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MODIFICATION OF RESPONSES TO SYMPATHETIC NERVE STIMULATION BY THE RENIN-ANGIOTENSIN SYSTEM IN RATS

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1 Angiotensin I (AI) and AII elicited a dose-dependent potentiation of contractions by rat vas deferens produced by low frequency nerve stimulation without enhancing the contraction produced by exogenous noradrenaline. The AII-induced presynaptic potentiation was blocked by the specific antagonist cysteine^{8}-AII.

2 The vasoconstrictor response to periarterial stimulation of rat isolated perfused kidney was potentiated by AII and there was a lesser enhancement of the effect of exogenous noradrenaline.

3 The response to stimulation of complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat was enhanced by angiotensin or vasopressin in direct proportion to the increase in prestimulus muscular tone. The blood pressure in the pithed rats is primarily maintained by the renin-angiotensin system since the converting-enzyme inhibitor (SQ-20881) or bilateral nephrectomy caused further substantial lowering of systemic blood pressure after spinal cord destruction and after treatment with curare and atropine.

Introduction

Angiotensin (AII), has been shown to augment the effects of sympathetic nerve stimulation in several isolated perfused preparations (Benelli, Della Bella & Gandini, 1964; Zimmerman, 1967; Zimmerman & Gisslen, 1968; Kadowitz, Sweet & Brody, 1971, 1972; Bell, 1972; Turker, 1973). Whether the augmentation produced by AII is at presynaptic or postsynaptic sites is usually assessed by comparing the potentiation of stimulation (i.e. release of transmitter from nerve terminals) to the enhancement of the response to exogenous noradrenaline which acts directly on smooth muscle receptors. The reported effects of AII on exogenously administered noradrenaline have been conflicting. For example, AII infusion has been found to potentiate (Zimmerman, 1973) or to have no effect on (Kadowitz *et al.*, 1972) exogenously administered noradrenaline in the perfused dog hindpaw preparation. Similarly AII has been reported to potentiate (Sweet, Ferrario, Khosla & Bumpus, 1973) or to have no effect (Kadowitz *et al.*, 1972) on the response to intraarterially administered tyramine (which releases noradrenaline from nerve terminals) in the dog hindpaw preparation.

The purpose of this paper is to assess the influence of AII and AI on the responses to sympathetic nerve stimulation of non-vascular and vascular smooth muscle preparations in the rat. The effect of AII on the vasoconstrictor response to spinal cord stimulation in pithed rats is also studied.

Methods

Peptides originally isolated from snake venom have been shown to inhibit the conversion of AI to AII as well as to prolong and enhance the action of bradykinin. These peptides have come to be known as bradykinin potentiating factors (BPF). The converting enzyme inhibitor (BPF) used in these experiments was SQ-20881 (Pyr-Tyr-Pro-Arg-Pro-Gln-Ile-Pro-Pro) and was kindly supplied by the Squibb Research Institute. Angiotensin II (Hypertensin, Ciba), noradrenaline (norepinephrine, Winthrop), vasopressin (Sigma), and oxytocin (Parke-Davis) were obtained commercially.

Rat vas deferens preparation

Male rats (Zivic-Miller, 175-235 g) were killed by a blow to the head and cervical dislocation. The

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branch of the hypogastric artery that supplies the vas deferens was ligated approximately 1 cm away from its insertion into the vas deferens. The vas deferens and attached artery were removed from the rat and carefully mounted in a 20 ml organ-bath under 1 g of tension. The bathing medium was Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 at 37° C (pH 7.4). The artery was carefully threaded through the stimulating electrode. Tension developed in the vas deferens was recorded isotonically with a Harvard rotary motion transducer attached to a Harvard physiological recorder.

In all experiments a Grass stimulator was used to supply square wave stimuli of supramaximal voltage and 1 ms duration. The nerve was stimulated at 2 Hz for 20 s at intervals of 3 minutes. After stable responses to this stimulus had been obtained, augmentation of the response was determined by the addition of increasing amounts of peptide after each stimulus. After each dose-response curve the medium was changed twice and subsequent curves were obtained at intervals of 20-30 minutes. A tendency for angiotensin to produce less augmentation over a period of 2 to 3 h was usually observed. Augmentation is expressed as a percentage of the original contraction for a given preparation.

