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# Interactions between angiotensin II, sympathetic nerve-mediated pressor response and cyclo-oxygenase products in the pithed rat

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**1** The influence of angiotensin II (AII) on resting blood pressure and on sympathetic nerve-mediated pressor responses in the pithed rat was investigated either by inhibiting the renin-angiotensin system or by infusing AII.

**2** Plasma AII levels in the pithed rat were approximately 20 fold higher than in normotensive rats.

**3** Infusion of a subpressor dose of AII ($50\,ng\,kg^{-1}\,min^{-1}$) had no effect on sympathetic nerve mediated pressor responses but a pressor dose of AII, ($200\,ng\,kg^{-1}\,min^{-1}$) facilitated nerve-mediated pressor responses.

**4** The angiotensin converting enzyme inhibitor, teprotide, and the AII-receptor antagonist, saralasin, lowered the diastolic blood pressure and attenuated sympathetic nerve-mediated pressor responses. There was no difference in the effects of teprotide at $1\,mg\,kg^{-1}$ and $10\,mg\,kg^{-1}$. Infusion of sodium nitroprusside at concentrations producing a fall in diastolic blood pressure of similar magnitude to that produced by teprotide and saralasin significantly attenuated nerve-mediated pressor responses.

**5** After teprotide, AII $50\,mg\,kg^{-1}\,min^{-1}$ increased diastolic blood pressure. The inhibitory effect of teprotide on nerve-mediated pressor responses was antagonized by this infusion of AII only if the rats were pretreated with the cyclo-oxygenase inhibitor, flurbiprofen.

**6** It is concluded that AII is a major determinant of vascular tone in the pithed rat and that inhibition of the renin-angiotensin system attenuates sympathetic nerve-mediated pressor responses at least in part through the fall in blood pressure *per se*. The demonstration of this is complicated by an excessive release of vasodilator prostaglandins possibly due to the infused AII. Since plasma AII levels are high, the effects of blockade of the renin-angiotensin system will be exaggerated and so the importance of AII as a modulator of sympathetic responses will be overestimated in this model.

## Introduction

The pithed rat has been used to assess the role of angiotensin II (AII) in maintaining a vascular sympathetic tone (Hatton & Clough, 1982; Antonaccio, 1985). AII is a direct pressor agent, a regulator of sodium balance and is thought to modulate the sympathetic nervous system at several sites. It stimulates the vasomotor centre (for reviews see Ferrario *et al.*, 1972; Dickenson & Ferrario, 1974; Reid, 1984), sympathetic ganglion cells (McCubbin, 1974) and stimulates the release of adrenal catecholamines (Peach, 1974). Also, it is thought to have a modulatory role at the sympathetic neuro-effector junction; prejunctionally, it can facilitate release and inhibit reuptake of noradrenaline (for reviews see Starke, 1977; Westfall, 1977); postjunctionally it has been reported to sensitize vascular smooth muscle to exogenous noradrenaline (Zimmerman, 1973; Johnson *et al.*, 1974; Kawasaki *et al.*, 1982).

The effectiveness of AII as a modulator of sympathetic neurotransmission has been studied in the pithed rat by blocking AII production with angiotensin converting enzyme (ACE) inhibitors or by competitive AII-receptor blockade with, for example, saralasin, but conclusions vary. Some controversy surrounds the question of whether infusion of AII can exactly reverse the effect of ACE inhibition. The

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present study attempts to clarify some aspects of this. For example, it is not always clear whether the ACE-inhibitors employed act via an AII-dependent mechanism. Some groups have suggested that the effects of captopril can be reversed in the pithed rat by AII infusion (Hatton & Clough, 1982; Oldham & Scotcher, 1985). However Bull & Drew (1984) and our own preliminary results (Grant & McGrath, 1984a) using the same preparation, showed that infusion of AII failed to reverse the effects of ACE-inhibition to a significant extent, whereas a partial recovery was noted by Kaufman & Vollmer (1985). An important factor, which may partly explain these discrepancies, seems to be the release of prostaglandins from the kidneys since de Jonge *et al.* (1983) have shown that restoration by AII is more straightforward if rats are nephrectomised or given indomethacin.

