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# Potentiation of purinergic transmission by angiotensin in prostatic rat vas deferens

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**1** Angiotensin II (AII) elicited only a minute, if any, direct contractile response in smooth muscle cells of prostatic rat vas deferens, but it potentiated contractile responses to field stimulation.

**2** Angiotensin-potentiated contractile response to field stimulation was concentration-dependent, and the order of potency was AII > AIII ≈ AI. The $EC_{50}$ of AII was 8.11 ± 2.79 nM.

**3** AII did not modify the contractile response of exogenous noradrenaline (NA) on non-stimulated prostatic vas deferens. Furthermore, the concentration-response curve for AII-potentiated contractile responses to field stimulation in reserpine-treated rats did not significantly differ from the control group.

**4** Desensitization of purinoceptors with 30 μM α,β-methylene-ATP almost completely abolished the potentiation of the contractile response to field stimulation by AII.

**5** The response to AII in the prostatic rat vas deferens was blocked by the $AT_1$ selective antagonist losartan, but not by the $AT_2$ selective antagonist CGP 42112. Losartan acted as a competitive antagonist with a $pA_2$ value of 8.75.

**6** In conclusion, AII potentiated purinergic transmission in the prostatic rat vas deferens via the $AT_1$ receptor.

**Keywords:** Angiotensin II; losartan; CGP 42112; purinergic transmission; $AT_1$ receptor; rat vas deferens

## Introduction

Angiotensin is a hormone which is also a potent vasoconstrictor. Substantial evidence suggests that angiotensin acts as a neurotransmitter in the central nervous system regulating various physiological functions, such as drinking behaviour and neurogenic pressor response (Regoli *et al.*, 1974; Peach, 1977; Phillips, 1987). Recently, a transgenic mice model indicated that the renin-angiotensin system may play an important role in male reproduction (Krege *et al.*, 1995).

Similar to arterial vessels, the rat vas deferens is innervated by sympathetic nerves, through the hypogastric plexuses (Snell, 1992). Anatomically, the rat vas deferens can be divided into prostatic and epididymal halves, which are known to respond differently to a variety of pharmacological agents (Sneddon & Machaly, 1992; Donoso & Huidobro-Toro, 1989). The prostatic half of the rat vas deferens responds to electric field stimulation with a fast, predominantly purinergic, component followed by a slow and minor noradrenergic component (Brown *et al.*, 1983). It has been well documented that angiotensin facilitates noradrenergic neurotransmission (Zimmerman, 1981; Story & Ziogas, 1987). Several lines of evidence also suggest an interaction between angiotensin and purinergic neurotransmission (Ellis & Burnstock, 1989; Weihprecht *et al.*, 1994). The present study has been undertaken to examine how angiotensin interacts with the noradrenergic and purinergic components of transmission in the regulation of smooth muscle contraction in prostatic rat vas deferens. It was found that angiotensin exerted only a minute, if any, direct contractile effect in the prostatic smooth muscle cells. Furthermore, angiotensin II selectively potentiated purinergic transmission while having no significant effect on noradrenergic transmission in the prostatic rat vas deferens.

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## Methods

### *Tissue preparation for bioassay*

Adult Sprague-Dawley rats (200-250 g) were killed by cervical dislocation and the vasa deferentia were isolated. They were freed of surrounding fat and connective tissue. The lumen was washed with Krebs buffer. Segments of 0.95-1.05 cm were then cut from the prostatic end. The tissue segments were suspended in 7 ml organ baths containing oxygenated (95% $O_2$, 5% $CO_2$) Krebs buffer (pH 7.5). The composition of the Krebs buffer was (mM): NaCl 126.97, KCl 2.55, $CaCl_2$ 1.33, $KH_2PO_4$ 1.18, $MgSO_4$ 0.39, $NaHCO_3$ 25.00 D-glucose, 1.00. The bath was maintained at 37°C.

### *Field stimulation of the isolated prostatic vas deferens*

The lower end of the tissue was fixed to a hook while the upper end was connected to an isometric force transducer (Grass UC3). The tissues were allowed to equilibrate under 1 g tension for 30 min. The tissue was stimulated transmurally with two platinum ring electrodes at a supramaximal voltage (0.1 Hz, 1 ms) by means of an electrical stimulator (Grass S88 stimulator). The contractions were recorded isometrically on a Beckman R511A polygraph.

