Facilitation by procaterol, a β -adrenoceptor agonist, of noradrenaline release in the pithed rat independently of angiotensin II formation

¹P. Kotsonis & H. Majewski

Prince Henry's Institute of Medical Research, P.O. Box 5152, Clayton 3168, Victoria, Australia

1 The effects of the β_2 -adrenoceptor agonist, procaterol, on sympathetic neuroeffector transmission were studied in the pithed adrenal demedullated rat to determine if generation of angiotensin II was involved in its effect. Pressor responses were elicited by either electrical stimulation (20 V, 2 Hz) of the entire spinal sympathetic outflow or methoxamine (0.1 mg kg^{-1} , i.v.).

2 Sodium nitroprusside (3 and $5 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) produced hypotension and the pressor responses to both sympathetic nerve stimulation and methoxamine were reduced. This indicates that decreasing blood pressure in pithed rats reduces pressor responses. Procaterol (10 and $30 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v.) also produced hypotension but did not alter pressor responses to sympathetic nerve stimulation. Nevertheless, procaterol (10 and $30 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v.) did reduce pressor responses to methoxamine. Together these results suggest that procaterol may have enhanced sympathetic neurotransmitter release. This was confirmed in another series of experiments where procaterol ($30 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v.) increased plasma noradrenaline levels during sympathetic nerve stimulation.

3 Captopril (5 mg kg^{-1} , i.v.) produced hypotension and as expected reduced pressor responses to sympathetic nerve stimulation. When the hypotensive effect of captopril was abolished by concomitant vasopressin infusion ($1.5\text{--}4.5 \text{ i mu kg}^{-1} \text{ min}^{-1}$, i.v.), pressor responses to sympathetic nerve stimulation were restored to pre-captopril levels. In this situation procaterol (10 and $30 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v.) reduced basal blood pressure and did not alter pressor responses to sympathetic nerve stimulation whereas the pressor responses were reduced by an equihypotensive infusion of sodium nitroprusside (3 and $5 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.). The lack of reduction of pressor responses after procaterol in the presence of captopril is indirect evidence that procaterol may have enhanced noradrenaline release independently of angiotensin II.

4 In another series of experiments, plasma noradrenaline levels elicited by sympathetic nerve stimulation were not altered by captopril (5 mg kg^{-1} , i.v.). In the presence of captopril (5 mg kg^{-1} , i.v.), procaterol ($30 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v.) no longer enhanced plasma noradrenaline levels during sympathetic nerve stimulation. However, since the dose of captopril is well above that required to block angiotensin converting enzyme (ACE) the effect may be non-specific. Therefore, the selective AT_1 receptor antagonist, losartan (10 mg kg^{-1} , i.v.), was also used. Losartan (10 mg kg^{-1} , i.v.) did not alter plasma noradrenaline levels during sympathetic nerve stimulation, and in the presence of losartan procaterol ($30 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v.) enhanced plasma noradrenaline levels during sympathetic nerve stimulation. This result further suggests that β -adrenoceptor facilitation of noradrenaline release from sympathetic nerves in the pithed rat occurs by a mechanism independent of angiotensin II generation.

Keywords: β -Adrenoceptors; angiotensin II; renin-angiotensin system; noradrenaline release; losartan; captopril; sympathetic nerves

Introduction

Sympathetic nerves possess prejunctional β_2 -adrenoceptors, activation of which results in enhanced action potential-evoked transmitter release (for reviews see Majewski, 1983; Borkowski, 1988). The endogenous activator appears to be adrenaline which enhances action potential-evoked noradrenaline release both *in vitro* (Stjärne & Brundin, 1975; Majewski *et al.*, 1981) and *in vivo* (Majewski *et al.*, 1985). In general the level of facilitation of noradrenaline release is small (10–50%, Stjärne & Brundin, 1975; Majewski *et al.*, 1981) but recent experiments in conscious rats suggests that larger effects are possible (100–200%, Coppes *et al.*, 1993). This action of adrenaline has been implicated in the development of hypertension and adrenaline has been found not only to induce hypertension through a β -adrenoceptor-mediated mechanism (see Majewski & Rand, 1986; Floras, 1992) but also like other β_2 -adrenoceptor agonists, increases vasoconstrictor responses to sympathetic nerve stimulation in isolated blood vessels (Kawasaki *et al.*, 1984) and in whole animals (Borkowski & Quinn, 1984; Schlicker *et al.*, 1988).

Up until the mid 1980's it was assumed that the β -adrenoceptors responsible for enhancing transmitter release were located on the nerve varicosities. Indeed, functional studies demonstrated their presence on sympathetic nerves of axonal sprouts (Weinstock *et al.*, 1978). However, Kawasaki *et al.* (1984) showed that a large proportion of the enhanced transmitter release after β -adrenoceptor activation was blocked by the angiotensin converting enzyme inhibitor, captopril, in the rat perfused mesentery. It was suggested that activation of β -adrenoceptor could induce the formation of angiotensin II locally and that angiotensin II enhanced noradrenaline release by activating a prejunctional angiotensin II receptor mechanism which is well described (for review see Zimmerman, 1981). Subsequently, β -adrenoceptor agonists were shown to evoke an overflow of angiotensin II-like immunoreactivity from rat perfused mesentery and this was antagonized by the angiotensin converting enzyme inhibitor, captopril (Nakamaru *et al.*, 1986). Further studies have shown that the facilitatory effects of β -adrenoceptor agonists on noradrenaline release can be inhibited by converting enzyme inhibitors and angiotensin II receptor blocking drugs (rat perfused mesenteric bed: Kawasaki *et al.*, 1984;

¹ Author for correspondence.