ACE-inhibitors have also been reported to attenuate pressor responses to exogenous noradrenaline *in vivo* (e.g. Antonaccio & Kerwin, 1981; Hatton & Clough, 1982; Grant & McGrath, 1984a; 1985), so it seems likely that at least part of the influence of AII is postjunctional. However, it has been suggested that attenuation of responses by ACE-inhibitors may be due to the fall in blood pressure *per se* evoked by these agents (de Jonge *et al.*, 1982), suggesting that the action is not only postjunctional but is a non-specific consequence of reducing vascular smooth muscle tone. It is therefore important to clarify whether the fall in blood pressure alone or some more specific action is needed to explain why antagonism of the renin-angiotensin system attenuates sympathetic nerve-mediated responses.

In this study we attempted a re-assessment of the interaction between AII and the vasopressor nerve-mediated response. Our initial aims were to determine whether the pithed rat was a suitable model in which to investigate the chronic hypertensive effects of AII (Brown *et al.*, 1981) and whether continuous infusion of sub-pressor doses of AII would facilitate sympathetic vasopressor resonses, perhaps by a prejunctional action. We re-investigated the effects of ACE inhibition as a check on the state of the renin-angiotensin system in our model since protocols vary considerably among those using pithed rat preparations. Our results suggest that responses to exogenous AII are complicated by AII-induced vasodilator products of cyclo-oxygenase.

## Methods

Male Wistar rats (245–265 g) were pithed under halothane anaesthesia by the method of Gillespie *et al.* (1970). Carotid arterial pressure was recorded and the heart rate was derived from this by an instantaneous rate-meter. Rats were ventilated at 2.5 ml per stroke (60 strokes $min^{-1}$) with a 40% $O_2$:60% $N_2$ gas mixture to produce physiological arterial blood gas tensions (Grant *et al.*, 1984; Grant & McGrath, 1984b). The sympathetic vasopressor outflow in the lower thoracic region was stimulated via the unshielded 1 cm tip of the pithing rod at 40 V and 0.05 ms pulse width. Peak changes in diastolic blood pressure to a range of frequencies (0.5–20 Hz) were studied with a fixed number of pulses (10 pulses). These stimuli did not significantly affect heart rate.

Bolus administration of drugs was via the left jugular vein in a fixed volume of 1 ml $kg^{-1}$ (i.e. 0.25 ml for a 250 g rat) washed in by a similar volume of saline. Infusions of drugs were given via the right jugular vein at a rate of 1.5 ml $h^{-1}$. Pressor responses to nerve stimulation were tested 5 min after bolus administration of teprotide, 10 min after starting continuous infusion of saralasin and 5 min after starting continuous infusion of nitroprusside. In some experiments AII was infused to determine whether it could overcome the inhibitory effects of teprotide; AII was infused continuously and responses to nerve stimulation were tested after 20 min; some rats were pretreated with flurbiprofen (5 mg $kg^{-1}$, i.v.) approximately 15 min before beginning control nerve stimulation, i.e. approximately 25 min after pithing.

All drugs were dissolved in 0.9% w/v saline, except nitroprusside which was dissolved in distilled $H_2O$. Results were analysed by Student's $t$ test (paired and unpaired).

### *Comparison of plasma angiotensin II levels in pithed and normotensive rats*

*Obtaining blood samples in pithed rats* Rats were pithed as previously described and ventilated on 40% $O_2$/60% $N_2$ gas mixture and with a tidal volume of 2.5 ml (60 strokes $min^{-1}$). Samples of arterial blood (abdominal aorta) were drawn into a 1 ml ice cold syringe containing 0.1 ml of 'AII-inhibitor solution' (EDTA, *O*-phenanthroline) to prevent breakdown of AII in the sample.

*Obtaining blood samples in unanaesthetized normotensive rats* Rats were stunned and the spinal cord quickly broken. The abdomen was opened and blood was drawn from the abdominal aorta with the same protocol as for pithed rats.

All blood samples were centrifuged at 3000 r.p.m., 5°C for 25 min. The plasma was decanted off and frozen to minimize any *in vitro* generation of AII. After thawing the samples, plasma AII was determined by radioimmunoassay by a modification (Morton *et al.*, 1979) of the method of Powell-Jackson & McGregor (1976).

The drugs used were angiotensin amide (Hypertensin) (Ciba), bradykinin triacetate (Sigma), [Sar$^1$],[Ala$^8$]-AII (saralasin) (Sigma), flurbiprofen (Boots), teprotide (SQ 20,881) (Squibb), sodium nitroprusside (Roche).