### *Concentration-response curves to angiotensin*

Concentration-response curves for angiotensin (1 nM-10 μM) were constructed by the methods of either single measurement or cumulative measurement. For single measurement, the tissue was electrically stimulated until contraction became reproducible and a concentration of angiotensin was then administered. The peptide was left in contact with the tissue until a peak increase in contraction was attained, or for at the most two minutes if there was no change in contraction magnitude after administration. Field stimulation was then stopped, the tissue was washed twice with Krebs buffer and six minutes left before another round of drug application. For cumulative measurement, the tissue was stimulated to obtain a

reproducible contraction. Angiotensin was administered as a stepwise increase of drug concentration as soon as a stable response was obtained. Stock solutions (5 mM) of various peptides were made in distilled water and aliquots were kept at −20°C before use. Dilutions were made up fresh in Krebs buffer for each assay.

### *Effects of angiotensin in the presence of protease inhibitors*

Once field-stimulation contraction became reproducible, angiotensin was administered as described above except that a mixture of protease inhibitors (chymostatin 5 $\mu g\ ml^{-1}$; bacitracin 50 $\mu M$; leupeptin 10 $\mu M$; pepstatin 10 $\mu M$) or SQ 20881 (500 nM) were added one minute before angiotensin. Stock solutions of chymostatin (5 $mg\ ml^{-1}$), bacitracin (50 mM) and leupeptin (10 mM) were dissolved in distilled water; while pepstatin (10 mM) was dissolved in dimethyl sulphoxide. The stock solutions were stored at 4°C.

### *Effect of losartan and CGP 42112*

Antagonistic effects of losartan and CGP 42112 were studied by pre-incubation of the resting tissue with the drugs for 5 min before the onset of field stimulation. The affinity of the antagonist was evaluated by a method of Schild (1957) and angiotensin II (1 nM − 10 $\mu M$) was administered as a single dose. Effect of losartan or CGP 42112 on field-stimulation concentration was determined by direct application of the drug to the organ bath during electrical stimulation without prior preincubation. Appropriately diluted solutions of losartan and CGP 42112 were made in distilled water and kept at 4°C and −20°C, respectively. They were used within one month of preparation.

### *Interaction of angiotensin II with exogenous noradrenaline*

Angiotensin II was co-administered with noradrenaline to the resting prostatic rat vas deferens. For measuring noradrenaline-induced contraction, either the peak contraction or the maximal contraction obtained within 5 minutes after addition of noradrenaline was adopted.

### *Concentration-response curve to angiotensin II after reserpine treatment*

Reserpine (0.5 $mg\ ml^{-1}$) was prepared as a fine suspension in distilled water containing 0.4% ascorbic acid with continuous shaking overnight. Reserpine, 1 $mg\ kg^{-1}$, was given to rats intraperitoneally as described by Gillespie and McGrath (1974). Rats were killed 24 h after reserpine treatment. Prostatic rat vas deferens was isolated as described above and concentration-response curves to angiotensin II determined by the method of single measurement.

### *Concentration-response curve to angiotensin II after desensitization of $P_2$-purinoceptors*

$P_2$-purinoceptors were desensitized by exposing the resting muscle to 30 $\mu M$ $\alpha,\beta$-methylene ATP for 2 min as described by Lee and Cheung (1989). Concentration-response curves to angiotensin II in the prostatic rat vas deferens were then performed by the method of single measurement. $\alpha,\beta$-Methylene ATP remained in contact with the tissue during the construction of the concentration-response curve.

### *Drugs and sources*

Angiotensins, p-aminophenylalanine[6] angiotensin II and SQ 20881 (nicotinic acid-Tyr-($N^{\alpha}$-benzyloxycarbonyl-Arg)Lys-His-Pro-Ile) were purchased from Peninsula Inc. Losartan and CGP 42112 (pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) were gifts from Du Pont Merck and Ciba-Geigy respectively. Noradrenaline, $\alpha,\beta$-methylene-ATP, reserpine, chymostatin, bacitracin, leupeptin and pepstatin were purchased from Sigma Chemicals.

### *Statistical analysis*

Statistical analyses were done by either multiple variance analysis or Student's $t$ test. A probability of $P<0.05$ was considered significant. Values are presented as means ± s.e.mean of $n$ separate experiments.

## Results

### *Concentration-response curves to angiotensins in prostatic rat vas deferens*

Angiotensin II markedly augmented the electric field-stimulation contraction (Figure 1b). However, in the resting muscle, angiotensin II elicited only minute, if any, contraction (Figure 1a).

