

Angiotensin II enhances noradrenaline release from sympathetic nerves of the rat prostate via a novel angiotensin receptor: implications for the pathophysiology of benign prostatic hyperplasia

M E Fabiani¹, M Sourial¹, W G Thomas³, C I Johnston^{1,3}
and A G Frauman²

¹Department of Medicine, University of Melbourne, Austin & Repatriation Medical Centre, Heidelberg VIC 3084, Australia

²Clinical Pharmacology & Therapeutics Unit, Department of Medicine, University of Melbourne, Austin & Repatriation Medical Centre, Heidelberg VIC 3084, Australia

³Baker Medical Research Institute, St Kilda Road Central, Melbourne VIC 8008, Australia

(Requests for offprints should be addressed to M E Fabiani, Department of Medicine, University of Melbourne, Austin & Repatriation Medical Centre, Heidelberg VIC 3084, Australia; Email: m.fabiani@austin.unimelb.edu.au)

Abstract

The renin–angiotensin system (RAS) is present in the human prostate and may be activated in benign prostatic hyperplasia (BPH), possibly contributing to the pathophysiology of this disorder by enhancing local sympathetic tone and cell growth. The functional role of the RAS in the prostate, however, is unknown. The present study was undertaken to determine whether angiotensin (Ang) II enhances sympathetic transmission in the prostate. The neuronal stores of the rat prostate were labelled with [³H]noradrenaline (NA). Ang II and Ang I enhanced [³H]NA release in a concentration-dependent manner. The Ang II receptor subtype 1 (AT₁ receptor) antagonist losartan and the AT₂ receptor antagonist PD123319 inhibited this facilitatory effect of Ang II and Ang I, whereas the other AT₂ receptor antagonist, CGP42112, was without effect. Bradykinin also increased [³H]NA release, which was inhibited by the B₂ receptor antagonist

Hoe140. The angiotensin–converting enzyme inhibitor captopril inhibited the effect of Ang I, but potentiated that of bradykinin. Interestingly, captopril alone produced an increase in [³H]NA release which was inhibited by Hoe140. Losartan, but not PD123319 or CGP42112, inhibited [¹²⁵I]–Ang II binding in Chinese hamster ovary cells transfected with the AT_{1a} or AT_{1b} receptor. In contrast, in cells expressing the AT₂ receptor, PD123319 and CGP42112, but not losartan, inhibited [¹²⁵I]–Ang II binding. In conclusion, Ang II enhances the release of NA from sympathetic nerves of the rat prostate via a novel functional receptor distinct from the cloned AT_{1a}, AT_{1b} or AT₂. These data provide direct evidence in support of a functional role for the local RAS in modulating sympathetic transmission in the prostate, which may have important implications for the pathophysiology of BPH.

Journal of Endocrinology (2001) **171**, 97–108

Introduction

Benign prostatic hyperplasia/hypertrophy (BPH) is a highly prevalent disorder that represents the most common cause of urinary obstruction in the ageing male population and frequently co-exists with hypertension (Garraway *et al.* 1991, Maruenda *et al.* 1999). BPH is characterised by increased cellular proliferation of stromal elements and enhanced sympathetic smooth muscle tone (Isaacs & Coffey 1989, McNeal 1990, Madsen & Bruskewitz 1995). In patients with BPH, approximately 50% of total urethral pressure is due to increased sympathetic smooth muscle tone, which can be ameliorated by α_1 -adrenoceptor blockers such as prazosin, terazosin or doxazosin (Furuya *et al.* 1982, Kirby 1989, Madsen & Bruskewitz 1995). The prostate is innervated by sympathetic nerves which, upon stimulation, release the chemical transmitter noradrenaline

(NA) and evoke smooth muscle contraction, mediated by α_1 -adrenoceptors of the α_{1A} (α_{1c}) subtype (Forray *et al.* 1994, Marshall *et al.* 1995). Moreover, there is some evidence to suggest that local sympathetic activity may also influence prostate growth (McVary *et al.* 1994). For example, it has been reported that unilateral sympathectomy of the lower urinary tract reduces ventral prostate weight in rats (McVary *et al.* 1994). Thus enhanced sympathetic activity is a major factor in the development of BPH, influencing smooth muscle tone and, possibly, growth of the prostate.

The renin–angiotensin system (RAS) is a hormonal cascade that has an important role in regulating blood pressure and cardiovascular homeostasis (Peach 1977, Johnston 1990). Similarly, hyperactivity of the RAS has been implicated in the development of hypertension and other cardiovascular disorders (Nicholls *et al.* 1998).