## Results

### *Effects of subpressor and pressor concentrations of AII on sympathetic nerve stimulation*

In drug-free pithed rats, infusions of AII at rates of 50 ng kg$^{-1}$ min$^{-1}$ and below were found to be subpressor, i.e. subthreshold for a direct increase in diastolic blood pressure (BP) and, if continued for up to 1 h, had no significant effect on pressor responses to nerve stimulation at 0.5 Hz, 1 Hz and 2 Hz ($n = 6$) (Figure 1) or at 5 Hz, 10 Hz or 20 Hz (results not shown). In contrast, after teprotide (1 mg kg$^{-1}$), which reduced diastolic BP, a similar infusion of AII produced a significant rise in diastolic BP (Figure 3).

Infusion of AII (200 ng kg$^{-1}$ min$^{-1}$) produced a maximum pressor response of 22.0 ± 2.3 mmHg ($n = 6$), which was usually not maintained although the diastolic BP remained elevated above initial baseline for the duration of the experiment. Infusion of AII (200 ng kg$^{-1}$ min$^{-1}$) produced a significant potentiation of pressor responses to nerve stimulation at all frequencies studied (0.5–20 Hz) by approximately 50% ($n = 6$) when compared to control (Figure 2).

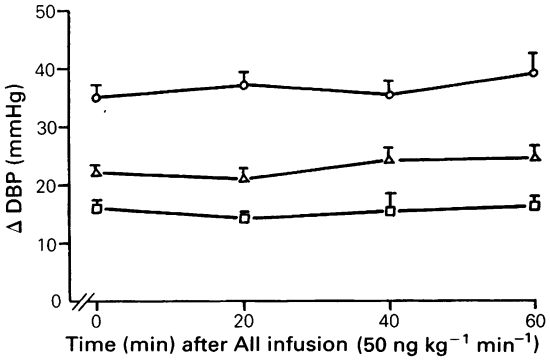


**Figure 1** The effects of subpressor angiotensin II (AII) on pressor responses to sympathetic nerve stimulation ($T_6$–$T_8$, 40 V, 0.05 ms pulse width, 10 pulses) in the pithed rat. The peak change in diastolic blood pressure (ΔDBP) to sympathetic nerve stimulation at three frequencies, 0.5 Hz (□), 1 Hz (△) and 2 Hz (○), was plotted against the time (min) after beginning infusion of AII (50 ng kg$^{-1}$ min$^{-1}$) ($n = 6$ at each frequency). The responses to nerve stimulation 20, 40 and 60 min after the onset of AII infusion were compared to control responses before AII infusion using Student's $t$ test (paired). The results show that infusion of AII for a 60 min period had no significant effect on pressor responses to nerve stimulation. Bars indicate s.e.mean.

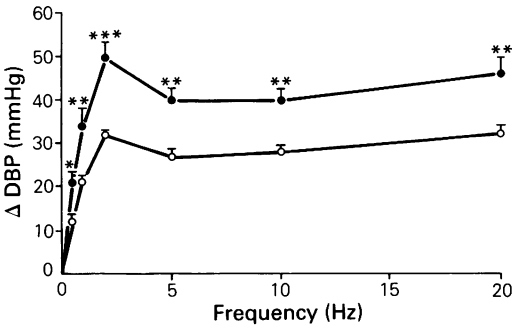


**Figure 2** The effects of pressor angiotensin II (AII) on pressor responses to sympathetic nerve stimulation ($T_6$–$T_8$, 40 V 0.05 ms pulse width, 10 pulses) in the pithed rat. The peak change in diastolic blood pressure (ΔDBP) to sympathetic nerve stimulation was plotted against the frequency of nerve stimulation (0.5–20 Hz). Two frequency-response curves were obtained from one animal, either (i) control (○) or (ii) after continuous infusion of AII (200 ng kg$^{-1}$ min$^{-1}$) (●) ($n = 6$ for each group). Responses after AII infusion were compared to control by Student's paired $t$ test (*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; ***$P < 0.001$). Bars indicate s.e.mean.