Potentiation of field-stimulation contraction by angiotensins was concentration-dependent (Figure 1c). The concentration-response curves of angiotensins displayed similar Hill slopes but differed in their potencies (Table 1). The order of potency was angiotensin II > angiotensin III ≈ angiotensin I (Table 1). The maximal response of the tissue to angiotensin I

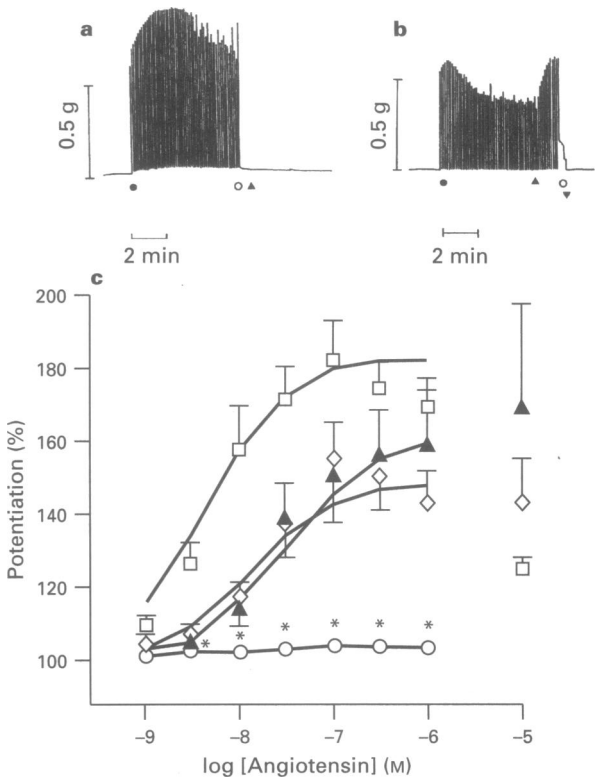


**Figure 1** Effects of angiotensin II (AII) on (a) unstimulated and (b) electrically stimulated prostatic rat vas deferens. (●) Train of electrical pulses applied; (○) stop of electrical stimulation; (▲) addition of 1 $\mu M$ (a) or 10 nM (b) AII; (▼) change of organ bath buffer. (c) Concentration-response curves for angiotensins and an analogue for the potentiation of field-stimulation contractions of the prostatic rat vas deferens. (◇) AI; (□) AII; (▲) AIII; (○) p-aminophenylalanine[6] angiotensin II. The curves were constructed by the single measurement as described in the Methods. Multiple variance analysis with a model of unbalanced repeated measures was done by using BMDP/Dynamic Program 5V. Significant difference ($P<0.004$) was found between groups for drug × concentration linear interaction. Kruskal-Wallis test with multiple comparison was used to compare groups for each concentrations, and * indicates $P<0.05$ when compared to AII.

was smaller than that to angiotensin II (Figure 1c). Tachyphylaxis was observed for angiotensin II with concentrations above 1 mM. Furthermore, p-aminophenylalanine[6] angiotensin II, an $AT_2$ specific agonist (Speth & Kwan, 1990), produced no activity in the prostatic half (Figure 1c).

The concentration-responses to angiotensin II determined by single measurement and cumulative measurement were different. With cumulative measurement, the maximal response to angiotensin II was lower (Figure 2), and angiotensin II was less potent ($EC_{50}$: $8.11 \pm 2.79$ nM; $n=7$ vs. $18.43 \pm 2.73$ nM; $n=7$, $P<0.05$, in single and cumulative measurements, respectively). Moveover the concentration-response curves of cumulative measurement displayed a steeper Hill slope than that of single measurement ($1.46 \pm 0.13$; $n=7$, vs $1.11 \pm 0.08$; $n=7$, $P<0.03$, respectively).

### *Effect of angiotensin II in the presence of protease inhibitors*

In the presence of SQ 20881 (500 nM), a potent inhibitor of angiotensin converting enzyme (Ondetti *et al.*, 1977), potentiation of field-stimulated contraction by angiotensin I (10 nM) was reduced significantly ($118 \pm 4\%$; $n=7$ vs. $102 \pm 1\%$; $n=5$, $P<0.05$). In contrast, the response to angiotensin II (3 nM) was not different in the presence or absence of SQ 20881 ($113 \pm 2\%$; $n=4$ vs. $114 \pm 1\%$; $n=4$).

Angiotensin II (AII) can be proteolytically broken down thus reducing its apparent potency. However, in the presence of a mixture of protease inhibitors (chymostatin, 5 $\mu g\ ml^{-1}$; bacitracin, 50 $\mu$M; leupeptin 10 $\mu$M; pepstatin 10 $\mu$M), there was no significant difference in the maximal response to angiotensin II ($182 \pm 11\%$; $n=7$, vs $172 \pm 10\%$; $n=4$ at 100 nM of AII in the absence and presence of inhibitors, respectively), nor in the potency ($EC_{50}$: $8.11 \pm 2.79$ nM; $n=7$, vs $9.19 \pm 3.18$ nM; $n=4$).