Angiotensin II (Ang II) is the principal effector peptide of the RAS and is formed by the sequential cleavage of the precursor macromolecule, angiotensinogen, to the inactive decapeptide Ang I by renin, and then hydrolysed to the active octapeptide Ang II by angiotensin-converting enzyme (ACE) (Johnston 1990). It should be noted, however, that ACE is not a specific enzyme and can degrade bradykinin and a host of other peptides to inactive fragments (Fabiani *et al.* 2000). In addition to its formation within the circulation, Ang II can also be generated locally in many target tissues such as the kidney, heart, brain and blood vessels, and may therefore also mediate autocrine or paracrine effects (Campbell 1987, Dzau 1988, Johnston 1992).

The effects of Ang II are subserved by at least two distinct receptor subtypes, denoted AT₁ and AT₂ (Griendling *et al.* 1996, Unger *et al.* 1996, Fabiani 1999). In rodents, but not higher species or humans, two further isoforms of the AT₁ receptor have been identified and termed AT_{1a} and AT_{1b} (Iwai & Inagami 1992, Yoshida *et al.* 1992). The characterisation of Ang II receptors into two major classes was promulgated by the development of selective non-peptide antagonists such as losartan and PD123319 (Timmermans *et al.* 1993). Ang II receptors sensitive to losartan were designated AT₁, whereas those sensitive to PD123319 were designated AT₂ (Timmermans *et al.* 1993). Ang II exerts a variety of biological effects that serve to modulate cardiovascular function and structure, including vasoconstriction, stimulation of aldosterone release and promotion of cell growth/hypertrophy, all of which are mediated by the AT₁ receptor (Chung *et al.* 1998, Fabiani 1999). The functional role of the AT₂ receptor is less well understood, but may be involved in anti-proliferation, apoptosis, differentiation and, possibly, vasodilatation (Chung *et al.* 1998, Csikos *et al.* 1998, Fabiani 1999).

Of particular note, the RAS can interact with the sympathetic nervous system in a stimulatory manner at several different levels of the neuronal network (Saxena 1992). Ang II is well known to facilitate the release of NA from sympathetic nerve terminals in many tissues including the heart, kidney and blood vessels (Story & Ziogas 1987). Moreover, Ang II is able to amplify the post-junctional actions of NA and other excitatory mediators on cardiac and smooth muscle cells (Purdy & Weber 1988). This facilitatory effect on sympathetic transmission represents one of the most potent actions of Ang II, usually requiring concentrations that are much lower than those required to produce direct vasoconstriction or cardiac inotropic and chronotropic effects (Story & Ziogas 1987).

Despite the known role of sympathetic activity in BPH, as described above, the functional role of the RAS in the prostate is unknown. Furthermore, although it was reported several years ago that the biochemical activity of ACE is significantly greater in BPH than in normal prostate or a variety of other tissue homogenates

(Yokoyama *et al.* 1980, Van Sande *et al.* 1985), no further work has since been undertaken to investigate the physiological or pathophysiological role of the RAS in the prostate. Our group has shown more recently that both the protein and mRNA expression of ACE (Nassis *et al.* 2000, 2001), in addition to Ang II immunoreactivity (Dinh *et al.* 2001a), are increased in BPH. Furthermore, we have observed that AT₁ receptors predominate in the human prostate and are down-regulated in BPH (Dinh *et al.* 2001a,b). Taken together, these findings suggest that the local RAS is activated in BPH, which may have pathophysiological consequences.

Nothing is known about the functional interaction of the RAS with sympathetic nerves in the prostate. We hypothesise that a local tissue-based RAS is functionally active and facilitates sympathetic neuroeffector transmission in the prostate, such that hyperactivity of the local RAS contributes to the development of BPH by enhancing local sympathetic activity. In view of the possibility that the RAS may be activated in BPH, the present study was undertaken to determine whether Ang II, the main effector molecule of the RAS, facilitates sympathetic transmission in the prostate. Specifically, we aimed to examine the effects of exogenous and locally generated Ang II on NA release from sympathetic nerves of the rat prostate and endeavoured to characterise the Ang II receptor subtype(s) involved.

Materials and Methods

Rat prostate preparation

Male Sprague–Dawley rats (250–350 g) were killed by decapitation and then exsanguinated. An incision was made in the lower abdominal region and the prostate identified and dissected free. The isolated prostate was transferred to a Petri dish containing pre-warmed physiological salt solution (PSS) and continuously gassed with carbogen (95% O₂–5% CO₂). Excess fatty tissue was removed and the prostate cut into two even sections. Each piece of prostate tissue was tied at both ends with fine silk threads.

