Angiotensin II augments sympathetically induced venoconstriction in man

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(Received 17 November 1987/5 February 1988; accepted 23 February 1988)

SUMMARY
1. The constriction produced by a single deep breath was measured simultaneously in two adjacent hand veins in normal volunteers. One vein was infused with angiotensin II (ANG II) while the other acted as a control.
2. At a dose lower than that required to produce direct venous constriction (1 pmol/min), ANG II significantly augmented the constriction caused by a deep breath in eight subjects \( (P<0.01) \). The same dose had no effect on the venoconstriction caused by infused noradrenaline (NA) in a further six subjects.
3. It is concluded that ANG II at low doses may cause venoconstriction indirectly by augmenting sympathetically induced venous tone via a presynaptic mechanism. This observation may help to explain the apparent venodilating property of angiotensin-converting enzyme inhibitors in clinical situations where the renin–angiotensin system is stimulated.

Key words: angiotensin II, sympathetic nervous system, veins.

Abbreviations: ANG II, angiotensin II; NA, noradrenaline.

INTRODUCTION
Angiotensin II (ANG II) is a potent arteriolar constrictor. The mechanism of this action in arterioles is thought to be through direct stimulation of specific ANG II receptors on vascular smooth muscle [1], although there is also evidence that ANG II may act in some circumstances by facilitation of sympathetically mediated constriction [2, 3].

When ANG II is infused directly into relaxed veins, either no effect is demonstrated [4] or if constriction occurs it does so only at high doses with marked and rapid tachyphylaxis [5]. Angiotensin-converting enzyme inhibitors, however, produce a large reduction of right atrial pressure when administered acutely to patients with heart failure and renal artery stenosis [6]. This suggests that, in these circumstances, these agents exhibit a venodilator action, presumably by reversing a venoconstrictor action of ANG II.

The purpose of the present study was to determine in healthy subjects whether ANG II, at a concentration which has no direct constrictor effect on relaxed veins, can affect venous constriction caused by sympathetic activity. The deep breath reflex was used as a stimulus for the production of transient venoconstriction [7, 8]. As the response to a deep breath may vary, an adjacent vein which was not infused with ANG II was studied simultaneously and served as a control.

METHODS
Protocol
Experiments were performed on healthy male volunteers aged 25–58 years. Saline (0.9% NaCl; Travenol, Thetford, Norfolk, U.K.), ANG II (Calbiochem, La Jolla, CA, U.S.A.), noradrenaline (NA; Levophed; Winthrop, Guildford, Surrey, U.K.) and phentolamine (Rogitine; Ciba, Horsham, W. Sussex, U.K.) were infused into a dorsal hand vein via a 23 SWG steel cannula (Butterfly-23, Abbott, U.K.) with a total infusate flow of 0.5 ml/min using a constant-rate infusion pump (Harvard, 944A). The hand was immobilized above heart level. The sizes of the infused vein and an adjacent control vein, when constricted by inflating an upper arm cuff to a pressure of 30 mmHg, were measured in arbitrary units by the method of Collier et al. [9] modified to include the use of light-weight levers and a control vein measurement. Light-weight levers rested on the summit of the veins and the fulcrum of each lever was connected to an electronic transducer (Harvard heart/smooth muscle transducer).
and pen recorder. The sizes of the veins were estimated by downward displacement of the levers after deflation of the upper arm cuff. Venoconstriction was shown by a downward displacement of the lever during cuff congestion. The laboratory temperature was maintained to within 1°C during each experiment at a temperature between 26 and 30°C.

In a preliminary study in two subjects, phentolamine was infused into the experimental vein at 25 nmol/min for 15 min. The effect of this on the constriction produced by a single deep breath was measured.

In the first set of experiments the effect of increasing doses of ANG II was studied in six subjects. Saline then ANG II (0.04, 0.1, 0.4, 1.0 and 4.0 pmol/min) were each infused into the cannulated vein for 10 min. In both the experimental and control vein the constriction resulting from a single deep breath was measured after 6 min of each infusion period and vein size after 8 and 10 min, allowing the percentage constriction resulting from the deep breath to be calculated.

In a second set of experiments a more detailed study of the effect of ANG II at 1 pmol/min was made in eight subjects. Saline was infused for 20 min into one vein, followed by ANG II at 1 pmol/min for 20 min and then saline again for 20 min. The size of both veins was measured after 5, 7, 18 and 20 min of each infusion period; the mean of all four measurements was taken as the vein size. The constriction to a single deep breath was measured after 10 and 16 min of each infusion, and the mean of these two measurements was used to calculate the percentage constriction.

In a third set of experiments the effect of ANG II at a dose of 1 pmol/min on veins preconstricted with infused NA was studied in six subjects. Saline was infused, followed by NA at a dose of 200–800 pmol/min to achieve approximately 50% constriction. ANG II was then infused in addition to the same dose of NA, followed by NA alone. Finally, NA at a higher dose of 1600 pmol/min was infused to achieve maximum constriction. Each infusion was continued for 10 min and vein size was measured after 8 and 10 min of each infusion period; the mean of these two measurements was used for analysis. A control vein was not used in this experiment.

**Statistics**

For each infusion period the ratio of percentage constriction caused by a deep breath between infused and control veins, was calculated. This ratio was compared for each period using a Wilcoxon signed rank test. In the study of the effect of ANG II in a vein pre-constricted with NA, the size of the vein was compared for each infusion period, also using a Wilcoxon signed rank test.

**RESULTS**

**Effect of phentolamine on the deep breath reflex**

Infusion of phentolamine at 25 nmol/min resulted in complete abolition of the deep breath venoconstrictor response in the infused vein, but left the response in the control vein unchanged.

