated vasoconstriction, β_2 -adrenoceptor-mediated vasodilation and, possibly, increased cardiac output. The question then is: could the continuing pressor response after α MNA infusion be due to β_2 -adrenoceptor mediated facilitation of noradrenaline release rather than continuing α_2 -adrenoceptor stimulation? It is unlikely since re-released α MNA should also stimulate presynaptic α_2 -adrenoceptors which inhibit noradrenaline release (Langer, 1974). In fact, since plasma noradrenaline concentration remained unchanged throughout the study, there is no evidence to support presynaptic receptor stimulation in either direction.

Clearly the evidence we present is circumstantial but the findings are consistent with perisynaptic location of α_2 -adrenoceptors which could contribute to blood pressure control in man.

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