### *Comparison of plasma AII levels in pithed rats and unanaesthetized normotensive rats*

Table 1 shows a comparison of plasma AII levels in the arterial blood of pithed rats and unanaesthetized normotensive rats. The table shows that plasma AII levels are approximately 20 fold greater in pithed rats compared with those in unanaesthetized normotensive rats.

**Table 1** Comparison of plasma angiotensin II (AII) concentrations (pg ml$^{-1}$) in arterial blood from normotensive and pithed rats

| *Group* | *Plasma AII* (pg ml$^{-1}$) | n *value* |
|---|---|---|
| Normotensive rats | 17 ± 3.1 | 4 |
| Pithed rats | 401 ± 25 (***) | 7 |

The results show that there was an approximately 20 fold increase in plasma AII levels in pithed rats. Results are expressed as mean concentrations of AII (pg ml$^{-1}$) ± s.e.mean. Comparisons were made between the two groups by Student's unpaired $t$ test (***$P < 0.001$). The number of experiments in both groups is shown in the right hand column.

*Effects of teprotide and AII infusion on pressor responses to sympathetic nerve stimulation*

*In the absence of cyclo-oxygenase inhibitor* Pressor responses to nerve stimulation consisted of an initial, rapid increase in diastolic BP followed by a more prolonged secondary response which is due to release of adrenal catecholamines (Gillespie *et al.* 1970; Brown *et al.*, 1981; Flavahan *et al.*, 1985). Over the range of 0.5–2 Hz, pressor responses to a train of a fixed number of pulses (10) increased with the increasing frequency of stimulation. Responses at 5 and 10 Hz (and also at 20 Hz in later experiments) were less than those at 2 Hz.

Teprotide (1 mg kg$^{-1}$) produced a sustained fall in diastolic blood pressure of 11.0 ± 0.4 mmHg ($n$ = 6) from an initial level of 38 ± 2.3 mmHg. Infusion of AII (50 ng kg$^{-1}$ min$^{-1}$) reversed the blood pressure lowering effect of teprotide: after a combination of teprotide and AII infusion the basal diastolic blood pressure was not significantly reduced compared to control although the mean was slightly lower than in control (Figure 3).

Teprotide (1 mg kg$^{-1}$) significantly inhibited pressor responses to nerve stimulation at all frequencies studied (0.5–10 Hz) by approximately 50% compared with control ($n$ = 6). Continuous infusion of AII(50 ng kg$^{-1}$ min$^{-1}$) failed to overcome the inhibitory effect of teprotide against nerve stimulation. After this infusion of AII, responses were still significantly reduced compared to control responses (Figure 3). In a separate series of experiments the effects of teprotide at two doses, 1 mg kg$^{-1}$ and 10 mg kg$^{-1}$ were compared. No significant difference was found either in the reduction in basal blood pressure or attenuation of the nerve-mediated response (Figure 4).

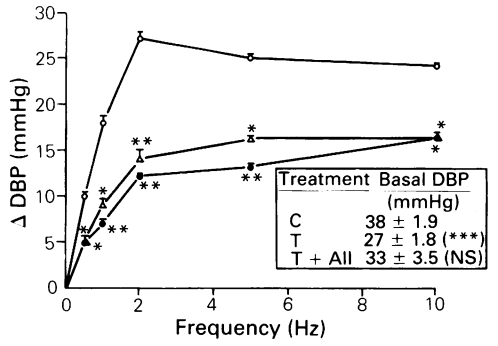


**Figure 3** The effects of teprotide on pressor responses to sympathetic nerve stimulation ($T_6$–$T_8$, 40 V, 0.05 ms pulse width, 10 pulses) in the pithed rat. The peak change in diastolic blood pressure (ΔDBP) to sympathetic nerve stimulation is plotted against the frequency of stimulation (0.5–10 Hz). Three frequency-response curves were obtained from one animal: (i) control (○), (ii) after teprotide (1 mg kg$^{-1}$) (●) or (iii) after angiotensin II (AII) (50 ng kg$^{-1}$ min$^{-1}$) in the presence of teprotide (1 mg kg$^{-1}$) (Δ) ($n$ = 6 for each group). Responses in groups (ii) and (iii) were compared to control by Student's paired $t$ test (*$0.01 < P < 0.05$; **$0.001 < P < 0.01$; *** $P < 0.001$). Bars indicate s.e.mean. The accompanying table shows the control basal diastolic blood pressure (DBP) (C), DBP after teprotide (10 mg kg$^{-1}$) (T) and DBP after teprotide (1 mg kg$^{-1}$) and AII infusion (50 ng kg$^{-1}$ min$^{-1}$) (T + AII).