### *Interaction of angiotensin II with exogenous noradrenaline*

It has been suggested that angiotensin II enhances the postsynaptic response of noradrenaline (Ellis & Burnstock, 1989). To evaluate whether angiotensin II potentiated the contractile response to field stimulation by enhancing postjunctional responses, the effect of angiotensin II on contraction evoked by exogenous noradrenaline was examined. Noradrenaline (30 $\mu$M) induced a slow contractile response in the prostatic rat vas deferens (Figure 3). Neither the time course nor the magnitude of the noradrenaline-induced contractile response was altered by angiotensin II (100 nM) ($7.23 \pm 1.49$ g tension $g^{-1}$ tissue; $n=5$, vs $7.70 \pm 1.24$ g tension $g^{-1}$ tissue; $n=5$ in the absence and presence of AII, respectively).

### *Concentration-response curves to angiotensin II after reserpine treatment*

The contractile response of rat vas deferens to field stimulation consists of two components, a fast non-adrenergic twitch response followed by a slow sustained noradrenergic response (McGrath, 1978; Brown *et al.*, 1983). To inhibit the noradrenergic component, rats were treated with reserpine at a dose of 1 mg $kg^{-1}$ body weight. The success of reserpinization was shown by the significant reduction in neurogenic contraction ($12.12 \pm 2.14$ g tension $g^{-1}$ tissue; $n=17$ vs $2.09 \pm 0.87$ g tension $g^{-1}$ tissue; $n=9$, $P<0.01$). The concentration-dependent potentiation to field stimulation by angiotensin II was not significantly different from the control group (Figure 4a). Moreover, the potency ($EC_{50}$) of angiotensin II as a potentiator was not different significantly from the control group

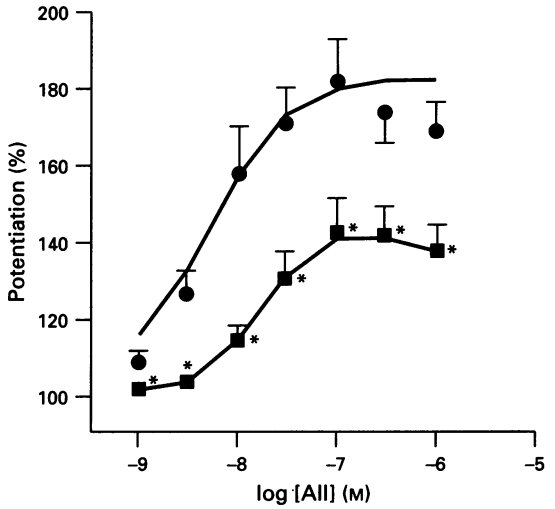


**Figure 2** Concentration-response curves for angiotensin II (AII)-potentiation of field-stimulated contraction in prostatic rat vas deferens. The curves were constructed by the single measurement (●) or cumulative measurement (■) as described in Methods. Multiple variance analysis with a model of unbalanced repeated measures was done by using BMDP/Dynamic Program 5V. Significant difference ($P<0.001$) was found between groups for drug × concentration linear interaction. Mann-Whitney test was used to compare between groups for each concentration, and * indicates $P<0.05$.

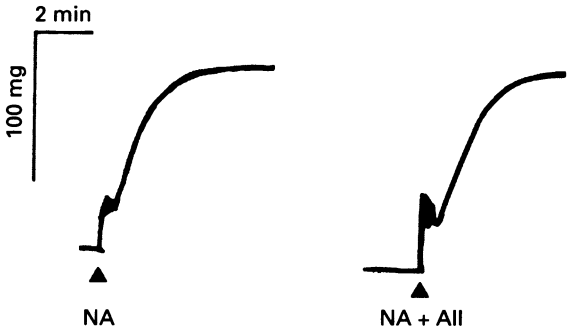


**Figure 3** Typical contractile responses to exogenous noradrenaline (NA; 30 $\mu$M) and angiotensin II (AII; 100 nM). NA was applied repeatedly to obtain reproducible contractile responses before administration of AII. (▲) NA or AII addition.

**Table 1** Potencies of angiotensins on the prostatic half of rat vas deferens

| *Peptides* | *Hill slopes* | *$EC_{50}$* (nM) | *RA* (%) | n |
|---|---|---|---|---|
| AI | $1.23 \pm 0.15$ | $21.68 \pm 6.43$ | 37.41 | 7 |
| AII | $1.11 \pm 0.08$ | $8.11 \pm 2.79$ | 100 | 7 |
| AIII | $0.93 \pm 0.15$ | $33.93 \pm 10.29$ | 23.90 | 7 |

$EC_{50}$ is the concentration of peptide required to induce 50% of the maximal potentiation of electrical field-stimulated contraction of the prostatic rat vas deferens. The $EC_{50}$ values were determined by a graphical method from individual curves. Values are means ± s.e.mean of $n$ separate experiments. RA: relative affinity expressed as the percentage of that of AII. *$P<0.05$ indicates significant difference from the $EC_{50}$ of AII by two-tail, Student's $t$ test.

