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Interactions between angiotensin II and α-adrenoceptor agonists mediating pressor responses in the pithed rat

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1 The aim of the study was to investigate the interactions between angiotensin II (AII) and adrenoceptor-mediated pressor responses in the pithed rat. Emphasis was placed on the effects of AII on blood pressure *per se* and the possibility of differential effects on α_1- and α_2-adrenoceptor-mediated pressor responses.

2 A low concentration of the angiotensin converting enzyme (ACE) inhibitor, teprotide ($1\,mg\,kg^{-1}$) lowered the resting diastolic blood pressure (BP) and attenuated only the second phase components of pressor responses to both α_1- and α_2-adrenoceptor agonists. Infusion of AII ($50\,ng\,kg^{-1}\,min^{-1}$) did not reverse the attenuating effect of teprotide and did not reliably restore the basal diastolic BP.

3 Although teprotide ($10\,mg\,kg^{-1}$) did not produce a greater fall in diastolic BP than did the low dose ($1\,mg\,kg^{-1}$), it attenuated the peak and second phase pressor responses to α_1- and α_2-adrenoceptor agonists but had no effect on pressor responses to AII or 5-hydroxytryptamine (5-HT). Infusion of AII reversed the effects of teprotide ($10\,mg\,kg^{-1}$) provided that rats were pretreated with flurbiprofen ($5\,mg\,kg^{-1}$), confirming that the depressor effects of the higher dose of teprotide are AII-dependent but that demonstration of this was complicated by products of cyclo-oxygenase.

4 The AII-receptor antagonist, saralasin ($4\,\mu g\,kg^{-1}\,min^{-1}$) attenuated α_1- and α_2-adrenoceptor-mediated pressor responses in a manner similar to that of teprotide ($10\,mg\,kg^{-1}$), suggesting that in this pithed rat model the α-adrenoceptor-mediated responses were selectively facilitated by endogenous AII.

5 Infusion of AII ($50\,ng\,kg^{-1}\,min^{-1}$) over a 60 min period did not produce a pressor response in the absence of other drugs but did facilitate pressor responses to α-adrenoceptor agonists. This confirms that AII can modulate α-adrenoceptor-mediated responses independently of basal blood pressure.

6 Overall the results indicate a facilitatory role for endogenous AII on α-adrenoceptor-mediated pressor responses. This is discussed in relation to the failure to demonstrate this convincingly under similar conditions on sympathetic nerve-mediated pressor responses.

Introduction

The pithed rat has been used to assess the mechanisms by which angiotensin II (AII) might maintain vascular tone (Hatton & Clough, 1982; Antonaccio, 1985). The effectiveness of AII as a modulator of adrenoceptor-mediated pressor responses has been studied by blocking AII production with angiotensin converting enzyme (ACE) inhibitors or by competitive AII-receptor blockade with, for example, saralasin, but interpretations of the mechanisms involved vary. For example, there may be a specific interaction at vascular smooth muscle (e.g. Antonaccio & Kerwin, 1981; Hatton & Clough, 1982); alternatively, attenuation of responses by ACE-inhibitors

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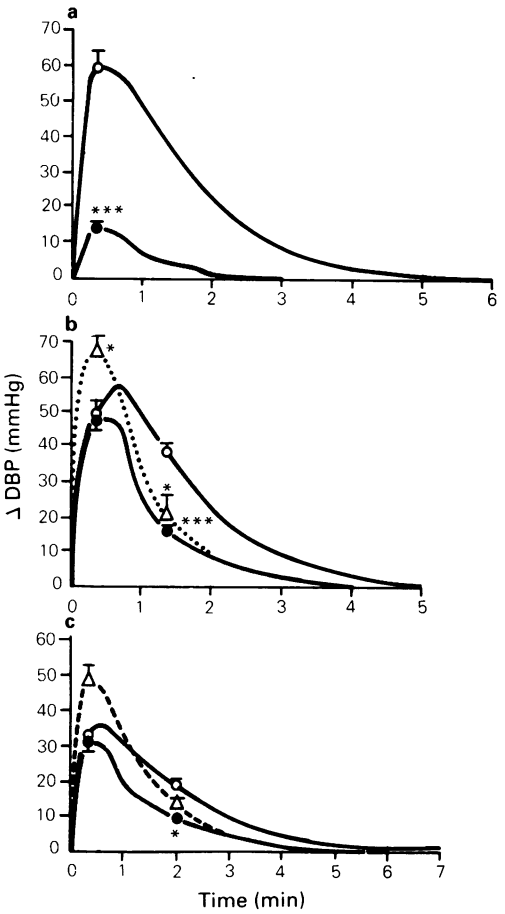

Figure 1 The effects of teprotide (1 mg kg^{-1}) and the subsequent infusion of angiotensin II (AII, 50 ng kg^{-1}) on the pressor responses to bolus injections of (a) angiotensin I (AI) (0.5 μg kg^{-1}), (b) noradrenaline (1 μg kg^{-1}) or (c) tyramine (0.2 mg kg^{-1}) in the pithed rat. Ordinate scale: change in diastolic blood pressure: abscissa scale: time (min) after administration. Only one response was obtained from each animal for AI but responses in all three conditions were obtained in each animal for noradrenaline and tyramine: either control (○), after treatment with teprotide (●) or after treatment with teprotide and subsequent infusion of AII (△, broken lines). Data at 15 s intervals are plotted but symbols are shown only at selected points (mean responses, $n = 6$ for each group). Peak responses after treatments were compared with control by Student's unpaired t test (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$). Bars indicate s.e.mean.

may be due to the fall in blood pressure *per se* evoked by these agents (de Jonge *et al.*, 1982). It is therefore important to clarify whether the fall in blood pressure is the only factor or whether some more specific action is needed to explain why antagonism of the renin-angiotensin system attenuates adrenoceptor-mediated pressor responses.