Rat isolated perfused kidney preparation

Rats (Zivic-Miller, 200-300 g) were anaesthetized with sodium pentobarbitone (30 mg/kg, i.p.). After opening the abdominal cavity, the animal was heparinized (250 units/kg) and a polyethylene cannula (PE-50, Clay Adams) was tied into the renal artery. The kidney was flushed with Krebs-Henseleit solution after cutting the renal vein. The kidney was removed from the animal. The cannula and renal artery were pulled through the electrode, thereby permitting periarterial nerve stimulation. The kidney was placed in a warming jacket and perfused with Krebs-Henseleit solution at a constant flow rate of 4 ml/minute. Changes in perfusion pressure were indicative of alterations in renal vascular tone. It was observed that the potentiation of nerve stimulation was reduced in preparations which had been perfused for longer periods. Therefore, all results described were obtained from kidneys within 60 min of the start of perfusion.

Pithed rat preparation

Female rats (Zivic-Miller, 225-275 g) were anaesthetized with sodium pentobarbitone (30 mg/kg, i.p.). The trachea (PE 240), both jugular veins (PE 50), and a carotid artery (PE 50) were cannulated. The animal was pithed with a steel rod and the complete sympathetic outflow stimulated as described by Gillespie & Muir (1969). After pithing, the rats were respired artificially and were treated with (+)-tubocurare (Squibb) (1.2 mg/kg) intravenously and atropine sulphate (1.5 mg/kg) intravenously. Changes in blood pressure were recorded from the carotid artery (Physiograph P-1000 linear-core pressure transducer). Nephrectomy was accomplished by ligation of the renal artery and vein; trauma to the adrenal glands was minimal. The rise in blood pressure elicited by stimulation of the entire sympathetic outflow was determined by stimulating the steel rod with square wave impulses of 50 V, 1 ms duration, for 30 s at varying frequencies. Stimulation was carried out at 5 min intervals.

Results

Rat vas deferens preparation

AI and AII produced dose-related increases in the responses of the rat vas deferens preparation to low frequency stimulation (2 Hz). The addition of AII at these concentrations caused a transient contraction which was not sustained and tension returned quickly to base line, usually within 3 minutes. The relative potencies of AI and AII in potentiating sympathetic nerve stimulation are shown in Figure 1. AI had approximately 10% of the activity of AII. The activity of AI appears not to be due to conversion in the tissue to AII since the effect of AI was not affected (Fig. 2) by the presence of a converting enzyme inhibitor (BPF) (Ferreira, Green, Alabaster, Bakhle & Vane, 1970).

The potentiation of nerve stimulation by AII was blocked (Fig. 3) by the specific angiotensin antagonist cysteine^8-AII (Needleman, Johnson, Vine, Flanigan & Marshall, 1972). A similar inhibition was also observed with Ile^8-AII. The effects of AII on responses to sympathetic nerve stimulation in the isolated perfused dog hindpaw and perfused kidney (Zimmerman, 1973; Sweet *et al.*, 1973) have been shown to be antagonized by specific angiotensin antagonists which block the spasmogenic effects of AII on smooth muscle.

AII clearly exerts its potentiating activity in the vas deferens at presynaptic sites since addition of AII (20 ng/ml) to the bathing medium, a concentration which doubles responses to nerve stimulation, had no effect on the dose-response curve to exogenous noradrenaline (Figure 4).

Experiments were carried out to determine if other spasmogenic peptide hormones produced similar increases in the response to sympathetic nerve stimulation. Vasopressin and oxytocin were found not to affect the responses of this preparation.