(8.56±2.13 nM; $n=9$ vs 18.55±6.56 nM; $n=9$). However, it is noteworthy that the potentiation response to angiotensin II and the $EC_{50}$ values varied over a wide range in the reserpine-treated group (Figure 4b). The significance of this observation is unclear.

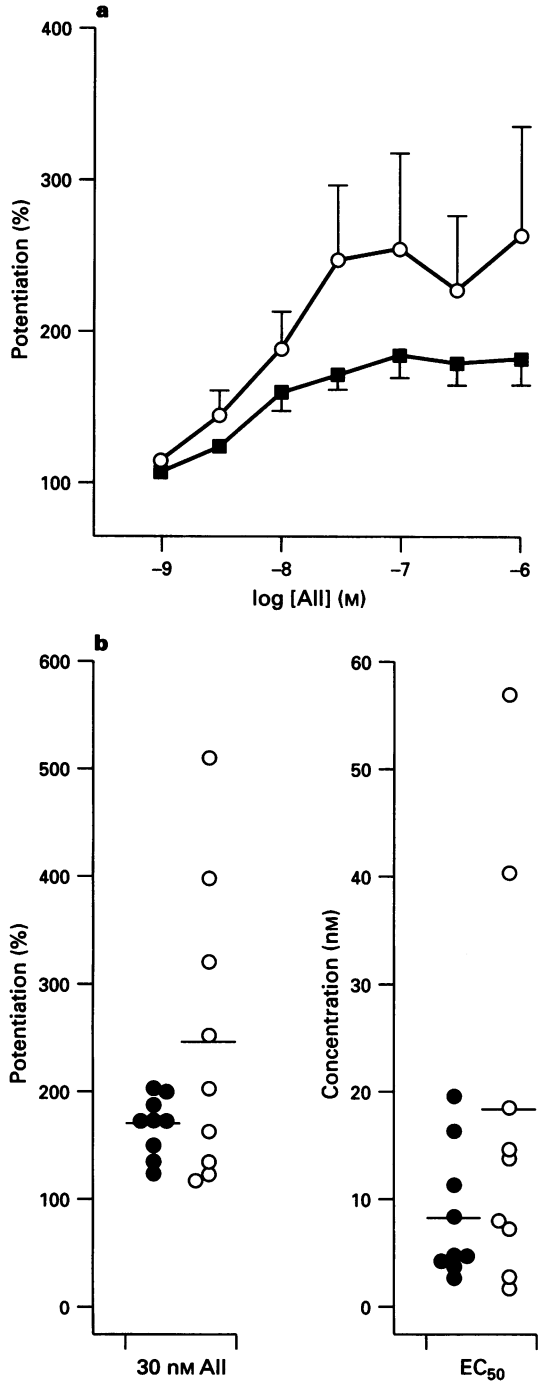


**Figure 4** (a) Concentration-response curves for angiotensin II (AII)-mediated responses of electrically field-stimulated prostatic rat vas deferens with (○) or without reserpine treatment (■). Multiple variance analysis with a model of unbalanced repeated measures was done by using BMDP/Dynamic Program 5V. Insignificant difference was found between the two groups for drug × concentration linear interaction. Mann-Whitney test was used to compare each concentration. Values are means±s.e.mean of 9 separate experiments. (b) Scatter plots of AII (30 nM)-potentiation of field-stimulated contraction and the $EC_{50}$ values of AII concentration-dependent curves in control (●) and reserpine treated (○) rats. Each point in the chart represents a data point from a separate experiment. Mean values are shown by the horizontal bars.

### *Concentration-response curves to angiotensin II after desensitization of $P_2$-purinoceptors*

The electric field-stimulation basal contraction was diminished significantly after desensitization of $P_2$-purinoceptors (12.12±2.14 g tension $g^{-1}$ tissue; $n=17$, vs 3.93±1.30 g tension $g^{-1}$ tissue; $n=8$, $P<0.02$). In addition, potentiation of contraction to field stimulation of angiotensin II (1 nM−1 μM) was almost completely suppressed (Figure 5).