Nakamaru *et al.*, 1986; Draper *et al.*, 1989; and rat vena cava: Göthert & Kollecker, 1986; Ziogas & Story, 1991). However, no interaction between β -adrenoceptor-mediated facilitation of noradrenaline release and angiotensin II was seen in rat kidney (Rump & Majewski, 1987), rat anococcygeus muscle (Li *et al.*, 1988), rat atria (Mian *et al.*, 1989), rat tail artery and mouse atria (Rajanayagam *et al.*, 1989).

In vivo evidence for angiotensin II being involved in the release-enhancing effect of β -adrenoceptor agonists has come from experiments in pithed rats. Brokowski & Quinn (1984) using spontaneously hypertensive rats have shown that β -adrenoceptor agonists enhance vasoconstrictor responses to sympathetic nerve stimulation. Subsequently, Schlöcker *et al.* (1988) proposed that this effect was in fact due to the generation of angiotensin II since the effect was reduced by both the angiotensin converting enzyme inhibitor, captopril, and the angiotensin receptor antagonist, saralasin. However, interpretation of pressor response data in the pithed rat is complicated since a number of studies have found that in the pithed rat, sympathetically induced vasopressor responses are critically affected by basal arterial pressure (Oldham & Scotcher, 1986) and anti-angiotensin drugs such as captopril depress sympathetic vasoconstrictor response largely due to the hypotension (e.g. Hatton & Clough, 1982).

The aim of the present study was to investigate further β -adrenoceptor/angiotensin II modulation of sympathetic neurotransmission in the pithed rat. This was done firstly, by negating the hypotensive effects of captopril by use of infusions of vasopressin so that the effects of captopril on pressor responses could be clearly assessed independently of blood pressure changes and secondly, by measuring plasma noradrenaline levels to provide an independent biochemical index of noradrenaline release.

Methods

Adrenal demedullation

Male Sprague-Dawley rats (240–350 g) were anaesthetized with an intraperitoneal (i.p.) injection of sodium methohexitone (25 mg kg⁻¹) and sodium amylobarbitone (63 mg kg⁻¹) and the adrenal demedullation was performed via bilateral flank incisions. Animals were then allowed to recover and were given normal food and water rations.

Pithed rats

Three to ten days after adrenal demedullation the rats were anaesthetized with an intraperitoneal (i.p.) injection of sodium methohexitone (25 mg kg⁻¹) and sodium amylobarbitone (63 mg kg⁻¹). The left carotid artery was cannulated with polyethylene tubing for the recording of arterial blood pressure and heart rate. The tubing was inserted as far as the aortic arch. The trachea was then cannulated. The right jugular vein was also cannulated with polyethylene tubing which was inserted as far as the right atrium. The rats were pithed through the orbit of the eye with a stainless steel rod (1.5 mm in diameter) which was passed down the spinal cord. A stainless steel needle, which acted as an indifferent electrode, was placed subcutaneously in the lower back and positioned parallel to the spinal cord.

After pithing, the rats were artificially respired with air (60 breaths min⁻¹ and a volume of 2 ml 100 g⁻¹ body weight). Tubocurarine (1 mg, i.v.) and atropine (1 mg kg⁻¹, i.v.) were given after pithing to prevent skeletal muscle contraction and muscarinic receptor activation during electrical stimulation of the entire spinal outflow, respectively. The rats were then allowed a 10–15 min equilibration period during which time blood pressure stabilised. Body temperature was maintained at 36°C.

After the equilibration period, the spinal cord was stimulated electrically through the pithing rod at a frequency

of 2 Hz with 0.5 ms square wave pulses at a voltage of 20 V across the electrodes for 20 s. A 5 min period was allowed between stimulations to enable the diastolic pressure to return to pre-stimulation values. Seven consecutive equilibration stimulations were performed by which time the pressor responses were stable and reproducible. Then seven test stimulations (S1–S7) were performed with the first test stimulation (S1) taken as the pre-saline or drug control value. Drug or saline (0.9% NaCl, control) was infused after the first stimulation (S1) until the end of the experiment (S2–S7).

In another series of experiments, pressor responses were elicited with a bolus injection (i.v., left jugular vein) of methoxamine (0.1 mg kg⁻¹, in a volume of 0.1 ml, flushed with 0.1 ml saline). A 10 min period was allowed between each bolus injection of methoxamine to enable diastolic pressure to return to pre-stimulation values. Six consecutive equilibration methoxamine administrations were given by which time the pressor responses were stable and reproducible. Then seven test administrations were performed with the first administration (M1) taken as the pre-saline or drug control value. Drug or saline (0.9% NaCl, control) was infused after the first administration (M1) until the end of the experiment (M2–M7).

Plasma noradrenaline levels during sympathetic nerve stimulation

Animals were prepared as described and the spinal cord was stimulated electrically through the pithing rod at a frequency of 2 Hz with 0.5 ms square wave pulses at a voltage of 20 V across the electrodes for 90 s. Fifteen minutes was allowed between stimulations to ensure that diastolic blood pressure had returned to pre-stimulation values. There were two test stimulations (S1 and S2). Arterial blood (1.0 ml) was taken from the carotid artery over a 1 min period (starting 60 s after the onset of electrical stimulation) and immediately replaced with an equal volume blood taken from an anaesthetized donor rat. This ensured haemodynamic stability. The drug under investigation was administered either 5 or 10 min before the start of the second period of electrical stimulation. Upon completion of the experiment additional blood was taken for the determination of recoveries and pH adjustment for alumina chromatography.