Some controversy has surrounded the question of whether infusion of AII could 'antagonize' the effect of ACE inhibition. In an earlier study of the sympathetic nerve-mediated pressor responses, we were unable to demonstrate convincingly that endogenous AII modulated the response except indirectly and non-specifically through its effect on basal blood pressure (Grant & McGrath, 1988). This also showed that intravenous infusions of AII could reverse the effects of ACE inhibitors but only provided that a cyclo-oxygenase inhibitor was administered. The present study attempts to clarify whether pressor responses to agonists behave in the same way.

Noradrenaline increases blood pressure in the pithed rat by interacting with two types of postjunctional α-adrenoceptors (α_1 and α_2) (Starke, 1981; McGrath, 1982). Following experiments employing converting enzyme inhibitors, the AII-receptor antagonist, saralasin, and a limited number of α-adrenoceptor agonists, it was suggested that pressor responses to α_2-adrenoceptor-mediated stimulation in the pithed rat are modulated by AII, whilst α_1-adrenoceptor-mediated responses are relatively unaffected (de Jonge *et al.*, 1982; Timmermans *et al.*, 1982). Another aim of this study was, therefore, to investigate whether the modulatory effects of AII were dependent on adrenoceptor subtype. The results suggest that there is a real interaction between endogenous AII and pressor responses to α-adrenoceptor agonists which is specific for them compared with other pressor stimuli but there is no differentiation between α_1- and α_2-subtypes.

Preliminary communications of these results have been published (Grant & McGrath, 1984a; 1985).

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).

Rapid intravenous injections (i.v.) of drugs were made 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}.

Table 1 Effects of a low dose teprotide (T, 1 mg kg^{-1}) and the subsequent infusion of angiotensin II (50 ng kg^{-1} min^{-1}, T + AII) on the basal diastolic blood pressure in the series of experiments testing responses to noradrenaline (NA), phenylephrine (Phe) and xylazine (Xyl)

	Basal diastolic BP		
Treatment	*NA*	*Phe*	*Xyl*
C	40 ± 1.4	43 ± 1.2	44 ± 1.1
T	27 ± 1.5 (***)	28 ± 1.2 (***)	29 ± 1.4 (***)
T + AII	41 ± 7.1 (NS)	42 ± 7.0 (NS)	41 ± 8.8 (NS)

Comparison of the effects of treatments compared with controls (C) were made with a paired t test. (NS = not significant, $P > 0.05$; *** $P < 0.001$).
Means ± s.e.mean shown.

Pressor responses to intravenous injections of agonists were tested 5 min after i.v. administration of teprotide or 10 min after starting continuous infusion of saralasin. In some experiments AII was infused to determine whether it could antagonize the inhibitory effects of teprotide. AII was infused continuously and responses to agonists were tested after 10 and 20 min or in a separate series of experiments without teprotide, at 20, 40 and 60 min; some rats were pretreated with flurbiprofen (5 mg kg^{-1}, i.v.) approximately 15 min prior to beginning control nerve stimulation, i.e. approximately 25 min after pithing.

All drugs were dissolved in 0.9% w/v saline. Results were analysed by Student's t test (paired and unpaired). All blood pressure and heart rate values are expressed as mean plus or minus standard error of the mean.

The drugs used were [Asp^1], [Val^5]-angiotensin II (AII) (Ciba), angiotensin I (Sigma), azepexole (BHT-933) (Thomae), indanidine (Sgd 101/75) (Siegfried), methoxamine HCl (Burroughs-Wellcome), (−)-noradrenaline bitartrate (Sigma), (−)-phenylephrine HCl (Sigma), [Sar^1],[Ala^8]-angiotensin II (Saralasin) (Sigma), 5-hydroxytryptamine creatinine sulphate (5-HT) (Sigma), teptrotide (SQ 20,881) (Squibb), tyramine HCl (Sigma), xylazine HCl (Bayer).

Results

Effects of a low dose of teprotide on pressor responses to α-adrenoceptor agonists given rapidly i.v.

Teprotide (1 mg kg^{-1}) produced a mean fall in diastolic blood pressure (BP) of 14 ± 0.4 mmHg ($n = 24$) (combined data from experiments with noradrenaline, phenylephrine, xylazine and AI). As shown in Figure 1a, teprotide (1 mg kg^{-1}) attenuated the pressor response to AI (0.5 μg kg^{-1}) by 77% ($P < 0.001$, $n = 6$). Since pressor responses to AI depend on its conversion to AII by ACE, this indicates a marked inhibition of ACE. Infusion of AII (50 ng kg^{-1} min^{-1}) in the presence of teprotide restored the basal diastolic BP to levels that were not significantly different from controls (see Table 1).

Doses of agonists were chosen to give peak pressor responses which were submaximal and within the range 40–60 mmHg, except for tyramine which gave slightly smaller responses.

Noradrenaline The control pressor response to noradrenaline (1 μg kg^{-1}) was biphasic: a fast initial increase in diastolic BP was followed by a more prolonged secondary phase response. The whole pressor response lasted approximately 4–5 min. Teprotide (1 mg kg^{-1}) had little effect on the early peak response measured at 20 s but significantly attenuated the late phase of the response to noradrenaline measured at 80 s ($P < 0.001$, $n = 6$). Infusion of AII (50 ng kg^{-1} min^{-1}) failed to reverse the effects of teprotide on the late phase of the response but significantly potentiated the early peak ($P < 0.05$, $n = 6$) (Figure 1b).

Phenylephrine The control pressor response to phenylephrine (3 μg kg^{-1}) was biphasic: a fast initial increase in diastolic BP was followed by a more prolonged secondary response. The whole response lasted approximately 4 min. Teprotide (1 mg kg^{-1}) had no effect on the early peak but significantly attenuated the late phase of the response to phenylephrine measured at 80 s ($0.001 < P < 0.01$, $n = 6$). Infusion of AII (50 ng kg^{-1} min^{-1}) failed to reverse the inhibitory effect of teprotide on the late phase of the response but produced a small, significant potentiation of the early peak measured at 10 s ($0.001 < P < 0.01$, $n = 6$) (Figure 2a).