### *Effect of losartan and CGP 42112*

To further characterize which receptor type mediates the angiotensin II response, the effect of selective angiotensin receptor antagonists was tested. CGP 42112 is a peptide antagonist selective for the $AT_2$ receptor and losartan is a non-peptide antagonist selective for the $AT_1$ receptor (Timmermans *et al.*, 1993). CGP 42112 (15 nM) did not potentiate electrical field-stimulated basal contraction when applied alone (111±5% of control; $n=4$); nor did it inhibit angiotensin II (100 nM)-potentiated contraction to field-stimulation (Figure 6). Losartan (100 nM) also did not potentiate electrical field-stimulated basal contraction when applied alone (103±2% of control; $n=4$). In contrast, the potentiation effect of angiotensin II (100 nM) was significantly inhibited by losartan (100 nM) (Figure 6).

Onset of the antagonistic action of losartan was rapid and reached a maximal response with 5 min of pre-incubation (Table 2). Moreover, losartan (3−100 nM) induced rightward shifts of the angiotensin II response curve in a concentration-dependent manner (Figure 7a). It exhibited a $pA_2$ of 8.75 from the Schild plot with a slope of −0.79 (Figure 7b).

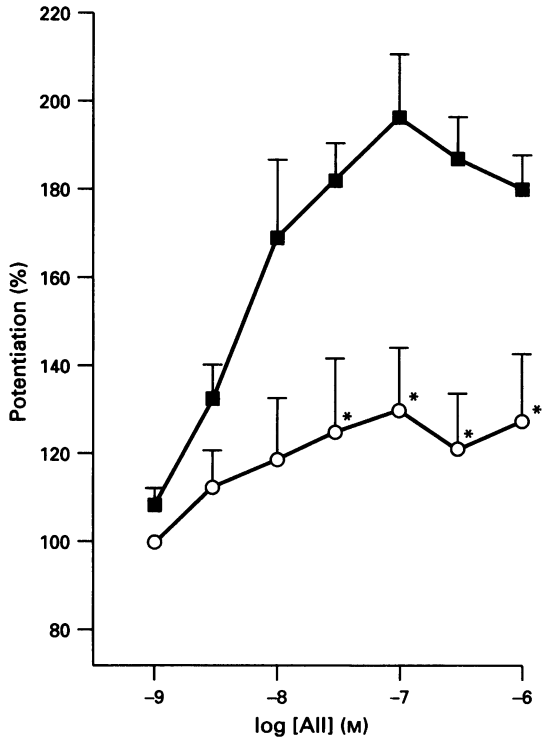


**Figure 5** Concentration-response curves of angiotensin II (AII)-potentiated contraction to field stimulation of the prostatic rat vas deferens. (■) Control; (○) pretreatment with 30 μM α,β-methylene ATP to desensitize $P_2$-purinoceptors before application of angiotensin II. Concentration-response curves were constructed by the single measurement. Multiple variance analysis with a model of unbalanced repeated measures was done by using BMDP/Dynamic Program 5V. Significant difference ($P<0.0001$) was found between the two groups for drug × concentration linear interaction. Mann-Whitney test was used to compare each concentration, and * indicates $P<0.05$ when compared with control. Values are means±s.e.mean of 4 separate experiments.

## Discussion

The observation that angiotensin II was more potent than angiotensin III suggests the presence of an $AT_1$ receptor mediating the potentiation of electrical field-stimulation contraction by angiotensin. This is further supported by the lack of effect of the angiotensin analogue, p-aminophenylalanine[6] angiotensin II, which is proposed to be an $AT_2$-selective agonist (Speth & Kwan, 1990). In addition, potentiation of contraction to field stimulation by angiotensin II was inhibited by losartan but not by CGP 42112. Losartan acted as a competitive antagonist shifting the angiotensin II dose-response curves to the right in a concentration-dependent manner. Schild analysis indicated a $pA_2$ value of 8.75. This value agrees with previously obtained $pA_2$ values which range from 8.48 to 9.03 (Hedge & Clarke, 1993; Tanabe *et al.*, 1993; Zhang *et al.*, 1993).

The response to angiotensin II was biphasic with maximal response at 100 nM and gradual decline in response to higher concentrations of angiotensin II. The potency and capacity of AII in potentiating the contraction to field stimulation were smaller when measured by cumulative than that of by single measurement. These results suggest that the $AT_1$ receptors are rapidly desensitized at least in prostatic rat vas deferens. Indeed, tachyphylaxis to angiotensin II has been documented in various isolated smooth muscle preparations (Robertson *et al.*, 1994). Furthermore, down regulation of angiotensin receptors has been described in hepatocytes (Bouscarel *et al.*, 1988) and N1E-115 cells (Reagan *et al.*, 1993). However, the possibility that tachyphylaxis in the rat vas deferens may be induced by neurotransmitters released during field stimulation cannot be excluded.