Plasma noradrenaline analysis

The blood sample was immediately transferred into an ice-cold centrifuge tube containing 0.3 ml of 0.9% NaCl (with 50 iu ml⁻¹ of heparin); 50 μ l Na₂EDTA (100 mg ml⁻¹) and 50 μ l Na₂SO₃ (125 mg ml⁻¹). The samples were then centrifuged (at 14,000 g for 20 min at 4°C) and an aliquot (0.85 ml) was transferred to a tube containing 0.1 ml 2 M HClO₄; 50 μ l Na₂EDTA (100 mg ml⁻¹) and 50 μ l Na₂SO₃ (125 mg ml⁻¹); these tubes were frozen (–40°C) for up to a week. After thawing, the samples were centrifuged again (at 14,000 g for 20 min at 4°C) and an aliquot (0.9 ml) of the final supernatant was adjusted to pH 8.3–8.5 with 2 M Tris acetate buffer ($\approx$ 170 μ l). The samples were poured onto alumina (200 mg) in a glass column stoppered with a glass fibre filter. The columns were then washed two times with 1 ml H₂O. Then 500 μ l of 0.1 M HClO₄ was added to the column and the eluate was recirculated once.

The amount of noradrenaline in 100 μ l HClO₄ eluate was determined by high-performance liquid chromatography (h.p.l.c.) coupled with electrochemical detection. A reverse phase column was used (EXCIL C18 5 μ m–Alt 230, Activon, Australia). The solvent consisted of Na₂HPO₄ buffer 0.06 M, pH 3.10 containing 450–650 mg l⁻¹ heptane sulphonic acid, 200 mg l⁻¹ Na₂EDTA and 10–15 ml methanol. The electrochemical detection was an ESA Coulochem 5100 with an ESA 5011 analytical cell (ESA, Bedford, MA, U.S.A.). The first electrode potential was 0.06 V and the detecting electrode was 0.30 V. Recoveries were performed by adding

500 pg noradrenaline to a blood sample at the end of the experiment. The mean recovery of noradrenaline was $89.2\% \pm 2.7\%$ ($n = 12$). Corrections were made for incomplete recovery.

Drugs and materials

Sodium methohexitone and sodium amylobarbitone (Lilly, Sydney, Australia); tubocurarine (Wellcome, Sydney, Australia); atropine sulphate (Astra, Ryde, Australia); sodium nitroprusside (Sigma, St Louis, U.S.A.); vasopressin (Parke-Davis, Caringbah, Australia). The following drugs were generously donated: captopril (Squibb, Melbourne, Australia); procaterol-HCl (Parke-Davis, Ann Arbor, Michigan, U.S.A.); methoxamine-HCl (Wellcome, Sydney, Australia); losartan (DuPont Merck Pharmaceutical Co., Wilmington, U.S.A.).

Captopril, sodium nitroprusside, vasopressin, losartan and methoxamine-HCl were freshly dissolved and diluted in 0.9% NaCl prior to use. In the case of methoxamine-HCl, saline solution also contained ascorbic acid (0.5 mg ml^{-1}). Procaterol-HCl was initially made up as a stock solution (1 mg ml^{-1}) in 0.01 M HCl ($\text{pH} \approx 2$) and diluted with 0.9% NaCl on the day of use. This did not alter the pH of 0.9% NaCl.

Statistical analysis of results

All data are expressed as the mean $\pm$ standard error of the mean (s.e.mean). All groups of data were analysed with two-way analysis of variance (ANOVA). Probability levels of less than 0.05 ($P < 0.05$) were taken to indicate a significant difference.

Results

Base parameters

The base cardiovascular parameters are shown in Table 1. In the series of experiments where plasma noradrenaline levels

in pithed adrenal demedullated rats were measured during sympathetic nerve stimulation the mean plasma noradrenaline levels were $424.3 \pm 23.5 \text{ pg ml}^{-1}$ ($n = 37$). In pithed adrenal demedullated rats, plasma adrenaline levels under basal conditions and during sympathetic nerve stimulation (2 Hz, 0.5 ms, 20 V, for 90 s) were below detectable levels ($< 10 \text{ pg ml}^{-1}$). In the absence of nerve stimulation, plasma noradrenaline levels were also undetectable.

Pressor responses to sympathetic nerve stimulation

The β_2 -adrenoceptor agonist, procaterol (10 and $30 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v.), did not affect pressor responses to sympathetic nerve stimulation (Figure 1b) but did reduce basal diastolic pressure (Figure 1a). To act as a control for the hypotensive effects of procaterol, the vasodilator sodium nitroprusside (SNP) was also used. In contrast to procaterol, SNP (3 and $5 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) significantly reduced pressor responses to sympathetic nerve stimulation (Figure 1d) whilst the reduction in basal diastolic pressure was not significantly different from that produced by procaterol (compare Figure 1a and c). Heart rate was not altered by either procaterol or SNP (not shown).

Pressor responses to methoxamine

Procaterol (10 and $30 \text{ ng kg}^{-1} \text{ min}^{-1}$, i.v.) significantly reduced blood pressure and pressor responses elicited by the α_1 -adrenoceptor agonist, methoxamine (Figure 2a and b). Equihypotensive infusions of SNP (3 and $5 \mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.) also reduced pressor responses to methoxamine (Figure 2c and d). The reduction in basal diastolic blood pressure and pressor responses to methoxamine by SNP was not significantly different from that produced by procaterol (compare Figure 2a and c as well as b and d).