Radiolabelling of noradrenergic transmitter stores with [³H]NA

The procedure used to radiolabel the noradrenergic transmitter stores was adopted from Fabiani & Story (1994, 1996). Prostate tissues were placed in small glass-jacketed organ baths and equilibrated for 10 min in 2 ml PSS, maintained at 37 °C and continuously gassed with carbogen. After this initial equilibration period, 5 µl [³H]NA (30–50 Ci/mmol) was added to each organ bath and the preparations allowed to incubate for 30 min. After incubation, prostate tissues were removed from the organ baths and immersed in a small volume of PSS to rinse any loosely bound radioactivity. Prostate preparations were then

mounted vertically between two platinum electrodes in acrylic flow chambers and superfused with PSS at a rate of 2 ml/min using an ISCO Wiz peristaltic pump (ISCO Inc., Lincoln, NE, USA). In order to further remove loosely bound radioactivity, the tissues were washed for 90 min before experimental procedures were commenced. After the first 30 min of the washout period, the preparations were subjected to a 30 s period of electrical field stimulation with a train of 1 ms monophasic square-wave pulses at a frequency of 5 Hz and a supramaximal voltage of 20 V, delivered by a Grass S88 stimulator (Grass Medical Instruments, Quincy, MA, USA). This brief 'priming stimulus' was intended to assist in the removal of any non-specifically bound radioactive material.

Stimulation of intrinsic sympathetic nerves

After the washout period, the intrinsic sympathetic nerves of the prostate preparations were subjected to two 60 s periods of electrical field stimulation (1 ms pulses, 5 Hz, 20 V). The first period of stimulation was given immediately after the 90 min washout period and the second period of stimulation given 30 min later.

The effects of Ang II, Ang I and various drugs on the resting and stimulation-induced effluxes of radioactivity were examined by adding the drugs to the PSS superfusing the prostate preparations 15 min before the second period of stimulation. The drugs then remained present for the duration of the experiment.

Determination of resting and stimulation-induced effluxes of radioactivity

The superfusate from the prostate preparations was collected at 3 min intervals by an automated ISCO Retriever IV fraction collector (ISCO Inc.). Each 3 min (6 ml) fraction of superfusate was mixed with 4 ml of scintillant (Ultima Gold, Packard Bioscience BV, Groningen, The Netherlands) and the radioactivity present determined by liquid scintillation counting. External automatic standardisation was utilised to correct for counting efficiency and the data were expressed in disintegrations per minute (d.p.m.).

The resting efflux of radioactivity from the prostate preparations was determined for each of the two periods of stimulation (R_1 and R_2) from the amount of radioactivity present in the fraction of superfusate collected immediately before stimulation. The stimulation-induced efflux of radioactivity for each of the two periods of stimulation (S_1 and S_2) was determined by subtracting the resting efflux from the amount of radioactivity present in each of the fractions of superfusate collected from the commencement of stimulation, and summing the differences:

$$S_1 = (F_1 + F_2 + F_3 \dots) - nR_1$$

$$S_2 = (F_1' + F_2' + F_3' \dots) - nR_2$$

where F and F' represent the radioactive content in each fraction collected after the first and second period of stimulation respectively, R_1 and R_2 represent the resting efflux for the first and second period of stimulation respectively and n represents the number of fractions. In each experiment, the resting and stimulation-induced effluxes for the second period of stimulation were expressed as percentages of the corresponding values for the first period of stimulation (% R_2/R_1 and % S_2/S_1 respectively).

Receptor constructs, expression and binding assay

The cloning of the rat AT_{1a} receptor and its incorporation into the mammalian expression vector, pRc/CMV, have been described previously (Thekkumkara *et al.* 1995). The rat AT_{1b} receptor, sub-cloned into pRc/CMV, was kindly provided by Dr T J Thekkumkara (Department of Medicine, University of Colorado, Denver, CO, USA). The rat AT_2 cDNA was kindly provided by Dr T J Murphy (Department of Pharmacology, Emory University School of Medicine, Atlanta, GA, USA) and subsequently sub-cloned into pRc/CMV using unique 5' (HindIII) and 3' (XbaI) restriction sites.