Xylazine The control response to xylazine (0.5 mg kg^{-1}) consisted of a fairly rapid initial increase in diastolic BP (slower than for noradrenaline or phenylephrine) followed by a rather prolonged second phase. The entire whole response lasted approximately 12–15 min. Teprotide

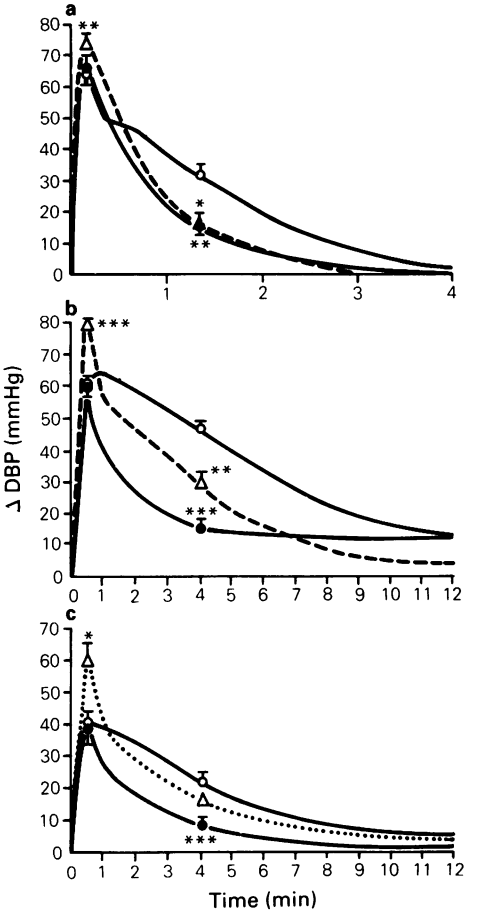

Figure 2 The effects of teprotide (1 mg kg^{-1}) and the subsequent infusion of angiotensin II (AII, 50 ng kg^{-1} min^{-1}) on the pressor responses to bolus injection of (a) phenylephrine (3 μg kg^{-1}), (b) xylazine (0.5 μg kg^{-1}) or (c) azepexole (BHT-933) (0.25 mg kg^{-1}) in the pithed rat. Ordinate scale: change in diastolic blood pressure, abscissa scale: time (min) after administration. Three responses were obtained from each animal: control (○), after treatment with teprotide (●) or after treatment with teprotide and subsequent infusion of AII (△, broken lines). Data at 15 s intervals are plotted but symbols are shown only at selected points (mean responses, $n = 6$ for each group). Peak responses after treatments were compared with control by Student's unpaired t test (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$). Bars indicate s.e.mean.

(1 mg kg^{-1}) had no effect on the initial peak response but markedly attenuated the late phase of the response measured at 4 min ($P < 0.001$, $n = 8$). Infusion of AII (50 ng kg^{-1} min^{-1}) partly reversed the inhibitory effect of teprotide on the late phase of the response but this may have been due to the large (35%) potentiation of the early peak response measured at 30 s ($P < 0.001$, $n = 8$) (Figure 2b).

Other sympathomimetic agents A similar pattern emerged when the effects of teprotide were studied on pressor responses to azepexole (BHT 933) (0.25 mg kg^{-1}) (Figure 2c) and the indirect sympathomimetic, tyramine (0.2 mg kg^{-1}) (Figure 1c). The late phases of the responses to these agonists were attenuated by teprotide (1 mg kg^{-1}) but the peak responses were unaffected. Infusion of AII (50 ng kg^{-1} min^{-1}) only partly antagonized the effects of converting enzyme inhibition on responses to azepexole and tyramine, potentiating the peak to beyond control levels but not fully restoring the late phase, i.e. qualitatively like xylazine.

Effects of a high dose of teprotide on pressor responses to α-adrenoceptor agonists given rapidly i.v.

Teprotide (10 mg kg^{-1}) produced a fall in diastolic BP of 13 ± 0.7 mmHg ($n = 20$) (combined data from experiments with AI, noradrenaline, phenylephrine and xylazine), which was not significantly different from the effect of teptrotide (1 mg kg^{-1}). However, infusion of AII (50 ng kg^{-1} min^{-1}) did not overcome this depressor effect (Table 2). Teprotide (10 mg kg^{-1}) attenuated the pressor response to AI (0.5 mg kg^{-1}) by 87% ($P < 0.001$, $n = 5$) (Figure 3a), indicating a marked inhibition of ACE. Teprotide (10 mg kg^{-1}) significantly attenuated the peak responses to noradrenaline (1 μg kg^{-1}; by 50%, $P < 0.001$, $n = 6$), xylazine (0.5 mg kg^{-1}; by 57%, $P < 0.001$, $n = 4$) and phenylephrine (3 μg kg^{-1}; by 60%, $P < 0.001$, $n = 5$) (Figure 3b,d,e), azepexole (0.25 mg kg^{-1}; by 29%, $P < 0.05$, $n = 4$) and indanidine (0.5 mg kg^{-1}, by 39%, $P < 0.05$, $n = 5$–7: in this case only, the data are unpaired, reproducible responses were unattainable due to tachyphylaxis to indanidine; $n = 7$ for control rats, $n = 5$ for teprotide-treated rats) (Figure 4).

Infusion of AII (50 ng kg^{-1} min^{-1}) failed to antagonize this inhibitory effect on peak responses to noradrenaline and phenylephrine; however, it did antagonize the effects of teprotide on the peak response to xylazine, azepexole and indanidine (Figure 3b,d,e; 4a,b).