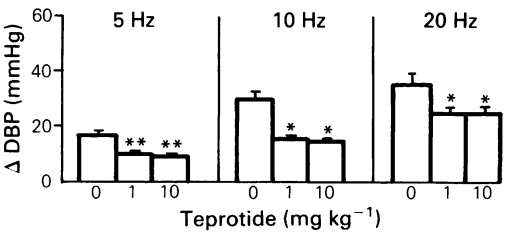


**Figure 4** The effects of two doses of teprotide on pressor responses to sympathetic nerve stimulation ($T_6$–$T_8$, 40 V, 0.05 ms pulse width, 10 pulses) in the pithed rat. The peak change in diastolic blood pressure (DBP) to sympathetic nerve stimulation is shown at three frequencies of stimulation (5, 10, 20 Hz) in controls and after teprotide 1 or 10 mg kg$^{-1}$, all three responses obtained in the same animals. Responses after teprotide were compared with controls by Student's paired $t$ test (*$0.01 < P < 0.05$; **$0.001 < P < 0.01$). Bars indicate s.e.mean.

*In the presence of a cyclo-oxygenase inhibitor* The effects of teprotide and AII infusion on pressor responses to nerve stimulation were studied in the presence of cyclo-oxygenase inhibitor, flurbiprofen (5 mg kg$^{-1}$), which was injected i.v. 15 min before beginning control nerve stimulation. Teprotide (1 mg kg$^{-1}$) produced a sustained fall in diastolic BP of 10.0 ± 0.2 mmHg ($n$ = 5) and significantly inhibited pressor responses to nerve stimulation at all frequencies studied (0.5–20 Hz) by approximately 30% ($n$ = 5): infusion of AII (50 ng kg$^{-1}$ min$^{-1}$) antagonized the blood pressure lowering effect of teprotide (Figure 5). These effects were similar to those found in the absence of flurbiprofen (compare Figure 3 and 5). However, infusion of AII (50 ng kg$^{-1}$ min$^{-1}$) now antagonized the inhibitory effect of teprotide in contrast to the failure of antagonism when no cyclo-oxygenase inhibitor was present.

*Effects of saralasin on sympathetic nerve stimulation*

Continuous infusion of saralasin (4 μg kg$^{-1}$ min$^{-1}$) produced a maintained fall in diastolic BP of

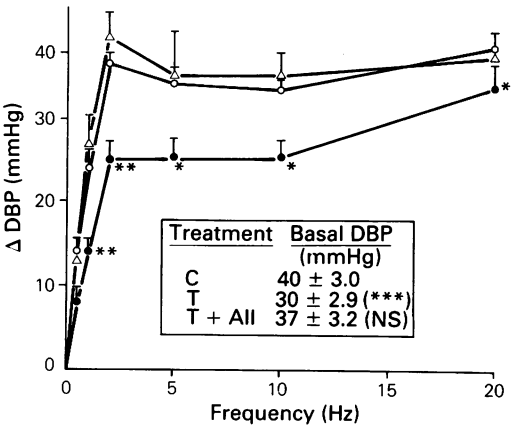


**Figure 5** The effects of teprotide on pressor responses to sympathetic nerve stimulation ($T_6$–$T_8$, 40 V 0.05 ms pulse width, 10 pulses) in pithed rats pretreated with the cyclo-oxygenase inhibitor, flurbiprofen (5 mg kg$^{-1}$). The change in diastolic blood pressure (DBP) to sympathetic nerve stimulation was plotted against the frequency of stimulation (0.5–20 Hz). Three frequency-response curves were obtained from the one animal: (i) control (○), (ii) after teprotide (1 mg kg$^{-1}$) (●) or (iii) after infusion of angiotensin II (AII, 50 ng kg$^{-1}$ min$^{-1}$) in the presence of teprotide (1 mg kg$^{-1}$) (△) ($n = 6$ for each group). Responses in groups (ii) and (iii) were compared to control using Student's paired $t$ test (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$). Bars indicate s.e.mean. The accompanying table shows the control basal diastolic blood pressure (DBP) (C), the DBP after teprotide (1 mg kg$^{-1}$) (T) and the DBP after teprotide (1 mg kg$^{-1}$) and AII infusion (50 ng kg$^{-1}$ min$^{-1}$) (T + AII).