Effects of captopril on pressor responses to sympathetic nerve stimulation

The effects of procaterol were examined in the presence of captopril (5 mg kg^{-1} , i.v.) which reduced basal diastolic pres-

Table 1 Absolute values for cardiovascular parameters at first electrical stimulation (S1) or methoxamine administration (M1)

	Initial basal diastolic pressure (mmHg)	Initial pressor response (Δ mmHg)	Initial heart rate (beats min^{-1})
<i>A Pithed rats: electrical stimulation (Figure 1)</i>			
Control ($n = 4$)	34 ± 1	43 ± 3	393 ± 18
Procaterol ($n = 4$)	32 ± 1	46 ± 6	395 ± 13
SNP ($n = 5$)	32 ± 2	42 ± 7	377 ± 20
<i>B Pithed rats: methoxamine (Figure 2)</i>			
Control ($n = 4$)	37 ± 6	48 ± 11	362 ± 21
Procaterol ($n = 3$)	38 ± 3	54 ± 7	371 ± 16
SNP ($n = 5$)	39 ± 4	55 ± 3	366 ± 10
<i>C Pithed rats: electrical stimulation captopril (Figure 3)</i>			
Control ($n = 5$)	33 ± 3	26 ± 3	340 ± 19
Procaterol ($n = 5$)	32 ± 2	27 ± 2	327 ± 10
<i>D Pithed rats: electrical stimulation captopril and vasopressin (Figure 4)</i>			
Control ($n = 5$)	37 ± 2	27 ± 2	346 ± 9
Procaterol ($n = 7$)	34 ± 2	30 ± 4	336 ± 7
SNP ($n = 5$)	34 ± 2	32 ± 2	372 ± 15
<i>E Pithed rats: electrical stimulation (Figure 5)</i>			
Control ($n = 7$)	39 ± 2	42 ± 6	319 ± 22
Procaterol ($n = 7$)	36 ± 1	38 ± 4	289 ± 6
Captopril ($n = 5$)	37 ± 3	33 ± 4	289 ± 6
Captopril + procaterol ($n = 6$)	34 ± 2	35 ± 4	277 ± 14
Losartan ($n = 6$)	39 ± 2	39 ± 7	322 ± 6
Losartan + procaterol ($n = 6$)	39 ± 3	39 ± 2	300 ± 3

In the experiments represented in Figure 1–5 consecutive measurements are made (S1–S7, M1–M7) and all results are expressed as a percentage of the initial value. The absolute values at S1 or M1 are shown above.

Figure 1 The effect of procaterol and sodium nitroprusside on basal diastolic pressure (a and c, respectively) and pressor responses (changes in diastolic pressure; b and d, respectively) to sympathetic nerve stimulation. There were 7 consecutive stimulations (S1–S7; 2 Hz, 0.5 ms, 20 V, for 20 s at 5 min intervals). Saline (0.9% NaCl, vehicle) or drug infusion began after the first stimulation (S1). All results are expressed as a percentage of S1: (○) indicate experiments where no drug was administered ($n = 4$) and (●) indicate either procaterol (a and b, Proc 10 and 30 ng kg⁻¹ min⁻¹, $n = 4$) or sodium nitroprusside (c and d, SNP 3 and 5 μg kg⁻¹ min⁻¹, $n = 5$) which were infused immediately after S1 and S4. Each symbol represents the mean with s.e.mean. A significant effect ($P < 0.05$, ANOVA) of procaterol or SNP at each dose is indicated by *. The hypotensive effects of SNP and procaterol were not significantly different ($P > 0.05$, ANOVA).

Figure 2 The effect of procaterol and sodium nitroprusside on basal diastolic pressure (a and c, respectively) and pressor responses (change in diastolic pressure; b and d, respectively) to methoxamine (0.1 mg kg⁻¹, i.v.). There were 7 consecutive methoxamine administrations (M1–M7; at 10 min intervals). Saline (0.9% NaCl, vehicle) or drug infusion began after the first methoxamine bolus (M1). All results are expressed as a percentage of M1: (○) indicate experiments where no drug was administered ($n = 4$) and (●) indicate either procaterol (a and b, Proc 10 and 30 ng kg⁻¹ min⁻¹, $n = 3$) or sodium nitroprusside (c and d, SNP 3 and 5 μg kg⁻¹ min⁻¹, $n = 5$) which were infused immediately after M1 and M4. Each symbol represents the mean with s.e.mean. A significant effect ($P < 0.05$, ANOVA) of procaterol or SNP at each dose is indicated by *. The hypotensive effects of SNP and procaterol were not significantly different ($P > 0.05$, ANOVA).

sure and pressor responses to sympathetic nerve stimulation (Figure 3a and b). In the presence of captopril, procaterol (10 and 30 ng kg⁻¹ min⁻¹, i.v.) had no effect on the already reduced basal diastolic pressure or pressor responses to sympathetic nerve stimulation (Figure 3a and b). Heart rate was not altered by captopril (not shown).