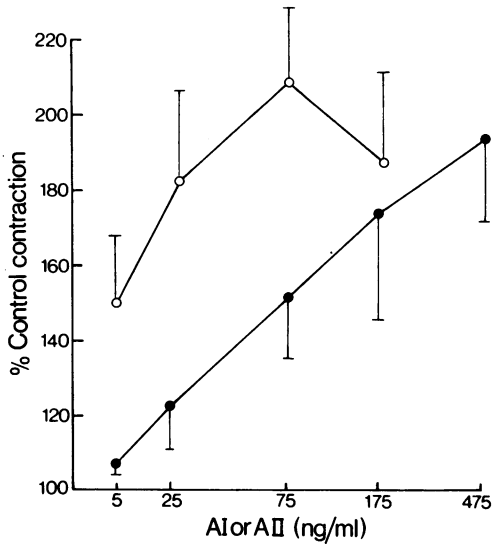

Fig. 1 Potentiation of contractions of rat vas deferens to nerve stimulation by increasing concentrations of angiotensin I (•) and angiotensin II (○). The increased response is expressed as a percentage of the response obtained prior to addition of peptide. Points represent the mean and s.e. of six preparations.

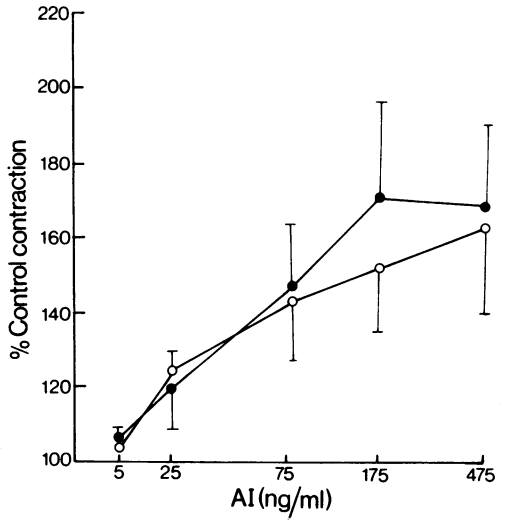

Fig. 2 Effect of an inhibitor of angiotensin converting enzyme (BPF, SQ-20881, 5 μg/ml) on the potentiation by angiotensin I (AI) of responses to nerve stimulation of rat vas deferens. BPF alone had no effect on responses of the preparation. (•) AI alone; (○) AI + BPF. Points represent the mean and s.e. of six preparations.

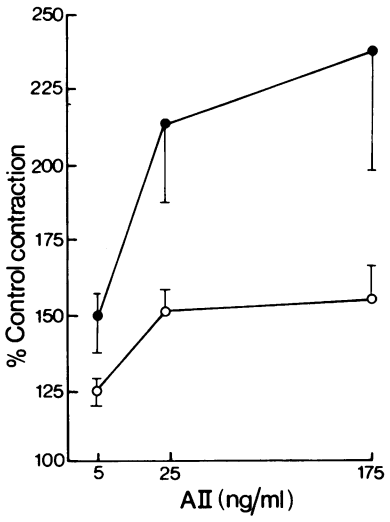

Fig. 3 Inhibition by cysteine[8] angiotensin II (Cys[8]-AII, 100 ng/ml) of the potentiation by angiotensin II (AII) of contraction of rat vas deferens produced by nerve stimulation. The antagonist had no effect on contractions when stimulation was done in the absence of AII. (•) AII alone; (○) AII plus Cys[8]-AII. Points represent the mean and s.e. of eight preparations.

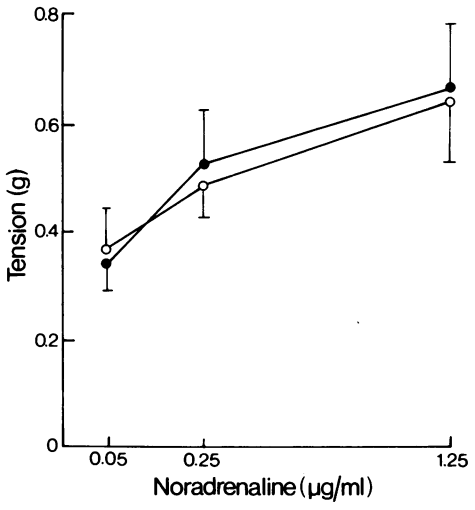

Fig. 4 Effect of angiotensin II (AII) on the contractions of the rat vas deferens produced by exogenous noradrenaline (NA). (•) Control (○) in presence of AII (20 ng/ml). Points represent the mean and s.e. of six preparations.