Effects of a high dose of teprotide on pressor responses to α-adrenoceptor agonists: pretreatment with flurbiprofen

Rats were pretreated with the cyclo-oxygenase inhibitor flurbiprofen (5 mg kg^{-1}) 15 min before other drugs were administered. This had no significant

Table 2 Effects of a high dose of teprotide (T, 10 mg kg^{-1}) and the subsequent infusion of angiotensin II (50 ng kg^{-1} min^{-1}, T + AII) on the basal diastolic blood pressure, in the series of experiments testing responses to noradrenaline (NA), phenylephrine (Phe) and xylazine (Xyl)

	Basal diastolic BP				
Treatment	*NA*	*NA + flur*	*Phe*	*Phe + flur*	*Xyl*
C	39 ± 0.8	39 ± 2.7	40 ± 0.8	39 ± 1.5	41 ± 3.7
T	26 ± 0.6 (***)	25 ± 4.3 (***)	24 ± 0.6 (***)	25 ± 2.9 (***)	26 ± 3.8 (***)
T + AII	27 ± 0.8 (***)	54 ± 3.3 (**)	29 ± 0.8 (***)	50 ± 2.6 (*)	31 ± 4.1 (*)

Note that AII infusion fails to restore diastolic BP unless rats are pretreated with fluribiprofen 5 mg kg^{-1} (+ flur). Comparison of the effects of treatments compared with controls (C) were made with a paired t test. (NS = not significant, $P > 0.05$; * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$. Means ± s.e. shown.

effect on the resting diastolic BP. Teprotide (10 mg kg^{-1}) produced a fall in diastolic BP of 14 ± 1.3 mmHg ($n = 9$) (combined data from experiments with noradrenaline and phenylephrine) and significantly attenuated the peak pressor responses to noradrenaline (1 μg kg^{-1}; by 47%, $P < 0.001$, $n = 5$), phenylephrine (3 μg kg^{-1}; by 58%, $P < 0.001$, $n = 4$) (Figure 3c and f) and xylazine (0.5 mg kg^{-1}; by 57%, $P < 0.001$, $n = 4$).

Infusion of AII (50 ng kg^{-1} min^{-1}) in the presence of teprotide increased the basal diastolic BP to values significantly greater than the control levels

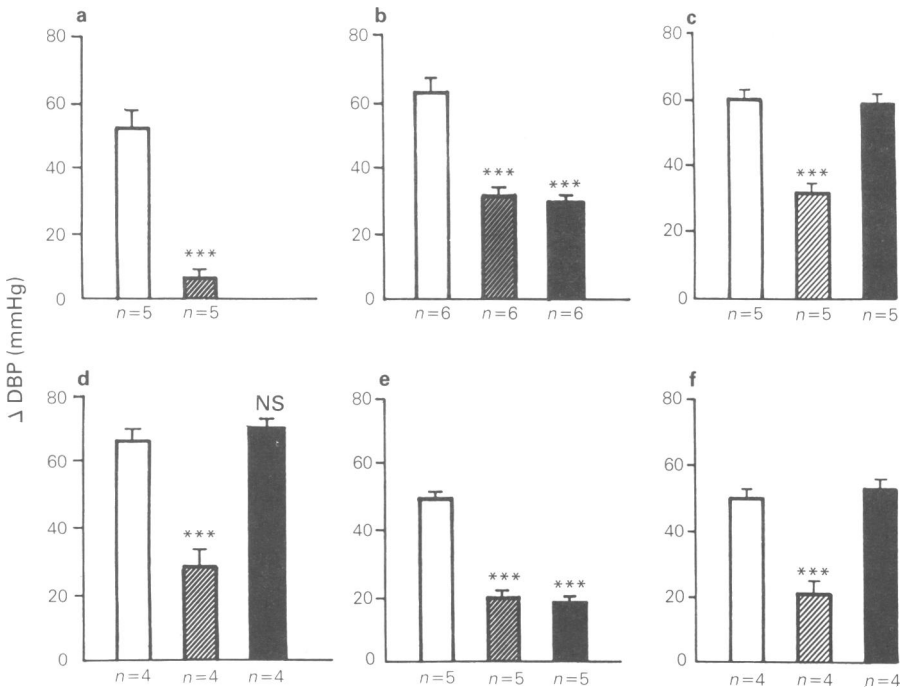

Figure 3 The effects of teprotide (10 mg kg^{-1}) and the subsequent infusion of angiotensin II (AII, 50 ng kg^{-1}) on the peak diastolic blood pressure responses to (a) angiotensin I (AI) (0.5 μg kg^{-1}), (b) noradrenaline (1 μg kg^{-1}), (c) noradrenaline (1 μg kg^{-1}) after flurbiprofen (5 mg kg^{-1}), (d) xylazine (0.5 mg kg^{-1}), (e) phenylephrine (3 μg mg^{-1}) and (f) phenylephrine (3 μg mg^{-1}) after flurbiprofen (5 mg kg^{-1}) in the pithed rat. For each agonist, except AI (a), three groups of responses were obtained in each animal: control (open column), after teptrotide (1 mg kg^{-1}) (hatched column), after teprotide (1 mg kg^{-1}) (solid column). Responses were compared with control by paired or unpaired t test (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$). Numbers under each column indicate the number of observations. Bars indicate s.e.mean.