In another series of experiments vasopressin (1.5–4.5 i mu kg⁻¹ min⁻¹, i.v.) was given to negate the hypotensive effects of captopril. The infusion rate of vasopressin was adjusted to each experiment immediately after captopril administration

(Figure 4a). In this case the pressor responses to sympathetic nerve stimulation were maintained at pre-captopril levels (Figure 4b) which contrasts the depressant action of captopril alone (Figure 3b). Procaterol (10 and 30 ng kg⁻¹ min⁻¹, i.v.) did not reduce pressor responses to sympathetic nerve stimulation after the combination of captopril and vasopressin (Figure 4b). In contrast, in the same situation, SNP (3 and 5 μ g kg⁻¹ min⁻¹, i.v.) reduced basal diastolic pressure (Figure 4c) and pressor responses to sympathetic nerve stimulation (Figure 4d).

Figure 3 The effect of procaterol on basal diastolic pressure (a) and pressor responses (change in diastolic pressure; b) to sympathetic nerve stimulation in animals given captopril (Capt, 5 mg kg⁻¹, i.v., immediately after S1). There were 7 consecutive stimulations (S1–S7; 2 Hz, 0.5 ms, 20 V, for 20 s at 5 min intervals). Saline (0.9% NaCl, vehicle) or drug infusion began after the first stimulation (S1). All results are expressed as a percentage of S1: (○) indicate experiments ($n = 5$) where captopril but not procaterol was administered; (●) indicate experiments where animals were given captopril (between S1 and S2) in addition to procaterol 10 ng kg⁻¹ min⁻¹ (after S1, Proc 10, $n = 5$) and procaterol 30 ng kg⁻¹ min⁻¹ (after S4, Proc 30, $n = 5$). Each symbol represents the mean with s.e.mean. There was no significant effect of procaterol ($P > 0.05$, ANOVA).

Figure 4 The effect of procaterol and sodium nitroprusside on basal diastolic pressure (a and c, respectively) and pressor responses (change in diastolic pressure; b and d, respectively) to sympathetic nerve stimulation in animals given captopril (Capt, 5 mg kg⁻¹, i.v., immediately after S1) and vasopressin (VP, 1.5–4.5 i mu kg⁻¹ min⁻¹, i.v., immediately after S1 until the end of the experiment). There were 7 consecutive stimulations (S1–S7; 2 Hz, 0.5 ms, 20 V, for 20 s at 5 min intervals). All results are expressed as a percentage of S1. Saline (0.9% NaCl, vehicle) or drug infusion began after the first stimulation (S1): (○) indicate experiments ($n = 5$) where captopril and vasopressin were administered between S1 and S2; (●) indicate experiments where in addition to captopril and vasopressin, either procaterol (a and b, Proc 10 and 30 ng kg⁻¹ min⁻¹, $n = 7$) or sodium nitroprusside (c and d, SNP 3 and 5 μ g kg⁻¹ min⁻¹, $n = 5$) were infused immediately after S1 and S4. Each symbol represents the mean with s.e.mean. A significant effect ($P < 0.05$, ANOVA) of procaterol or SNP at each dose is indicated by *. The hypotensive effects of SNP and procaterol were not significantly different ($P > 0.05$, ANOVA).

Figure 5 The effect procaterol infusion on plasma noradrenaline (NA) levels during sympathetic nerve stimulation (a). Also shown are the corresponding values of basal diastolic pressure (b) and pressor responses (change in diastolic pressure; c). There were 2 consecutive stimulations (S1 and S2; 2 Hz, 0.5 ms, 20 V, for 90 s at 15 min intervals). Saline (0.9% NaCl, vehicle) or drug infusion began after the first stimulation (S1). Drugs used were procaterol (30 ng kg⁻¹ min⁻¹, i.v.), captopril (5 mg kg⁻¹, i.v.) and losartan (10 mg kg⁻¹, i.v.). All results are expressed as a percentage of the first stimulation (S1). The mean with s.e.mean is shown. A significant effect of procaterol ($P < 0.05$, Student's *t* test) versus vehicle ($n = 7$) or appropriate drug is indicated by *. A significant effect of drug ($P < 0.05$, Dunnett's test) versus vehicle ($n = 7$) is indicated by †.

Effects of procaterol on plasma noradrenaline levels during sympathetic nerve stimulation

In this series of experiments plasma noradrenaline levels in arterial blood during sympathetic nerve stimulation (2 Hz, 0.5 ms, 20 V, for 90 s) were measured. Procaterol (30 ng kg⁻¹ min⁻¹, i.v.) significantly enhanced plasma noradrenaline levels compared to control but pressor responses to sympathetic nerve stimulation were not altered (Figure 5a and c).

Plasma noradrenaline levels during sympathetic nerve stimulation were not significantly altered by captopril (5 mg kg⁻¹, i.v., Figure 5a). However, in the presence of captopril, procaterol (30 ng kg⁻¹ min⁻¹, i.v.) no longer significantly enhanced plasma noradrenaline levels during sympathetic nerve stimulation (Figure 5a). The AT₁ angiotensin receptor antagonist, losartan (10 mg kg⁻¹, i.v.), did not alter plasma noradrenaline during sympathetic nerve stimulation (Figure

5a) and the enhancement of plasma noradrenaline levels during sympathetic nerve stimulation produced by procaterol was not reduced by losartan (Figure 5a). All of the drugs which produced hypotension (captopril and losartan) reduced the pressor responses to sympathetic nerve stimulation (Figure 5b and c).