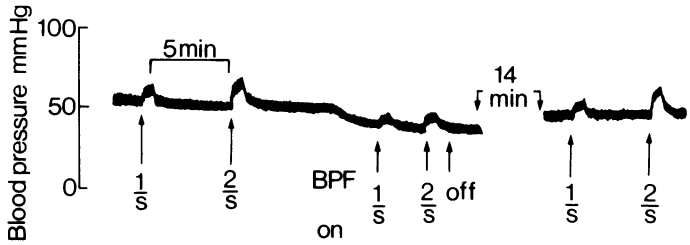

Fig. 5 Effect on blood pressure of infusion of an inhibitor of angiotensin converting enzyme (BPF) in a pithed rat. The inhibitor was infused at a rate of 12 μg/min via a jugular vein cannula. Nerve stimulation (S) was performed as described in the methods section. Numbers indicate stimulation frequency (Hz).

Rat isolated perfused kidney

Perfusion of the rat kidney with Krebs-Henseleit medium at 4 ml/min yielded a perfusion pressure of 72 ± 8 mmHg (n = 6). Infusion of a low concentration of AII (0.5 ng/min) into the medium as it entered the kidney yielded an average increase in perfusion pressure of 5 mmHg. The responses to periarterial nerve stimulation and exogenous noradrenaline were compared before and during the angiotensin infusion (Table 1). The responses to nerve stimulation and, to a lesser degree, exogenous noradrenaline were potentiated whether the results are expressed as absolute pressure rise (ΔPP mmHg) or as a percentage of initial perfusion pressure (ΔPP %). The enhancement by AII of the effects of both nerve stimulation and exogenous noradrenaline was blocked by simultaneous infusion of the specific antagonist Cys^8-AII.

Table 1 Effect of angiotensin on nerve stimulation and noradrenaline injection in the isolated perfused rat kidney

Pressor stimulus		*Pre-infusion*		*During infusion*	
		ΔPP mmHg	*ΔPP %*	*ΔPP mmHg*	*ΔPP %*
Exogenous noradrenaline 20 ng		40 ± 6	56	78 ± 8	101
Nerve stimulation (Hz)	2	11 ± 4	15	39 ± 8	55
	4	30 ± 6	42	100 ± 5	139

The rat kidney was perfused as described in the methods section. Pre-infusion perfusion pressure averaged 72 ± 8 mmHg (n = 8). Angiotensin II infused at 0.5 ng/min yielded an average of 5 mmHg increase in perfusion pressure. ΔPP indicates changes in perfusion pressure which are expressed either in absolute values as mmHg or as percentage change compared to the pre-infusion perfusion pressure.

Table 2 Effect of angiotensin on pressor responses to nerve stimulation in the nephrectomized pithed rat

Pressor stimulus	*Pre-infusion*		*During infusion*	
	ΔBP mmHg	*ΔBP %*	*ΔBP mmHg*	*ΔBP %*
Exogenous noradrenaline (i.v.)				
50 ng	13 ± 2	51 ± 7	17 ± 2	52 ± 3
150 ng	20 ± 3	77 ± 8	26 ± 3	80 ± 6
Nerve stim. (Hz)				
0.5	8 ± 2	29 ± 6	11.5 ± 2	32.5 ± 3.5
1	13 ± 4	51 ± 12	19.5 ± 3	56 ± 6
2	18 ± 6	74 ± 18	31 ± 5	89 ± 10
4	24 ± 4	105 ± 14	44 ± 7	130 ± 12

Angiotensin II (AII) was infused at a rate of 17 ng/min into rats prepared as described in the methods section. Pre-infusion blood pressures ranged from 18-32 mmHg. Blood pressures were raised 41 ± 4% by the AII infusions. Data are mean with s.e. from six rats.