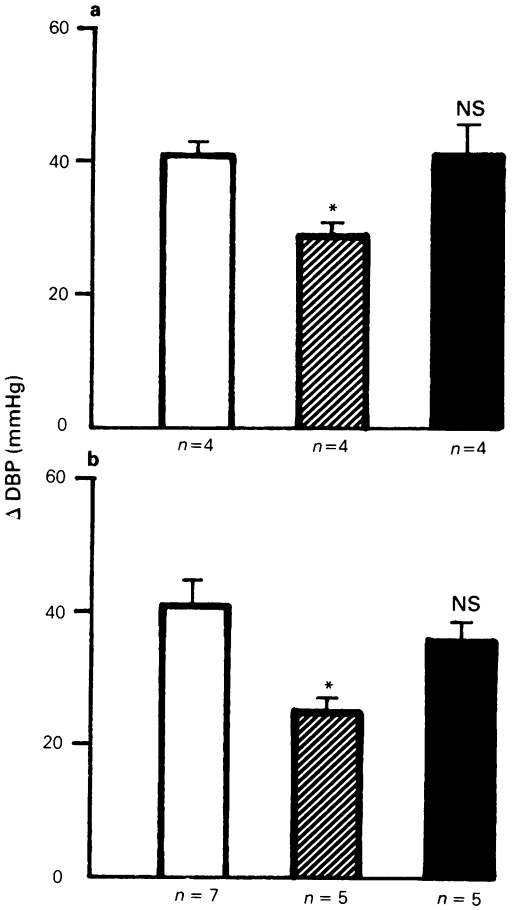

Figure 4 The effects of teprotide ($10\,mg\,kg^{-1}$) and the subsequent infusion of angiotensin II (AII, $50\,ng\,kg^{-1}\,min^{-1}$) on the peak diastolic blood pressure responses to (a) azepexole ($0.25\,mg\,kg^{-1}$), (b) indanidine ($0.5\,mg\,kg^{-1}$) in the pithed rat. In (a) three groups of response to azepexole were obtained in the one animal: control (open column), after teprotide ($10\,mg\,kg^{-1}$) (hatched column) or after AII ($50\,ng\,kg^{-1}\,min^{-1}$) in the presence of teprotide ($10\,mg\,kg^{-1}\,min^{-1}$) (solid column). In (b) three groups of response to indanidine were obtained, one response per animal (key similar to a). Responses were compared to control by Student's paired t test (a) or Student's unpaired t test (b). * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$). Numbers under each column indicate the number of observations. Bars indicate s.e.mean.

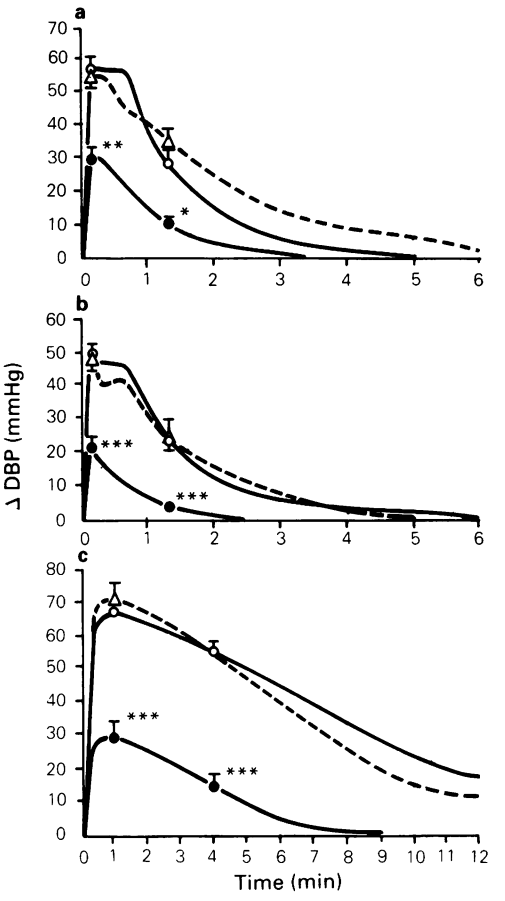

Figure 5 After pretreatment with flurbiprofen ($5\,mg\,kg^{-1}$): the effects of teprotide ($10\,mg\,kg^{-1}$) and the subsequent infusion of angiotensin II (AII) ($50\,ng\,kg^{-1}\,min^{-1}$) on the pressor responses to (a) noradrenaline ($1\,\mu g\,kg^{-1}$), (b) phenylephrine ($3\,\mu g\,kg^{-1}$) and (c) xylazine ($0.5\,\mu g\,kg^{-1}$) in the pithed rat. The figure shows plots of the change in diastolic blood pressure against the time (min) after administration. Three responses were obtained from each animal: control (○), after treatment with teprotide (●) or after treatment with teprotide and subsequent infusion of AII (△, broken lines). Data at 15s intervals are plotted but symbols are shown at selected points (mean responses, $n = 6$ for each group). Peak responses after treatments were compared with control by Student's unpaired t test (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$). Bars indicate s.e.mean.

(see Table 2). Furthermore, the inhibitory effects of teprotide against noradrenaline and phenylephrine were antagonized by the infusion of AII: pressor responses were not significantly different from controls without teprotide (Figure 3c and f). These two actions contrast with the experiments in the absence of a cyclo-oxygenase inhibitor, in which the pre-teprotide situation was not restored by AII infusion (Figure 3b and e: Table 2).

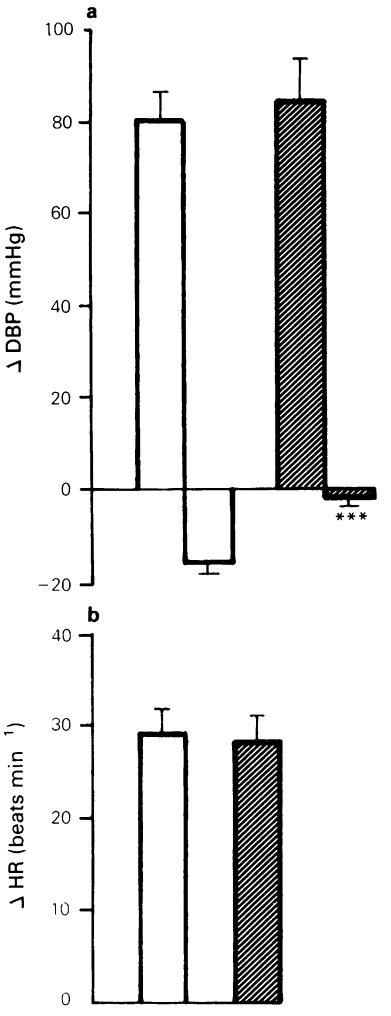

Figure 6 Effects of teprotide (10 mg kg^{-1}) on (a) peak diastolic pressor and (b) cardioaccelerator responses to 5-hydroxytryptamine (5-HT) (0.1 mg kg^{-1}) in the pithed rat. Two responses to 5-HT were obtained in each animal: control (open columns) and after treatment with teprotide (hatched columns). Since 5-HT produced a biphasic pressor response, the maximum pressor and depressor responses are shown in (a). Responses after treatment with teprotide were compared with control by Student's paired t test ($n = 4$) (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$). Bars indicate s.e.mean.