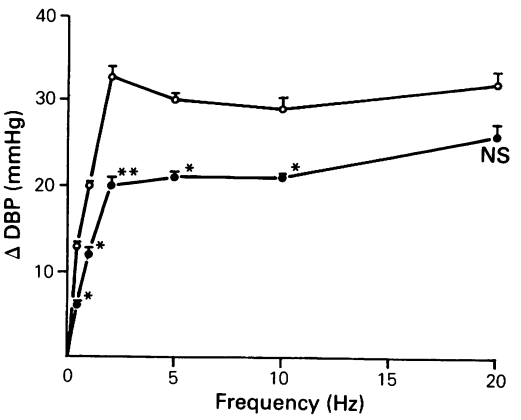


**Figure 6** The effects of infusion of saralasin on pressor responses to sympathetic nerve stimulation ($T_6$–$T_8$, 40 V, 0.05 ms pulse width, 10 pulses) in the pithed rat. The peak change in diastolic blood pressure (DBP) to sympathetic nerve stimulation was plotted against the frequency of stimulation (0.5–20 Hz). Two frequency-response curves were obtained from one animal: (i) control (○) or (ii) after continuous infusion of saralasin (4 μg kg$^{-1}$ min$^{-1}$) (●) ($n = 6$ for each group). Responses to nerve stimulation after saralasin infusion were compared to control by Student's paired $t$ test (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; NS, not significant). Bars indicate s.e.mean.

9.0 ± 0.9 mmHg ($n = 5$) and significantly inhibited pressor responses to nerve stimulation at all frequencies studied from 0.5 to 10 Hz by approximately 30% compared with controls ($n = 5$) (Figure 6).

*Effects of sodium nitroprusside on sympathetic nerve stimulation*

Sodium nitroprusside was continuously infused to produce a fall in diastolic BP of the same magnitude as that produced by teprotide and saralasin. The rate of infusion required to produce such a fall in diastolic BP varied between 1 and 8 μg kg$^{-1}$ min$^{-1}$. In each rat the infusion rate gradually increased until it was just sufficient to maintain a lowering of diastolic BP of approximately 10 mmHg. A fall in diastolic BP of 10.0 ± 0.7 mmHg ($n = 6$) evoked by nitroprusside infusion inhibited responses to nerve stimulation (0.5–20 Hz) by approximately 30% ($n = 6$) (Figure 7).

## Discussion

This study, like several previously, demonstrates that an ACE inhibitor can reduce the absolute size of the pressor response to sympathetic nerve stimulation. It makes clear that this is due to loss of endogenous AII by demonstrating circumstances (cyclo-oxygenase inhibition) which allow restoration of the pressor response by exogenous AII. However, we are not convinced that this demonstrates a facilitation of sympathetic transmission by AII.

Our results are consistent with the pressor nerve-mediated response being simply proportional to basal blood pressure, whether blood pressure is reduced by blockade of AII or raised by its infusion. Figure 8 shows that the only observation which does not fully concur with this is the failure to restore responses with AII after teprotide, and even this is corrected by flurbiprofen. If blood pressure were reduced to a similar degree (to that produced by teprotide or saralasin) by nitroprusside, which acts independently of AII to reduce blood pressure through