Pithed rat

In order to assess the influence of AII on nerve stimulation in the whole animal, responses in the pithed rat were studied. The basal blood pressure of the pithed rats tended to rise and repeated nerve stimulation at the same frequency or repeated administration of the same dose of noradrenaline produced larger increases in blood pressure as time elapsed. A large percentage of initial blood pressure and the subsequent rise observed in these animals was apparently being maintained by the renin-angiotensin system because: (1) a fall in blood pressure could be obtained in these animals by infusion of the converting enzyme inhibitor SQ 20881 (BPF, 50-75 $\mu g\ kg^{-1}\ min^{-1}$, Fig. 5); and (2) the fall in blood pressure observed upon BPF infusion as well as the increasing basal blood pressure were completely prevented if the kidneys of the rat were ligated.

The effects of nerve stimulation (spinal cord) and injection of noradrenaline in pithed animals pretreated with atropine and tubocurare were studied before and during infusion of AII (10-17 ng/minute). This level of AII caused about a 40% increase in blood pressure in these animals. The responses are shown in Table 2 and Figure 6. It can be seen that all stimuli produced a greater absolute increase in blood pressure during the infusion of AII, but when the pressure rises were calculated as a percentage of the AII shifted basal blood pressure, the responses were essentially the same before and after AII infusion. Hence, the data suggest that the 'potentiation' under these conditions simply reflects the effect of AII on basal vascular tone and is an 'apparent' potentiation rather than a 'real' potentiation (Redleaf & Tobian, 1958).

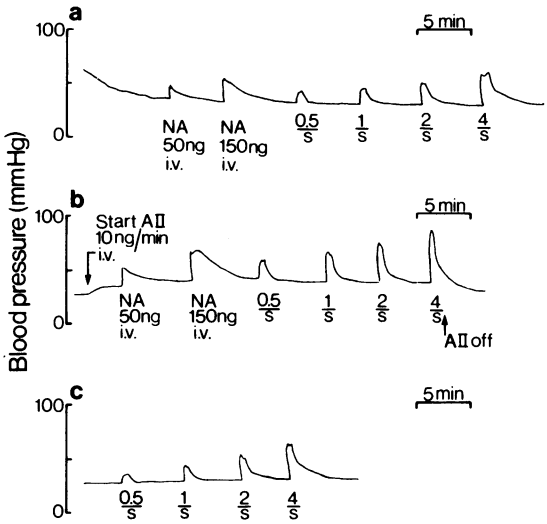

Fig. 6 Effect of infusion of angiotensin II (AII) in a nephrectomized pithed rat on response to nerve stimulation (S) and intravenous noradrenaline (NA). (a) Before AII infusion; (b) during AII infusion; (c) after AII infusion.

In order to test this hypothesis, the blood pressure in the pithed rats was raised by infusion of vasopressin, rather than AII. Vasopressin was chosen because it was without effect on the vas deferens. The blood pressure of the nephrectomized pithed rats was raised by the vasopressin infusion to a level similar to that in the previous AII experiments (Table 3). The same pattern was observed as seen with AII, the increased response in absolute terms disappeared if the pressure rise was calculated as a percentage of initial blood

Table 3 Effect on vasopressin on pressor responses in the nephrectomized pithed rat

Pressor stimulus	*Pre-infusion*		*During infusion*	
	ΔBP mmHg	*ΔBP %*	*ΔBP mmHg*	*ΔBP %*
Exogenous noradrenaline (i.v.)				
50 ng	12 ± 1	52 ± 5	25 ± 3	67 ± 8
150 ng	20 ± 1	83 ± 5	45 ± 7	117 ± 9
Nerve stim. (Hz)				
0.5	5 ± 1	22 ± 4	13 ± 2	34 ± 7
1	9 ± 2	40 ± 10	21 ± 3	53 ± 7
2	15 ± 3	61 ± 11	30 ± 4	77 ± 15
4	23 ± 3	95 ± 20	43 ± 8	114 ± 25

Vasopressin was infused at a rate of 3-12 μunits/min into rats prepared as described in the methods section. Pre-infusion blood pressures were 25 ± 1 mmHg. Blood pressures were raised to 38 ± 3 mmHg (54 ± 4%) by the vasopressin infusions. Data are mean with s.e. from seven rats.

pressure. The responses to AII or vasopressin were proportional to initial blood pressure over a wide range of initial blood pressures.