The effects of teprotide (10 mg kg^{-1}) and AII infusion (50 ng kg^{-1} min^{-1}) on the time courses of the pressor responses to bolus injections of noradrenaline (1 μg kg^{-1}), phenylephrine (3 μg kg^{-1}) and xylazine (0.5 mg kg^{-1}) in flurbiprofen-pretreated rats are shown in Figure 5. Responses were essentially restored to control values.

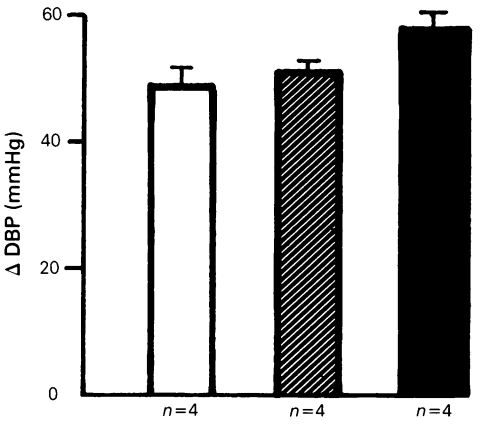

Figure 7 The effects of teprotide on the peak diastolic blood pressure responses to angiotensin II (AII) (400 ng kg^{-1} min^{-1}) in the pithed rat. Three groups of response to angiotensin were obtained, one response per animal: either control (open column), after teprotide (1 mg kg^{-1}) (hatched column) or after teprotide (10 mg kg^{-1}) (solid column). Responses were compared with control by Student's unpaired t test. No significant difference was found. Numbers under each column indicate the number of observations. Bars indicate s.e.mean.

Effects of teprotide on pressor responses to 5-hydroxytryptamine and angiotensin II

The effect on arterial blood pressure of a bolus of 5-HT (0.1 mg kg^{-1}) was biphasic, consisting of a rapid initial increase in diastolic BP followed by a small, transient decrease in diastolic BP to below initial baseline. Teprotide (10 mg kg^{-1}) decreased diastolic BP by 18 ± 1.9 mmHg but had no significant effect on the height of the pressor response to 5-HT (Figure 6). The small depressor response to 5-HT was significantly attenuated by teprotide ($P < 0.001$, $n = 4$) but the absolute value of the nadir of this response was similar to control. 5-HT (0.1 mg kg^{-1}) also increased heart rate by approximately 30 min^{-1}. This was not significantly altered by pretreatment with teprotide 10 mg kg^{-1} (Figure 6b). Both phases of this blood pressure response to 5-HT have been shown to be resistant to prazosin (1 mg kg^{-1}) but the pressor response is susceptible to mianserin (1 mg kg^{-1}) (Barnett *et al.*, 1980).

Similarly, pressor responses to the rapid i.v. injection of AII (400 ng kg^{-1}) were not significantly affected by teprotide (1 and 10 mg kg^{-1}) (Figure 7).

Effects of saralasin on pressor responses to α-adrenoceptor agonists

Continuous infusion of saralasin ($4\,\mu g\,kg^{-1}\,min^{-1}$) produced a mean fall in diastolic BP of 11 ± 0.6 mmHg ($n = 24$) (combined data from experiments with AII, noradrenaline, phenylephrine and xylazine). As shown in Figure 8a, saralasin $4\,\mu g\,kg^{-1}\,min^{-1}$ significantly inhibited the pressor response to AII ($400\,ng\,kg^{-1}$) by 72% ($P < 0.001$, $n = 9$), indicating that this dose of saralasin effectively blocks those AII receptors on vascular smooth muscle that initiate pressor responses in the pithed rat.

As shown in Figure 8b, c and d, saralasin ($4\,\mu g\,kg^{-1}\,min^{-1}$) significantly inhibited the peak pressor responses to noradrenaline ($1\,\mu g\,kg^{-1}$; by 35%, $0.001 < P < 0.01$), phenylephrine ($3\,\mu g\,kg^{-1}$; by 33%, $0.001 < P < 0.01$, $n = 4$) and xylazine ($0.5\,mg\,kg^{-1}$; by 52%, $P < 0.001$, $n = 6$), indicating that it blocks the AII receptors which are responsible for the modulation, by endogenous AII, of α-adrenoceptor-mediated pressor responses. After teprotide ($10\,mg\,kg^{-1}$), saralasin ($4\,\mu g\,kg^{-1}\,min^{-1}$) had no further effect on responses to pressor agonists (results not shown).

Effects of teprotide on pressor responses to noradrenaline infusion

Noradrenaline infusion ($1\,\mu g\,kg^{-1}\,min^{-1}$) produced a pressor response reaching a maximum of approx 60 mmHg, comparable to a $1\,\mu g\,kg^{-1}$ bolus. This response was inhibited to a significantly greater degree by teprotide ($10\,mg\,kg^{-1}$) than was the peak response to a rapid injection of noradrenaline (Figure 9).

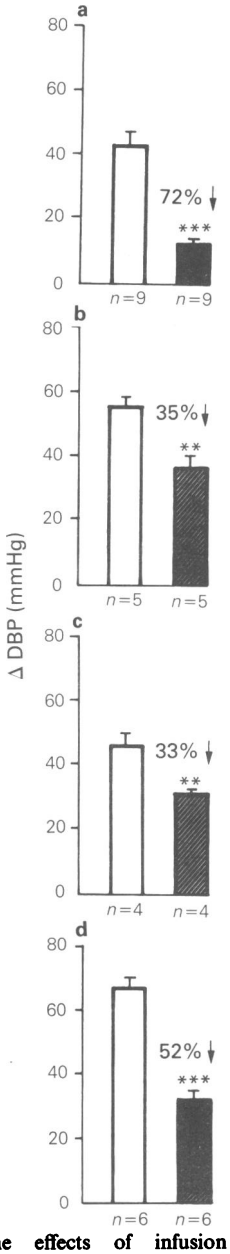

Figure 8 The effects of infusion of saralasin ($4\,\mu g\,kg^{-1}\,min^{-1}$) on peak diastolic blood pressure responses to (a) angiotensin II ($400\,ng\,kg^{-1}$), (b) noradrenaline ($1\,\mu g\,kg^{-1}$), (c) phenylephrine ($3\,\mu g\,kg^{-1}$) or (d) xylazine ($0.5\,mg\,kg^{-1}$) in the pithed rat. Two responses to one agonist were obtained in the same animal: control (open column), or after infusion of saralasin ($4\,\mu g\,kg^{-1}\,min^{-1}$) (hatched column). Responses after treatment with saralasin were compared with control by Student's paired t test (** $0.001 < P < 0.01$; *** $P < 0.001$). The numbers under each column indicate the number of observations. Bars indicate s.e.mean.