Discussion

There has been considerable debate about adrenaline activation of presynaptic β_2 -adrenoceptors and the development of hypertension through facilitation of noradrenaline release from sympathetic nerves (see Majewski & Rand, 1986; Floras, 1992). An intriguing possibility has arisen that β_2 -adrenoceptor facilitation of transmitter release is in fact mediated indirectly by local generation of angiotensin II (see Introduction). This hypothesis was the basis for the present study where we investigated the effect of the selective β_2 -adrenoceptor agonist, procaterol, on pressor responses to thoracolumbar preganglionic sympathetic nerve stimulation in the pithed rat which was adrenal demedullated several days prior to experimentation to remove endogenous sources of circulating and tissue adrenaline (Majewski & Murphy, 1989).

It has been shown previously that infusions of procaterol, which did not affect resting blood pressure, enhanced pressor responses to sympathetic nerve stimulation in pithed spontaneously hypertensive rats (SHR) (procaterol, 10–13 ng kg⁻¹ min⁻¹, Borkowski & Quinn, 1984) and pithed Wistar rats (procaterol, 13–21 ng kg⁻¹ min⁻¹, Schlicker *et al.*, 1988). These effects are attributed to activation of β_2 -adrenoceptors and enhanced sympathetic transmitter release since procaterol had no effect on pressor responses to exogenous noradrenaline (Borkowski & Quinn, 1984; Schlicker *et al.*, 1988). The studies of Borkowski & Quinn (1984) and Schlicker *et al.* (1988) imply that β_2 -adrenoceptors responsible for enhancing transmitter release are more sensitive to agonists than β_2 -adrenoceptors responsible for mediating vasodilatation. However, in our study we were unable to obtain an infusion of procaterol which altered pressor responses without affecting resting blood pressure. Low infusion rates of procaterol (1 and 3 ng kg⁻¹ min⁻¹) did not affect resting blood pressure and did not affect responses to sympathetic nerve stimulation (not shown). Higher infusion rates (10 and 30 ng kg⁻¹ min⁻¹), comparable to the previous studies of Borkowski & Quinn (1984) and Schlicker *et al.* (1988), reduced resting blood pressure but still had no effect on responses to sympathetic nerve stimulation.

Effects on resting blood pressure may be very important in pithed rats. Many studies have shown that reducing blood pressure with drugs in the pithed rat reduces the vasoconstrictor effects of drugs (e.g. Oldham & Scotcher, 1986; Hatton & Clough, 1982). In the present study the vasodilator sodium nitroprusside (SNP), which is a guanosine 3':5'-cyclic monophosphate (cyclic GMP)-dependent vasodilator, reduced pressor responses to sympathetic nerve stimulation. SNP does not inhibit noradrenaline release (Waldman & Murad, 1987) and the effect is probably due to a reduction in post-junctional vasoconstrictor sensitivity since pressor responses to methoxamine were also reduced by SNP. This contrasts with the case of procaterol which reduced pressor responses to methoxamine but not sympathetic nerve stimulation and suggests that there may have been an underlying facilitation of sympathetic transmitter release by procaterol to compensate for the reduced postjunctional effectiveness. Indeed, when Borkowski & Quinn (1984) used an infusion of procaterol which lowered blood pressure (100 ng kg⁻¹ min⁻¹) there was no effect on pressor responses to sympathetic nerve stimulation but there was a reduction in vasoconstrictor responses to noradrenaline, which is similar to the findings of the present study.

Procaterol (30 ng kg⁻¹ min⁻¹) enhanced the plasma nora-

drenaline levels elicited by sympathetic nerve stimulation by 89 pg ml^{-1} (21%), which indicates facilitation of noradrenaline release. This enhancement is unlikely to be due to a reduction in noradrenaline plasma clearance since in most studies, β -adrenoceptor agonists either have no effect (Majewski *et al.*, 1982) or enhance noradrenaline plasma clearance (Cryer *et al.*, 1980). In other *in vivo* models including the anaesthetized and pithed rabbit (Schmidt *et al.*, 1984) and in man (Musgrave *et al.*, 1984) β -adrenoceptor agonists also enhance noradrenaline release.

To investigate a possible role of locally generated angiotensin II in the effect of procaterol, captopril (5 mg kg^{-1}) was used in the present study to block angiotensin II formation from angiotensin I. In this case captopril reduced pressor responses to sympathetic nerve stimulation as has been previously reported (e.g. Oldham & Scotcher, 1986). This effect was presumably due to the hypotension induced by captopril since subsequent vasopressin infusion, which prevented the hypotension, restored pressor responses. This observation further highlights the importance of resting blood pressure on pressor response in this model. It should be noted that vasopressin acts directly on vascular smooth muscle (Share, 1988) and is thought to have no effects on sympathetic transmitter release (Johnson *et al.*, 1974). In the presence of captopril and vasopressin, SNP reduced pressor responses to sympathetic nerve stimulation yet procaterol, which produced a similar degree of hypotension, had no effect on pressor responses suggesting that neurogenic vasoconstriction was still being facilitated. This result contrasts with that of Schlicker *et al.* (1988) who found that the facilitatory effect of procaterol on neurogenic responses was inhibited by captopril and the angiotensin receptor antagonist, saralasin. The discrepancy may be because in the latter case the pressor responses were themselves reduced by the anti-angiotensin drugs and this was not corrected for as in the present study.