In contrast to the prostatic half, AII exerts a direct contractile response mediated by $AT_1$ receptors in resting muscle in epididymal rat vas deferens (Sum & Cheung, 1995). Comparing the concentration-dependent responses to angiotensin in epididymal and prostatic rat vas deferens, there are four fold and thirty-two fold differences in the potencies of AII and

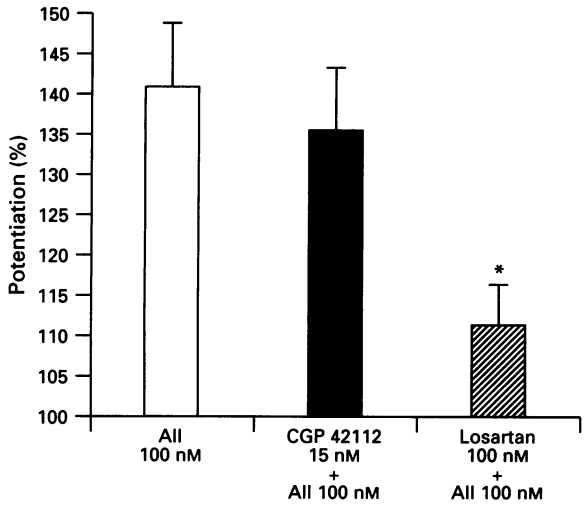


**Figure 6** Effects of CGP 42112 and losartan on angiotensin II (AII)-potentiation of the electrical field-stimulation contraction of the prostatic half of rat vas deferens. The antagonists were added to the organ bath 5 min before the onset of electrical stimulation. Values are means ± s.e.mean of 4–8 separate experiments. *$P<0.005$ indicates significant difference from the response to 100 nM AII by Student's $t$ test.

**Table 2** Time-dependence of the inhibition by losartan of angiotensin II (AII)-mediated potentiation of the contractile response

| *Pre-incubation time* | *Potentiation* (%) | n |
|---|---|---|
| Absence of losartan | 199.88 ± 17.32* | 4 |
| 5 min | 127.95 ± 11.53 | 4 |
| 10 min | 136.48 ± 10.73 | 4 |
| 20 min | 139.35 ± 17.13 | 4 |

Potentiation of electrical field-stimulated responses to 100 nM AII was determined by single measurement as described in the Methods, except the tissue was at rest for a 20 min interval before another round of drug application. The tissue was primed with 100 nM AII until a reproducible potentiation was obtained. Losartan (100 nM) was added before the onset of electrical field stimulation at the time indicated in the Table. *$P<0.05$ indicates significant difference from the 5-min losartan pretreatment by two tail Student's $t$ test.