Discussion

The evidence overall indicates that removal of endogenous AII in the pithed rat attenuates α-adrenoceptor-mediated responses irrespective of the adrenoceptor subtype activated. We are confident that the doses of the adrenoceptor agonists chosen for this study were selective for α_1- and α_2-adrenoceptors. For example, in the pithed rat the pressor response to the α_1-adrenoceptor agonist, phenylephrine, was almost abolished by prazosin ($1\,mg\,kg^{-1}$) but was not significantly affected by Wyeth 26703 ($1\,mg\,kg^{-1}$). In contrast, we have previously found (Grant & McGrath, unpublished observations) that responses to the α_2-adrenoceptor agonists, xylazine and azepexole, were markedly

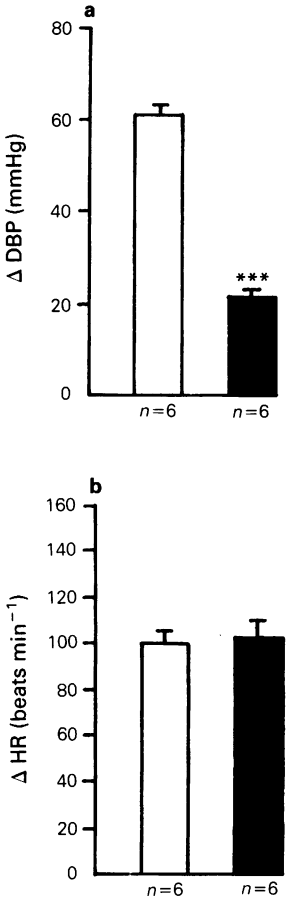

Figure 9 Effects of teprotide (10 mg kg^{-1}) on (a) peak diastolic pressor and (b) cardioaccelerator responses to noradrenaline infusion in the pithed rat. Two responses to noradrenaline infusion were obtained from each rat: control (open column), after teprotide (solid column). Responses were compared with control by Student's paired t test. (*** $P < 0.001$). Numbers under each column indicate the number of observations. Bars indicate s.e.mean.

attenuated by Wyeth 26703 but were not significantly affected by prazosin.

The lower concentration of the ACE-inhibitor teprotide (1 mg kg^{-1}) employed in this study had no effect on the peak responses to α-adrenoceptor agonists in the pithed rat but significantly attenuated the late, second component of pressor responses. This effect occurred irrespective of adrenoceptor subtype activated but it was more pronounced with the α_2-adrenoceptor agonists since these induced a more distinct second component. Since the pressor response to AI was markedly attenuated, this low dose of teprotide (1 mg kg^{-1}) presumably produced a clear inhibition of ACE.

The higher dose of teprotide (10 mg kg^{-1}) lowered diastolic BP to the same extent as the low dose and had only a small further effect on depression of the response to AI. However, this high dose not only inhibited the second phase components of the responses to α-adrenoceptor agonists but also significantly inhibited the peak responses. This effect occurred irrespective of adrenoceptor subtype since responses to the relatively selective α_1-adrenoceptor agonists phenylephrine and indanidine and to the relatively selective α_2-adrenoceptor agonists azepexole and xylazine were attenuated.

Also, we have demonstrated that pressor responses to noradrenaline, phenylephrine and xylazine were attenuated by the AII-receptor antagonist, saralasin, an effect similar to that of the high dose of teprotide.

It has been shown that responses to the preferential α_2-adrenoceptor agonist, clonidine, and the relatively selective α_1-adrenoceptor agonist, phenylephrine, are attenuated by captopril (Clough *et al.*, 1983). Also, Richer *et al.* (1984) showed that captopril and enalopril reduced, to the same extent, pressor and regional vasoconstrictor responses to the α-adrenoceptor agonists, cirazoline (α_1) and UK14304 (α_2) in pithed spontaneously hypertensive rats. In these latter studies, the rats were pretreated daily with ACE inhibitors for 7 days before pithing. All of these results suggest that ACE-inhibition does not selectively antagonize α_2-receptor-mediated responses and hence oppose the view of de Jonge *et al.* (1982), whose conclusions were based on studies using a limited number of α-adrenoceptor agonists.

At first it appeared that the inhibition of α-adrenoceptor-mediated responses by teprotide was not necessarily due to the removal of the influence of endogenous AII. We found that infusion of AII failed to reverse all of the effects of teprotide, viz. (1) After the low dose of teprotide, AII failed to restore the late phase of the responses to α-adrenoceptor agonists, yet it did produce potentiation of the early peak responses. (2) Infusion of AII failed to restore the effects of a high dose of teprotide on responses to α-adrenoceptor-agonists except in the cases of xylazine and azepexole. (3) Restoration, by AII infusion, of the fall in blood pressure which teprotide produced was not straightforward: with the lower dose of teprotide, the mean diastolic BP was returned to the control value but the effect in individual rats was very variable and with the higher dose of teprotide very little restoration could be produced.