When plasma noradrenaline levels were measured in the presence of captopril, it was found that procaterol no longer significantly enhanced plasma noradrenaline levels during sympathetic nerve stimulation, although there was a tendency for this to happen. In contrast, in the presence of the selective angiotensin II receptor (AT_1) blocking drug, losartan (10 mg kg^{-1}), procaterol still produced increases in noradrenaline release during sympathetic nerve stimulation. This dose was sufficient to block completely the pressor effects of infused angiotensin II in the pithed rat (Rechtman & Majewski, 1993). The discrepancy between the effects of captopril and losartan may be because the dose of captopril (5 mg kg^{-1}) (this dose blocked the facilitatory effects of procaterol on neurogenic responses in the study by Schlicker *et al.*, 1988) was high and and in excess of that required to block angiotensin converting enzyme activity and angiotensin II production (see Boura *et al.*, 1982; Clough *et al.*, 1982; where 0.01 and 0.1 mg kg^{-1} was sufficient, respectively). Indeed, in a previous study in the rat isolated kidney (Rump & Majewski, 1987) low concentrations of captopril ($0.1 \mu\text{M}$), which blocked angiotensin II formation from angiotensin I, had no effect on β -adrenoceptor facilitation of noradrenaline release whilst high concentrations of captopril ($5 \mu\text{M}$) did block β -adrenoceptor facilitation of noradrenaline release. In the same study (Rump & Majewski, 1987) the angiotensin receptor blocking agent, saralasin, did not affect β -adrenoceptor facilitation of noradrenaline release. Thus, high concentrations of captopril may affect noradrenaline release by angiotensin II-independent mechanisms. Whether this involves other substances affected by converting enzyme such as kinins, opioids and tachykinins, or other processes is unclear.

In conclusion, this study has demonstrated that pressor responses to sympathetic nerve stimulation are critically dependent on basal blood pressure in the pithed rat. It should be noted that the large reductions in pressor responses to sympathetic nerve stimulation by captopril and losartan were not accompanied by any change in plasma noradrenaline levels (compare Figure 5a and c) and thus are most likely to be due to the hypotension produced by these agents. If it is assumed that this can be taken into account by examining the changes in pressor responses to exogenous agonists or maintaining blood pressure with vasopressin infusion, then it appears that the β_2 -adrenoceptor agonist, procaterol, facilitates neurogenic vasoconstriction by a mechanism independent of angiotensin II since this was not affected by captopril. Biochemical measurements of increments in plasma noradrenaline during nerve stimulation also support the proposal that procaterol enhances stimulation-induced noradrenaline release. This effect is not blocked by losartan and thus does not involve angiotensin receptors. However, the effects of captopril are equivocal with an indication that it may block the facilitatory effect of procaterol on plasma noradrenaline. However, this may be unrelated to blockade of angiotensin II since the dose of captopril was high.

This work was supported by a grant from the National Health and Medical Research Council of Australia. P.K. is a National Heart Foundation of Australia Postgraduate Research Scholar.

References

- BOURA, A.L.A., HUI, S.-C.G., RECHTMAN, M.P. & WALTERS, W.A.W. (1982). Captopril inhibits pressor responses to peripheral sympathetic nerve activation in cats and rats. *Clin. Exp. Pharmacol. Physiol.*, **7**, 123–128.
- BORKOWSKI, K.R. (1988). Pre- and postjunctional β -adrenoceptors and hypertension. *J. Auton. Pharmacol.*, **8**, 153–171.
- BORKOWSKI, K.R. & QUINN, P. (1984). Facilitation by β -adrenoceptors of stimulation-induced vasoconstriction in pithed spontaneously hypertensive rats. *J. Auton. Pharmacol.*, **4**, 127–131.
- CLOUGH, D.P., COLLIS, M.C., CONWAY, J., HATTON, R. & KEDDIE, J.R. (1982). Interaction of angiotensin-converting enzyme inhibitors with the function of the sympathetic nervous system. *Am. J. Cardiol.*, **49**, 1410–1414.
- COPPES, R.P., SMIT, J., KHALI, N.N., BROUWER, F. & ZAAGSMA, J. (1993). Strong activation of vascular prejunctional β_2 -adrenoceptors in freely moving rats by adrenaline released as a co-transmitter. *Eur. J. Pharmacol.*, **243**, 272–279.
- CRYER, P.E., RIZZA, R.A., HAYMOND, M.W. & GERICH, J.E. (1980). Epinephrine and norepinephrine are cleared through beta-adrenergic but not alpha-adrenergic mechanisms in man. *J. Clin. Invest.*, **29** (suppl. 1), 1114–1118.
- DRAPER, A.J., MEGHJI, S. & REDFERN, P.H. (1989). Enhanced presynaptic facilitation of vascular adrenergic neurotransmission in spontaneously hypertensive rats. *J. Auton. Pharmacol.*, **9**, 103–111.
- FLORAS, J.S. (1992). Epinephrine and the genesis of hypertension. *Hypertens.*, **19**, 1–18.
- GÖTHERT, M. & KOLLECKER, P. (1986). Subendothelial β_2 -adrenoceptors in the rat vena cava: Facilitation of noradrenaline release via local stimulation of angiotensin II synthesis. *Naunyn-Schmied. Arch. Pharmacol.*, **334**, 156–165.
- HATTON, R. & CLOUGH, D.P. (1982). Captopril interferes with neurogenic vasoconstriction in the pithed rat by angiotensin-dependent mechanisms. *J. Cardiovasc. Pharmacol.*, **4**, 116–123.
- JOHNSON, E.M., MARSHALL, G.R. & NEEDLEMAN, P. (1974). Modification of the pressor responses to sympathetic nerve stimulation by the renin-angiotensin system in rats. *Br. J. Pharmacol.*, **51**, 541–546.