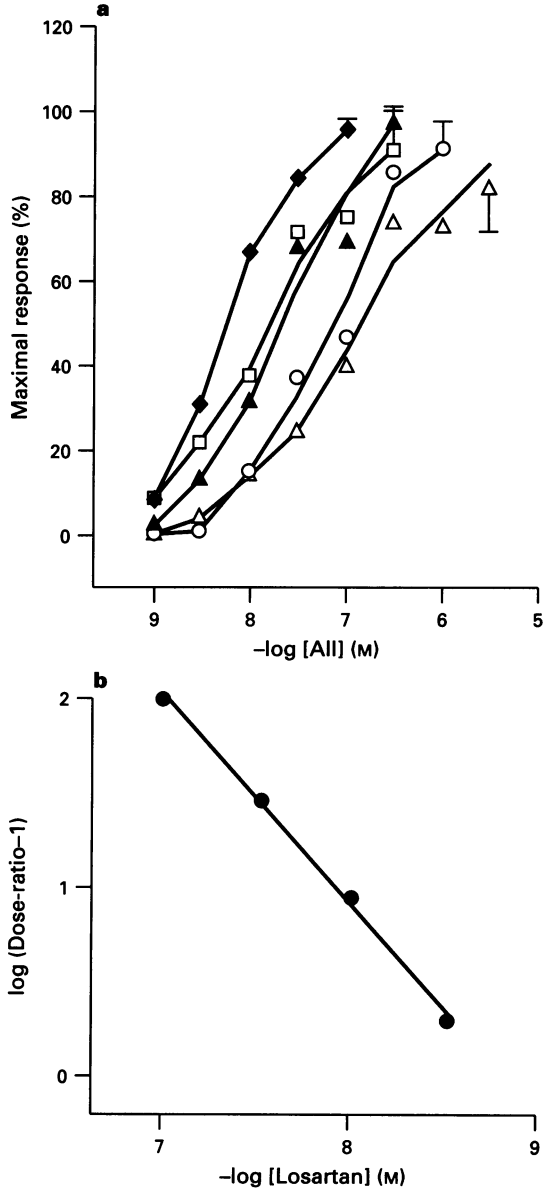


**Figure 7** (a) Concentration-dependent angiotensin II (AII)-potentiation of electrically field-stimulated contraction in the absence (◆) and presence of 3 nM (□), 10 nM (▲), 30 nM (○) and 100 nM (△) losartan. The concentration-response curves were constructed by single measurement as described in the Methods. Values are means ± s.e.mean of 4–7 separate experiments. For clarity only the error bar of maximal dose is shown. (b) Schild plot for the analysis of the antagonistic effect of losartan. Data were derived from (a) and fitted by using a linear regression programme, giving values of slope = −0.79; $pA_2 = 8.75$ and $r = 0.98$.

AIII, respectively (AII: 34.49±4.46 nM vs 8.11±2.79 nM; AIII: 1118.98±93.77 nM vs 33.93±10.29 nM; $EC_{50}$ in epididymal vs prostatic halves). Subtypes of $AT_1$ receptors have been found by cloning (Sasamura *et al.*, 1992; Kakar *et al.*, 1992). Angiotensin receptors coupling to distinct signal transduction mechanisms in neurones and astrocytes have been described (Sumners *et al.*, 1991). Hence, whether such a difference is due to multiple subtypes of $AT_1$ receptors or coupling to different signalling pathways requires further investigations.

The response to angiotensin I but not angiotensin II was reduced by SQ 20881, an angiotensin converting enzyme inhibitor (Ondetti *et al.*, 1977). This suggests that the effect of angiotensin I may be partly due to its conversion to angiotensin II. The characteristics and the distributions of the ACE-like activity in rat vas deferens are yet to be established. The smaller response to angiotensin I may be due to rate-limiting conversion of angiotensin I to angiotensin II.

As described by McGrath (1978), the contractile response of rat vas deferens to electrical field-stimulation consists of noradrenergic and non-adrenergic components. In the prostatic half, there is a predominant and fast non-adrenergic component followed by a slow and minor noradrenergic component (Sneddon *et al.*, 1984; Rohde *et al.*, 1986). Desensitization of $P_2$-purinoceptors by $\alpha,\beta$-methylene ATP significantly diminished the electrical field-stimulated response. This indicates that the $P_2$-purinoceptors mediate partly the non-adrenergic component of the electrical field-stimulated response. Angiotensin II was found to potentiate the $P_2$-purinoceptors-mediated response as shown by the nearly complete abolition of the angiotensin II response upon desensitization of $P_2$-purinoceptors. Whether angiotensin acts presynaptically to enhance the neurotransmitter release or postsynaptically to augment the effect of neurotransmitter is yet to be established.

In the reserpine-treated rat, the $EC_{50}$ of angiotensin II as well as the overall potentiation were not statistically different from the control group. Contraction induced by exogenous noradrenaline was not enhanced by angiotensin either. These results suggest that angiotensin II may not potentiate the postsynaptic action of noradrenaline in prostatic rat vas deferens. However, the possibility that angiotensin II may interact with the noradrenergic system presynaptically cannot be excluded.

In contrast to previous findings (Brown *et al.*, 1983) that the magnitude of electrical field-stimulated basal contraction of rat vas deferens was not altered by reserpine treatment, we found that the response diminished significantly. It has been shown that noradrenaline interacts synergistically with ATP to potentiate each other's response (Huidobro-Toro & Parada, 1988), and therefore the removal of either of these two components should result in the reduction of the electrical field-stimulated response. This is in agreement with our results that both desensitization of $P_2$-purinoceptors and reserpine treatment lead to a diminished response.

In the rabbit vas deferens, angiotensin inhibits the non-adrenergic twitch response but enhanced the noradrenergic sustained phasic response with an effect of angiotensin III greater than angiotensin II (Trachte, 1988). In guinea-pig vas deferens, angiotensin II was found to enhance the overflow of ATP stimulated at low frequency (2 Hz), but did not alter the ATP overflow when stimulated at high frequency (20 Hz). In contrast, angiotensin II enhanced the overflow of noradrenaline when stimulated at both high and low frequencies (Ellis & Burnstock, 1989). The differences in the effects of angiotensin in vas deferens of rat, rabbit and guinea-pig suggest species-specific modulation by angiotensin. However, the underlying mechanism that angiotensin II attains its species-specific effect is still an open question.

In conclusion, angiotensin II potentiates electrical field-stimulated contraction in prostatic rat vas deferens via $AT_1$ receptors. The purinergic response is potentiated by angiotensin II whereas the postsynaptic action of noradrenaline is not enhanced by angiotensin II.

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