However, this picture was radically altered by pretreating with the cyclo-oxygenase inhibitor, flurbiprofen. Responses to noradrenaline and phenylephrine (i.e. peak and late phase responses) could be

restored by infusion of AII and, even after the high dose of teprotide, the blood pressure was restored to control levels. Thus it appears that the effects of teprotide can be accounted for by ACE inhibition but AII infusion is complicated by its effects on prostaglandin release. The ability of exogenous AII to antagonize the effects of ACE inhibition may be hidden by the effects of prostaglandins released by exogenous AII, which act in physiological opposition to the vascular effects of AII and of α-adrenoceptor agonists. In an earlier study, where we found a similar interaction of cyclo-oxygenase products with nerve-mediated pressor responses, we could not tell whether the effect of cyclo-oxygenase products was pre- or postjunctional (Grant & McGrath, 1988). The present evidence suggests that this is postjunctional.

In retrospect, the infusion dose of AII which we employed ($50\,ng\,kg^{-1}\,min^{-1}$) seems to be more than is necessary to restore AII levels after teprotide. This became evident only after we used flurbiprofen and may account for the excessive potentiation of the initial peak responses. In our earlier study (Grant & McGrath, 1988), after teprotide ($1\,mg\,kg^{-1}$), we failed to restore the initial blood pressure even when infusing the higher dose of AII of $200\,\mu g\,kg^{-1}\,min^{-1}$.

It seems also that pressor responses to xylazine, azepexole and indanidine are relatively less markedly inhibited by prostaglandins, since AII infusion, without flurbiprofen, restored their pressor responses after their depression by teprotide. This implies that the facilitatory action of AII on the pressor responses to noradrenaline and phenylephrine is selectively 'antagonized' by prostaglandins.

We have no explanation for this other than to suggest that the natural phenylethanolamines act at other sites (or through alternative coupling processes) in addition to those activated by the other agonists and that the interaction of the various modulatory factors is different at these sites, e.g. vasodilator prostaglandins are more effective at the 'phenylethanolamine sites'. There is evidence that noradrenaline and phenylephrine possess peculiar properties distinct from those of other pressor α-adrenoceptor agonists since their pressor response on infusion is not maintained (Gillespie & Muir, 1967; O'Brien & McGrath, 1987). It is possible that they rely more heavily on release of intracellular Ca^{2+}. Whatever the explanation, this cautions against relying on non-phenylethanolamine agonists as tools for unravelling the physiological interactions between catecholamines, angiotensins and prostaglandins.

Of all the pressor stimuli tested in this study, the most susceptible to teprotide was that of infusion of noradrenaline and even the residual response in that case probably has little vasoconstrictor component, since a large increase in heart rate occurs, which is likely to increase cardiac output. In the absence of endogenous AII, therefore, it is possible that the infusion of NA may produce no vasoconstrictor response at all. This means that there is a considerable differential effect of AII between circulating catecholamines and vasopressor nerves, the former being much more clearly affected (Grant & McGrath, 1988).

Other evidence confirms that the effects of teprotide are AII-dependent. The infusion of the AII-receptor antagonist, saralasin, significantly inhibited the late phase and peak pressor responses to α-adrenoceptor agonists. The rate of infusion of saralasin employed in this study significantly attenuated pressor responses to AII. Similarly, Hatton & Clough (1982) and Clough *et al.* (1983) have shown that saralasin significantly attenuated pressor responses to noradrenaline, clonidine and phenylephrine. Also, we have shown that the effects of saralasin and teprotide ($10\,mg\,kg^{-1}$) are not additive against responses to α-adrenoceptor agonists (Grant & McGrath, unpublished observations).

In our equivalent study of pressor nerve-mediated responses (Grant & McGrath, 1988), we pursued the argument that pressor nerve responses decline in proportion of the effect of teprotide or saralasin on diastolic BP and that there is, therefore, no need to suggest a physiological interaction between AII and the neurotransmission process at the level of vascular smooth muscle. When this is applied to the studies with pressor agonists, the picture is less straightforward. First, a change in blood pressure did not necessarily affect the initial rapid response on injection of agonists. With the low dose of teprotide ($1\,mg\,kg^{-1}$), which did reduce nerve-induced responses, the first phase of the response to agonists was resistant to blockade; in contrast the second, more prolonged component declined to the extent that would have been expected from the fall in diastolic BP, as we found for the nerve-mediated responses. With the higher dose of teprotide, the responses to AII and 5-HT remained resistant but now, even the initial rapid responses to α-adrenoceptor agonists were attenuated. Thus a real, direct, specific interaction of endogenous AII with α-adrenoceptor agonists was uncovered with the higher dose of teprotide.

We conclude from this that in the pithed rat model, which was a high endogenous plasma AII level (Grant & McGrath, 1988), interference with the action of endogenous AII has a 2-stage effect on adrenoceptor mediated responses. The first stage involves removal of the effect of AII on basal blood pressure without necessarily any direct effect on adrenoceptor mechanisms. This action does not attenuate the early rapid responses to agonist bolus

injection. In a second stage, exemplified by the high dose of teprotide, a facilitatory influence of endogenous AII on both phases of adrenoceptor-mediated vasopressor responses is uncovered: even this action has no influence on the response to bolus injections of 5-HT or AII. Neither of these effects shows preference for either of the α-adrenoceptor subtypes.

We concluded in our previous paper (Grant & McGrath, 1988) that pressor nerve-mediated vasoconstriction is not affected by endogenous AII but in the present study, we conclude that vasoconstrictor responses to exogenously administered α-adrenoceptor agonists are facilitated by endogenous AII. This differential facilitation of agonist responses correlates also with the effects of prolonged infusion of subpressor doses of AII in the pithed rat model: nerve-mediated pressor responses were not affected (Grant & McGrath, 1988), whereas responses to α-adrenoceptor agonists were potentiated (this study). There are insufficient data for further analysis of this phenomenon in the present study but the evidence points quite clearly, in this model, to facilitation of the effects of agonists acting on vascular smooth muscle which does not extend to the actions of the neurotransmitters involved in sympathetic nerve-mediated vasoconstriction, although a portion of this response is mediated by α-adrenoceptors (Flavhan *et al.*, 1985). We are currently assessing whether our failure to find a modulatory effect of AII on sympathetic vasoconstriction (Bulloch & McGrath, 1986; 1988) might arise because the nerve-mediated response involves both purinergic and α-adrenergic components.

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