Chinese hamster ovary (CHO-K1) cells were transfected in 12-well plates with 0.6 µg/well of either AT_{1a} , AT_{1b} or AT_2 receptor plasmid DNA using lipofectAMINE (4.8 µl/well), as previously described (Thomas *et al.* 1998). At 48 h post-transfection, competition radioreceptor-binding assays were performed using 0.05 nM [125 I]-Ang II as tracer and increasing concentrations of Ang II, losartan (an AT_1 -selective ligand), PD123319 (an AT_2 -selective ligand) or CGP42112 (an AT_2 -selective ligand). Non-linear regression of the data was achieved using GraphPad Prism (Graphpad Software Inc., San Diego, CA, USA) and K_D and B_{max} were determined by the method of Swillens (1992).

Drugs, solutions and radiochemicals

The following drugs were used: angiotensin II (AUSPEP Pty Ltd, Parkville, VIC, Australia; Sigma Chemical Co., St Louis, MO, USA); angiotensin I (AUSPEP Pty Ltd; Sigma Chemical Co.); ω -conotoxin GVIA (AUSPEP Pty Ltd); tetrodotoxin (Sigma Chemical Co.); losartan (Merck & Co. Inc., Rathway, NJ, USA), PD123319 (Research Biochemicals International, Natick, MA, USA), captopril (Sigma Chemical Co.), indomethacin (Sigma Chemical Co.), CGP42112 (Novartis Pharma AG, Basel, Switzerland), idazoxan (Research Biochemicals International), atropine (Sigma Chemical Co.), bradykinin (Sigma Chemical Co.) and Hoe140 (Hoechst AG, Frankfurt, Germany).

Stock solutions of indomethacin were made in 2 M Na_2CO_3 . All other stock solutions and intermediate dilutions of drugs were made in distilled water. Final concentrations of drugs were achieved by the appropriate

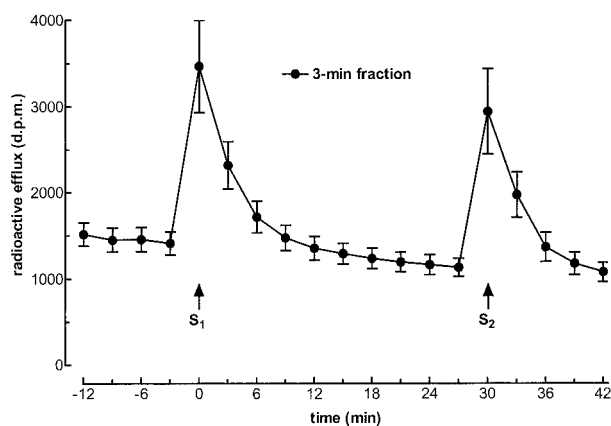


Figure 1 Effects of successive periods of electrical field stimulation on the radioactive efflux from isolated preparations of the rat prostate in which the noradrenergic transmitter stores had been loaded with [^3H]NA. The intrinsic sympathetic nerves of the prostate were subjected to two 60 s periods of electrical field stimulation (S_1 and S_2) at 5 Hz, given 30 min apart. The graph shows the radioactive content, expressed in disintegrations per minute (d.p.m.), from consecutive 3-min fractions of superfusate collected against time (min). Each point on the graph represents the mean \pm S.E.M. of six prostate preparations.

dilution in PSS, continuously gassed with carbogen and maintained at a temperature of 37 °C.

The PSS had the following composition (mM): NaCl, 118; KCl, 4.7; CaCl_2 , 2.5; MgSO_4 , 0.45; NaHCO_3 , 25; KH_2PO_4 , 1.03 and D-(+)-glucose, 11.1. EDTA (0.067 mM) and ascorbic acid (0.14 mM) were also present to prevent oxidation of NA.

(L)-[Ring 7, 8- ^3H]-noradrenaline (specific activity 30–50 Ci/mmol) was supplied by Amersham Pharmacia Biotech Pty Ltd (Buckinghamshire, England, UK).

Statistical analysis of results

Results are expressed as mean \pm S.E.M.; n represents the number of experiments. The levels of statistical significance of differences were determined by one-way analysis of variance (ANOVA) followed by Dunnett's test or Student–Newman–Keuls (SNK) test, where appropriate. All statistical analyses were performed using the statistical program SigmaStat for Windows (Jandel Scientific Software Inc., San Rafael, CA, USA). In all cases, probability levels less than 0.05 ($P < 0.05$) were taken to indicate statistical significance.