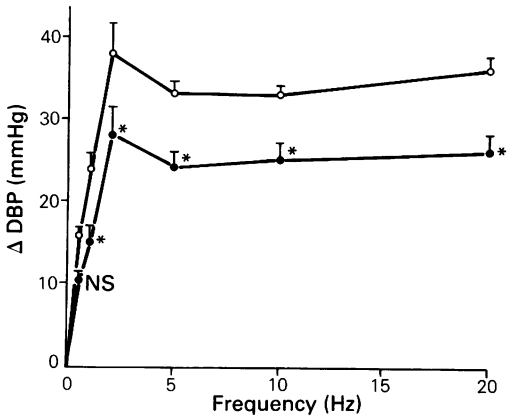


**Figure 7** The effects of infusion of sodium nitroprusside on pressor responses to sympathetic nerve stimulation ($T_6$–$T_8$, 40 V, 0.05 ms pulse width, 10 pulses) in the pithed rat. The peak change in diastolic blood pressure (DBP) to sympathetic nerve stimulation was plotted against the frequency of stimulation (0.5–20 Hz). Two frequency-response curves were obtained from one animal: (i) control (○) or (ii) after continuous infusion of nitroprusside (1–8 $\mu g\,kg^{-1}\,min^{-1}$) (●) which produced a mean fall in diastolic blood pressure of $10 \pm 0.7$ mmHg ($n = 6$ for each group). Responses to nerve stimulation after infusion of nitroprusside were compared to control by Student's paired $t$ test (* $0.01 < P < 0.05$; NS, not significant). Bars indicate s.e.mean.

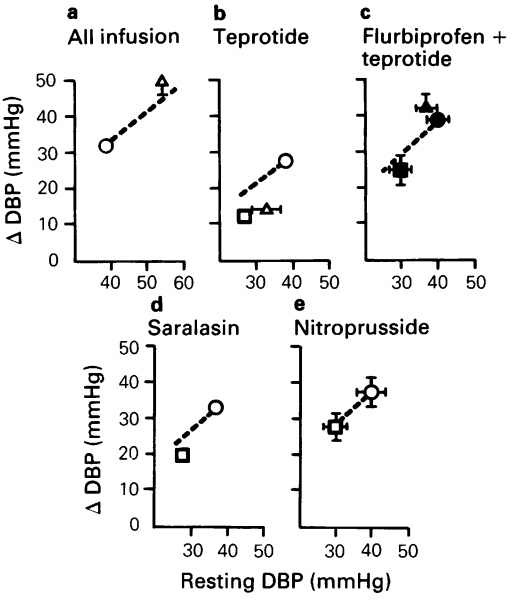


**Figure 8** Relationship between resting arterial diastolic blood pressure (DBP) and pressor response to sympathetic nerve stimulation at 2 Hz for 20 pulses (ΔDBP). Control, no drug treatment (○). (a) During infusion of AII ($200\,ng\,kg^{-1}\,min^{-1}$) (Δ); (b) teprotide ($1\,mg\,kg^{-1}$) (□), teprotide and AII infusion ($50\,ng\,kg^{-1}\,min^{-1}$) (Δ); (c) as (b) but all in the presence of flurbiprofen ($5\,mg\,kg^{-1}$), filled symbols; (d) saralasin ($4\,mg\,kg^{-1}\,min^{-1}$) (□); (e) nitroprusside (1–$8\,mg\,kg^{-1}\,min^{-1}$) (□).

Dashed lines indicate the hypothetical changes in the measured pressor response (caused by a fixed increase in peripheral resistance) which should occur as the resting DBP varies, without any pharmacological interaction between the agents employed. This is on the basis that the changes in resting DBP are due to changes in cardiac output while resting peripheral resistance remains constant. Points represent means ($n = 6$). Error bars indicate s.e.mean and are omitted when smaller than the symbols.

its direct intracellular action on vascular smooth muscle, then the reductions in pressor nerve-mediated responses would be quantitatively similar (Figure 8e). The work of Kaufman & Vollmer (1985) suggests that AII maintains cardiac output in the pithed rat model. Taking this work into account, the simplest conclusion is that in the present study, nerve-mediated responses were dependent only on blood pressure and that the changes in responses simply reflect changes in blood pressure due to the varying contribution to cardiac output. If this conclusion were correct, then it should be possible to restore sympathetic responses after ACE-inhibition by increasing the cardiac output via plasma expansion, for example.

The answer to our initial question is that the vasopressor nerve-mediated responses in pithed rat were not influenced by infusion of subpressor doses of AII over a 1 h period although this treatment did facilitate responses to pressor agonists (Grant & McGrath, 1988). The rest of the results suggest that the concentrations of AII in plasma are very high and that pressor nerve-mediated responses are largely a function of blood pressure e.g. 'pressor' infusion of AII increased nerve-mediated responses by no more than would be expected from the new baseline. Consequently, the model does not seem to be particularly suitable for the investigation of the effects of AII at the vascular neuro-effector junction, although it may help cast some light on the mechanisms involved in responses to blood-borne agonists (Grant & McGrath, 1988).