Discussion

Angiotensin II produced a presynaptic potentiation of response to sympathetic nerve stimulation in the vas deferens without affecting responses to exogenous noradrenaline. Our observations in the isolated vas deferens are generally consistent with those of Bell (1972) in the guinea-pig isolated vas deferens. The response to AI is not due to conversion in the tissue since it is unaffected by inhibitors of angiotensin converting enzyme. The angiotensin analogues, Cys^8-AII and Ile^8-AII, which have been shown to block the spasmogenic effects of AII (Needleman *et al.*, 1972) were also potent antagonists of the potentiating effects of AII on the rat vas deferens.

The kidney appears extremely sensitive to AII. Marked effects on nerve stimulation were observed at concentrations in the perfusing medium of 125 pg/ml. Zimmerman & Gisslen (1968) report responses in the *in situ* perfused dog kidney at concentrations as low as 60 pg/ml. In contrast to the completely presynaptic effects observed in the vas deferens, the effect of AII on nerve stimulation in the perfused rat kidney appear more complex. Infusion of AII also resulted in increases in responsiveness to exogenous noradrenaline, although the increase was less than that of nerve stimulation. A similar mixed effect was observed by Zimmerman & Gisslen (1968). The increase in response to exogenous noradrenaline in the perfused rat system is not due to an increase in perfusion pressure, because that increase (approximate 5 mmHg) was much too small. An alternative explanation of our observation would be that AII is exerting a cocaine-like effect (i.e. inhibition of reuptake of transmitter by presynaptic nerve endings). This would seem unlikely since AII has been shown not to exert such an effect in several other vascular systems (Zimmerman & Gisslen, 1968; Kadowitz *et al.*, 1971; Kadowitz *et al.*, 1972; Turker, 1973). A mixed type of enhancement was also reported in the rat perfused caudal artery which persisted in the presence of maximally effective concentrations of cocaine (Nicholas, 1970).

Our observation that an inhibitor of angiotensin converting enzyme (BPF) lowered the blood pressure in pithed rats, indicates that a portion of vascular tone in these animals is being maintained by the renin-angiotensin system. This was further shown by the lower and more stable basal pressures observed if the animal's kidneys were ligated prior to pithing. In view of the large fall in blood pressure observed after BPF, we suggest that the pithed rat may be a convenient system to test the effect of angiotensin antagonists and converting enzyme inhibitors *in vivo*.

The parallel increase in responses to exogenous noradrenaline and nerve stimulation in the pithed rat is in conflict with the results of Day & Owen (1969). The hyper-responsiveness to nerve stimulation and noradrenaline appears to be 'apparent' rather than 'real' (Redleaf & Tobian, 1958) and resulted from the decrease in cross-sectional area of resistance arterioles induced by infusion of the vasoconstrictor agent, which produces greater absolute changes in pressure for the same degree of shortening of individual muscle fibres (Redleaf & Tobian, 1958). To test whether the absolute increase in response was due to a completely postsynaptic effect, a pressor agent was administered which did not have any effect on the vas deferens. Vasopressin, infused at levels adjusted to produce comparable pressure increases to AII (which acts entirely at presynaptic sites in the vas deferens), yielded essentially the same results. These 'apparent' increases in responsiveness are operative through a wide range of pressures in the pithed rat.

The pattern that emerges is that the potentiated responses to sympathetic nerve stimulation during AII infusion can result from: (a) a presynaptic effect due to an increase in neurotransmitter release; (b) a postsynaptic effect due to an increase in apparent responsiveness of smooth muscle because of an increase in pre-stimulus muscular tone; or (c) an enhanced response due to alteration of the drug-effector (postsynaptic) site interaction (independent of nerve stimulation or of the smooth muscle tone). The relative importance of these effects vary from a completely presynaptic effect as observed in the vas deferens to what appears to be a completely postsynaptic effect when the parameter is blood pressure changes in the pithed rat. The vasculature of many isolated organ systems would appear to demonstrate a combination of these effects.

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Please send reprint requests to Dr P. Needleman.

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