Results

Effects of exogenous and locally-generated Ang II on [^3H]NA release from rat prostate

Control experiments Figure 1 shows the mean content of radioactivity (d.p.m.) in consecutive 3 min fractions of

superfusate collected from control rat prostates ($n=6$) loaded with [^3H]NA and subjected to two periods of stimulation. The mean resting efflux of radioactivity for the first period of electrical field stimulation (R_1) was 1414 ± 134 d.p.m. per 3 min collection ($n=6$). There was a gradual decrease in the resting efflux between the two periods of stimulation, such that the resting efflux preceding the second period of stimulation, expressed as a percentage of the first ($\%R_2/R_1$), had a mean value of $80.1 \pm 1.0\%$. Electrical field stimulation evoked an increase in the radioactive efflux from prostate preparations loaded with [^3H]NA. The mean stimulation-induced efflux evoked by the first period of stimulation (S_1) was 2954 ± 587 d.p.m. ($n=6$). The mean value of the stimulation-induced efflux for the second period of stimulation, expressed as a percentage of the first ($\%S_2/S_1$), was $87.0 \pm 3.0\%$.

Characterisation of stimulation-induced efflux

To confirm that the source of radioactivity released from the radiolabelled prostate by electrical field stimulation was due to the neuronal exocytotic release of [^3H]NA from sympathetic nerves, the effects of tetrodotoxin (neuronal Na^+ channel blocker), ω -conotoxin (neuronal N-type Ca^{2+} channel blocker) and Ca^{2+} -free PSS on stimulation-induced efflux were investigated. Tetrodotoxin (1 μM), ω -conotoxin (0.1 μM) and the removal of extracellular Ca^{2+} from the PSS, introduced 15 min before the second period of stimulation, virtually abolished the stimulation-induced efflux of radioactivity (Fig. 2; $P < 0.05$). The selective α_2 -adrenoceptor antagonist idazoxan was used to determine whether transmitter NA release was subject to auto-inhibition mediated by prejunctional α_2 -adrenoceptors. Idazoxan (0.1 μM), introduced into the PSS 15 min before the second period of stimulation, markedly enhanced stimulation-induced efflux, by approximately 170% above control (Fig. 2; $P < 0.05$).

Effects of Ang II and Ang I

As shown in Fig. 3, introduction of exogenous Ang II (0.001–1 μM) into the PSS, 15 min before the second period of stimulation, produced a concentration-dependent increase in the stimulation-induced efflux of [^3H]NA, with a maximal effect at 0.1 μM ($P < 0.05$). The precursor molecule Ang I was used to determine whether locally generated Ang II could also modulate transmitter NA release in the prostate. Similar to Ang II, Ang I (0.001–1 μM), introduced 15 min before the second period of stimulation, also enhanced the stimulation-induced efflux in a concentration-dependent manner, but was approximately 10-fold less potent than Ang II, with a maximal effect at 1 μM (Fig. 3; $P < 0.05$). The maximal increase in stimulation-induced efflux produced by both Ang II and Ang I was approximately 40% above control.

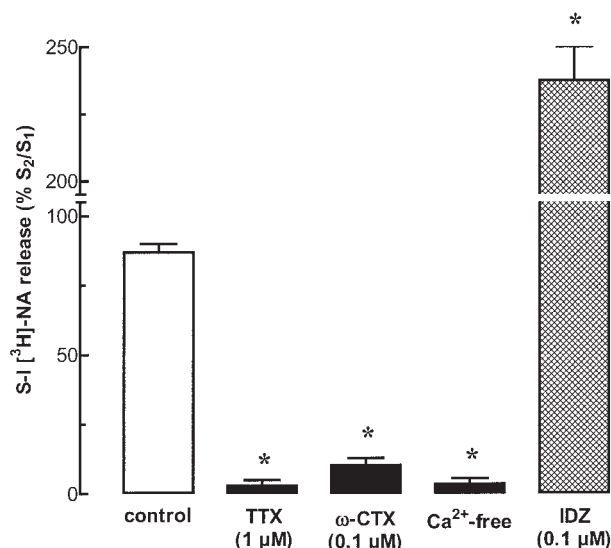


Figure 2 Effects of various neuronal drugs and the removal of extracellular Ca²⁺ on the stimulation-induced (S-I) release of [³H]NA from the rat prostate. The intrinsic sympathetic nerves of the prostate were subjected to two 60 s periods of electrical field stimulation at 5 Hz, given 30 min apart. Where indicated, tetrodotoxin (TTX, 1 μM), ω-conotoxin (ω-CTX, 0.1 μM) or idazoxan (IDZ, 0.1 μM) was added to the PSS superfusing the prostate preparations, or the PSS was replaced with Ca²⁺-free PSS, 15 min before the second period of stimulation and then remained present throughout. The stimulation-induced efflux for the second period of stimulation was expressed as a percentage of the value for the first period (% S₂/S₁). Each column represents the mean ± S.E.M. of four to six prostate preparations. *Significant difference from control (*P*<0.05, ANOVA, Dunnett's test).