One consequence of the high plasma concentration of AII is an apparent subsensitivity to low doses of AII. Infusion of AII at a rate of $50\,ng\,kg^{-1}\,min^{-1}$ did not produce an increase in diastolic BP in con-

trols, but following ACE inhibition (which reduced diastolic BP) it almost restored diastolic BP to control levels. Since the pressor effect of AII is related to the logarithm of plasma concentration, it is reasonable to conclude that in controls this rate of infusion produces a small arithmetic rise in plasma concentration which has an undetectable effect on vascular tone. After ACE inhibition, with a low initial AII level, the same infusion produces a rise in plasma concentration that is sufficient to raise tone. Consequently, the pithed rat provides a relatively insensitive assay for the pressor effect of AII unless AII production is blocked.

The high plasma concentrations of AII show that the influence of AII in this model is not representative of the normal physiological state. The low blood pressure is likely to activate renin release from the juxtaglomerular apparatus of the kidney via a local baroreceptor reflex. de Jonge *et al.* (1982) have shown that plasma renin activity is higher in pithed rats compared with normotensive unanaesthetized rats and the present study demonstrates the consequent high level of AII. Thus, the effects of removing the influence of endogenous AII will be exaggerated. However, high circulating levels of AII are found in some forms of renovascular hypertension and so the pithed rat may still be a useful model for investigating the pathophysiological influence of AII on adrenergic mechanisms.

The interactions between ACE inhibition, reinfusion of AII and cyclo-oxygenase inhibition suggest that infusion of AII releases prostaglandins. Alternatively, it is possible that vasodilator prostaglandins are released simply in response to tissue underperfusion in this model and contribute to vasodilatation. However, the cyclo-oxygenase inhibitor flurbiprofen had no effect on basal blood pressure in this model so it seems more likely that the prostaglandins are released by an AII-dependent mechanism. The site of AII-induced prostaglandin release could be either the kidneys or the vascular walls of the blood vessels involved in pressor responses. AII stimulates prostaglandin release (e.g. prostaglandin $PGE_2$) in the kidneys (Aitken & Vane, 1973; Needleman *et al.*, 1974; Blumberg *et al.*, 1977). Pressor responses to nerve stimulation in the pithed rat are reduced after nephrectomy, which lowers plasma renin and AII levels. In that case, infusion of AII, without cyclo-oxygenase inhibition, restored responses to the same level as in untreated controls (de Jonge *et al.*, 1983). This suggests that prostaglandins released by infusion of AII are renal in origin. These prostaglandins could antagonize the restorative effect of AII in several ways: for example by lowering blood pressure (via venodilatation and hence reduced cardiac output), by interfering with vasoconstrictor excitation-contraction coupling or by inhibiting the sympathetic nerves directly. Clearly, they disturb the straightforward relationship between blood pressure and pressor response (Figure 8).

It is important to be sure that teprotide is acting via an angiotensin-dependent mechanism. Since kininase II, the enzyme that metabolises bradykinin, and ACE are the same enzyme (Ferreira, 1965; Engel *et al.*, 1972; Dorer *et al.*, 1974; Erdos, 1975), the depressor and inhibitory effects of teprotide could be due to facilitation of bradykinin. However, intravenous infusion of bradykinin at up to $5\,\mu g\,kg^{-1}\,min^{-1}$ had no effect on pressor responses to nerve stimulation (results not shown), confirming the observation of Hatton & Clough (1982). It therefore seems unlikely that teprotide inhibits nerve responses by increasing plasma concentrations of bradykinin. We have confirmed that the competitive AII receptor antagonist, saralasin, also antagonized pressor responses to nerve stimulation (Hatton & Clough, 1982; Clough *et al.*, 1982; de Jonge *et al.*, 1983) and that after saralasin, teprotide ($1\,mg\,kg^{-1}$) had no additional effect on nerve-mediated pressor responses (Grant & McGrath, results not shown). It therefore appears that teprotide is acting via an AII-dependent mechanism in this study.

We conclude that most of the interaction between circulating AII and sympathetic pressor responses in this preparation can be explained by the effect of AII on basal blood pressure, possibly via venous vascular smooth muscle, but that the release of vasodilator products of cyclo-oxygenase by exogenous AII complicates the experimental demonstration of this. We have no evidence to support a specific interaction of circulating AII at the vasoconstrictor neuro-effector junction.

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