**Effect of a deep breath on veins receiving ANG II**

The size of the infused vein during saline infusion was 33 ± 2 (mean ± SEM), and during incrementally increasing doses of ANG II (0.04 to 4.0 pmol/min) was unchanged at 33 ± 2, 35 ± 3, 34 ± 3, 30 ± 2, 31 ± 2 and 32 ± 2 arbitrary units. The size of the control vein similarly did not change during the course of this experiment. The ratio of percentage constriction caused by a deep breath at each dose of ANG II is shown in Fig. 1. There was a significantly greater constriction after a deep breath in the vein infused with ANG II at doses of 1.0 and 4.0 pmol/min, but not when given at lower doses.

When ANG II was infused at a single dose of 1 pmol/min over a longer period of time, the percentage constriction caused by a deep breath in the two veins was significantly different from the control periods, both before (P < 0.01) and after (P < 0.05) ANG II infusion (Fig. 2 and Fig. 3a), suggesting that the peptide caused an enhanced constriction in the vein infused with ANG II. During the three infusion periods the resting sizes of the control and infused veins were unchanged (Fig. 3b).

**Effect of ANG II on the response to infused NA**

In veins constricted with NA, the addition of ANG II at a dose of 1 pmol/min had no significant effect on vein size in six experiments (Fig. 4). However, increasing the dose of NA in all experiments caused further constriction of the vein studied, demonstrating that the vein was capable of further venoconstriction.

**DISCUSSION**

This study demonstrates the ability of ANG II to augment venous constriction caused by a deep breath in normal volunteers. Enhancement of this reflex is achieved at
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Fig. 2. Tracing from one experiment showing augmentation of construction in an infused hand vein produced by a deep breath (DB) during ANG II administration compared with saline. The control vein shows no change. Cuff deflation (C) was performed to measure basal vein size, which did not change in either vein throughout the experiment. Reduction in vein size is shown in a downward direction.

Fig. 3. (a) Constriction resulting from a deep breath in infused vein (△) and control vein (●) before, during and after ANG II infusion (mean ± SEM). (b) Vein size in arbitrary units during the same infusion periods. Statistical significance: *P < 0.05, **P < 0.01 compared with previous infusion period.

Fig. 4. Vein size during infusions of saline, NA (200–800 pmol/min) NA + ANG II and higher dose NA [1600 pmol/min, NA(HD)]. Statistical significance: *P < 0.05 compared with previous infusion period.

doses which we have shown are below these required to cause a direct constrictor effect [5].

The deep breath venoconstrictor reflex has been shown by others to be neurogenically mediated, as it is abolished both by instillation of local anaesthetic and systemic infusion of ganglion-blocking agents [8]. We have shown that it is also blocked by phentolamine, suggesting that it is sympathetically mediated through an action on vascular α-receptors.

Previous studies in intact animals and isolated tissue preparations have demonstrated the capacity of ANG II to potentiate the effects of sympathetic activity peripherally [2, 3]. The mechanism may be through enhanced NA release at the nerve terminal [10] or by postsynaptic potentiation [11]. The present study suggests that in the dorsal hand vein a presynaptic mechanism is involved as there is no enhancement of the constrictor effect of infused NA. However, it is possible that exogenous NA is acting primarily on α2-receptors which are distant from sympathetic nerve terminals [12] and that the sensitivity of these receptors, as opposed to the α1 postsynaptic type, is not influenced by ANG II. Whereas the direct constric-
tion elicited in relaxed veins by higher doses of ANG II is rapidly attenuated [5], the augmentation of sympathetic constriction at lower doses in this study was undiminished after 16 min infusion when compared with that after 10 min infusion. This difference is consistent with the observation [13] that tachyphylaxis to ANG II occurs only in vessels that lack basal tone.

From theoretical considerations, the flow through a single dorsal hand vein is between 1 and 4 ml/min [14]. The dose of ANG II used in this study would be expected to produce local plasma concentrations greater than basal concentrations in normal man but similar to those produced when the renin-angiotensin system is stimulated, as in patients with diuretic-treated heart failure [15]. If augmentation of sympathetically mediated venoconstriction, shown here in superficial veins, is found in other venous beds in man and if, as our results suggest, the response does not exhibit tachyphylaxis, then the plasma concentration of ANG II in patients with heart failure may be within a range which, while having no direct vеноconstrictor action, may amplify sympathetically mediated vеноconstriction. Such ANG-II-induced augmentation of sympathetic function may explain the abnormally large rise in venous pressure which occurs with exercise in patients with heart failure, and is known to be mediated by increased sympathetic tone [16], although central effects of ANG II may also play a role [17]. Furthermore, interruption of such an ANG-II-induced vеноconstrictor mechanism may contribute to the reduction in right and left atrial pressure produced by angiotensin-converting enzyme inhibitors in patients with heart failure where the elevated concentration of ANG II is unlikely to have a direct vеноconstrictor effect. In addition such venodilatation may account for the marked first-dose hypotension that occurs with angiotensin-converting enzyme inhibitors in circumstances where ANG II plasma concentrations are raised [18].

In summary, this study shows that ANG II enhances the vеноconstriction produced by peripheral sympathetic activation, probably through a presynaptic mechanism. This action may play a role in the pathophysiology of heart failure and may account, at least in part, for the particular value of angiotensin-converting enzyme inhibitors in this condition.

ACKNOWLEDGMENTS

We thank Dr Warren Cooper and Merck, Sharpe and Dohme for providing financial assistance. N.B. is a Wellcome Research Training Fellow.

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