- KAWASAKI, H., CLINE, W.H. & SU, C. (1984). Involvement of the vascular renin-angiotensin system in beta adrenergic receptor-mediated facilitation of vascular neurotransmission in spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.*, **231**, 23–32.
- LI, C., MAJEWSKI, H. & RAND, M.J. (1988). Facilitation of noradrenaline release from sympathetic nerves in rat anococcygeus muscle by activation of prejunctional β -adrenoceptors and angiotensin receptors. *Br. J. Pharmacol.*, **95**, 385–392.
- MAJEWSKI, H. (1983). Modulation of noradrenaline release through activation of presynaptic β -adrenoceptors. *J. Auton. Pharmacol.*, **3**, 47–60.
- MAJEWSKI, H., HEDLER, L., SCHURR, C. & STARKE, K. (1985). Dual effect of adrenaline on noradrenaline release in the pithed rabbit. *J. Cardiovasc. Pharmacol.*, **7**, 251–257.
- MAJEWSKI, H., HEDLER, L. & STARKE, K. (1982). The noradrenaline release rate in the anaesthetized rabbit: facilitation by adrenaline. *Naunyn-Schmied. Arch. Pharmacol.*, **321**, 20–27.
- MAJEWSKI, H. & MURPHY, T.V. (1989). Beta-adrenoceptor blockade and sympathetic neurotransmission in the pithed rat. *J. Hypertens.*, **7**, 991–996.
- MAJEWSKI, H. & RAND, M.J. (1986). A possible role of epinephrine in the development of hypertension. *Med. Res. Rev.*, **6**, 467–486.
- MAJEWSKI, H., RAND, M.J. & TUNG, L.H. (1981). Activation of prejunctional β -adrenoceptors in rat atria by adrenaline applied exogenously or released as a co-transmitter. *Br. J. Pharmacol.*, **73**, 669–679.
- MIAN, M.A., MAJEWSKI, H. & RAND, M.J. (1989). Facilitation of noradrenaline release by isoprenaline in rat isolated atria does not involve angiotensin II formation. *Clin. Exp. Pharmacol. Physiol.*, **16**, 905–911.
- MUSGRAVE, I.F., BACHMAN, A.W. & GORDON, R.D. (1984). Increased plasma noradrenaline during adrenaline infusion in man. *J. Hypertens.*, **2** (suppl 3), 135–137.
- NAKAMURA, M., JACKSON, E.K. & INAGAMI, T. (1986). β -Adrenoceptor mediated release of angiotensin II from mesenteric arteries. *Am. J. Physiol.*, **250**, H144–H148.
- OLDHAM, A.A. & SCOTCHER, K.P. (1986). Interactions between angiotensin II, the sympathetic nervous system and blood pressure of the pithed rat. *Br. J. Pharmacol.*, **86**, 749P.
- RAJANAYAGAM, M.A.S., MUSGRAVE, I.F., RAND, M.J. & MAJEWSKI, H. (1989). Facilitation of noradrenaline release by isoprenaline is not mediated by angiotensin II in mouse atria and rat tail artery. *Arch. Int. Pharmacodyn. Ther.*, **299**, 185–199.
- RECHTMAN, M.P. & MAJEWSKI, H. (1993). A facilitatory effect of anti-angiotensin drugs on vagal bradycardia in the pithed rat and guinea-pig. *Br. J. Pharmacol.*, **110**, 289–296.
- RUMP, L.C. & MAJEWSKI, H. (1987). Beta-adrenoceptor facilitation of norepinephrine release is not dependent on local angiotensin II formation in the rat isolated kidney. *J. Pharmacol. Exp. Ther.*, **243**, 1107–1112.
- SCHLICKER, E., ERKENS, K. & GÖTHERT, M. (1988). Probable involvement of angiotensin II formation in the β_2 -adrenoceptor-mediated facilitation of the neurogenic vasopressor response in the pithed rat. *Naunyn-Schmied. Arch. Pharmacol.*, **338**, 536–542.
- SCHMIDT, H., SCHURR, C., HEDLER, L. & MAJEWSKI, H. (1984). Local modulation of noradrenaline release in vivo: presynaptic β_2 -adrenoceptors and endogenous adrenaline. *J. Cardiovasc. Pharmacol.*, **6**, 641–649.
- SHARE, L.A. (1988). Role of vasopressin in cardiovascular regulation. *Physiol. Rev.*, **68**, 1248–1284.
- STJÄRNE, L. & BRUNDIN, J. (1975). Dual adrenoceptor-mediated control of noradrenaline secretion from human vasoconstrictor nerves: facilitation by β -receptors and inhibition by α -receptors. *Acta Physiol. Scand.*, **94**, 139–141.
- WALDMAN, S.A. & MURAD, F. (1987). Cyclic GMP synthesis and function. *Pharmacol. Rev.*, **39**, 163–196.
- WEINSTOCK, M., THOA, N.B. & KOPIN, I.J. (1978). β -Adrenoceptors modulate noradrenaline release from axonal sprouts in cultured rat superior cervical ganglia. *Eur. J. Pharmacol.*, **47**, 297–302.
- ZIMMERMAN, B.G. (1981). Adrenergic facilitation by angiotensin: Does it serve a physiological function? *Clin. Sci.*, **60**, 343–348.
- ZIOGAS, J. & STORY, D.F. (1991). Angiotensin II generation in the rat vena cava: Stimulation of local synthesis by β -adrenoceptor activation. *Naunyn-Schmied. Arch. Pharmacol.*, **343**, 31–36.

(Received May 11, 1994
Revised July 1, 1994
Accepted July 4, 1994)