Effects of Ang II and Ang I in the presence of losartan The Ang II receptor antagonists losartan, PD123319 and CGP4112 were used to characterise the angiotensin receptor subtype(s) mediating the effects of Ang II and Ang I on stimulation-induced [³H]NA release. When introduced alone 15 min before the second period of stimulation, the AT₁ receptor antagonist losartan had no effect on stimulation-induced efflux. However, when introduced 15 min before the second period of stimulation in combination with Ang II (0.1 μM) or Ang I (1 μM), losartan (0.01 and 0.1 μM) significantly inhibited the increase in stimulation-induced efflux produced by Ang II and Ang I, in a concentration-dependent manner (Fig. 4; *P*<0.05).

Effects of Ang II and Ang I in the presence of PD123319 When introduced alone 15 min before the second period of stimulation, the AT₂ receptor antagonist PD123319 had no effect on the stimulation-induced efflux. However, as seen with losartan, when introduced in combination with Ang II (0.1 μM) or Ang I (1 μM) 15 min before the second period of stimulation, PD123319 (0.01 and 0.1 μM) also inhibited the increase in

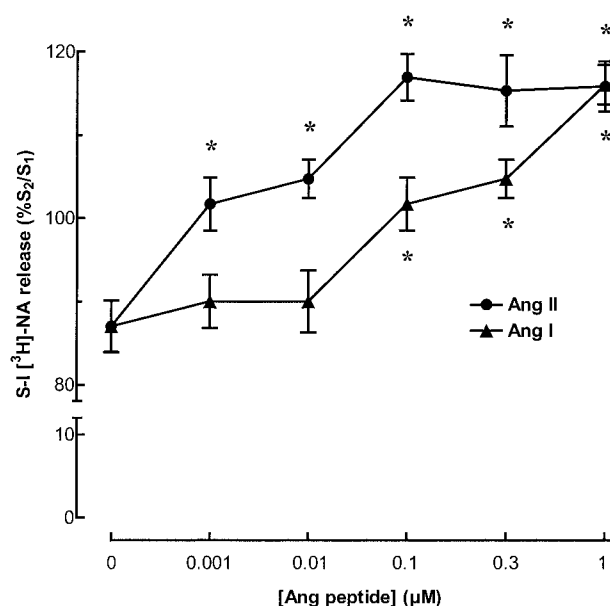


Figure 3 Effects of Ang II and Ang I on the stimulation-induced (S-I) release of [³H]NA from the rat prostate. The intrinsic sympathetic nerves of the prostate were subjected to two 60 s periods of electrical field stimulation at 5 Hz, given 30 min apart. Ang II or Ang I (0.001–1 μM) was added to the PSS superfusing the prostate preparations 15 min before the second period of stimulation and then remained present throughout. The stimulation-induced efflux for the second period of stimulation was expressed as a percentage of the value for the first period (% S₂/S₁). Each point on the graph represents the mean ± S.E.M. of four to nine prostate preparations. *Significant difference from control (0) (*P*<0.05, ANOVA, Dunnett's test).

stimulation-induced efflux produced by Ang II and Ang I, in a concentration-dependent manner (Fig. 5; *P*<0.05).

Effects of Ang II and Ang I in the presence of CGP42112 When introduced alone 15 min before the second period of stimulation, the AT₂ receptor ligand CGP42112, like PD123319 or losartan, had no effect on the stimulation-induced efflux. However, in contrast to PD123319 or losartan, CGP42112 (0.01 and 0.1 μM), introduced in combination with Ang II (0.1 μM) or Ang I (1 μM), did not alter the facilitatory effect of Ang II or Ang I on stimulation-induced efflux (Fig. 6; *P*>0.05).

Effects of Ang I in the presence of captopril The ACE inhibitor captopril was used to determine whether the effects of Ang I on stimulation-induced efflux were due to generation of Ang II via an ACE-dependent pathway. When introduced alone 15 min before the second period of stimulation, captopril (3 μM) produced a small but significant increase in stimulation-induced efflux (Fig. 7; *P*<0.05). However, when introduced in combination with Ang I (1 μM) 15 min before the second period

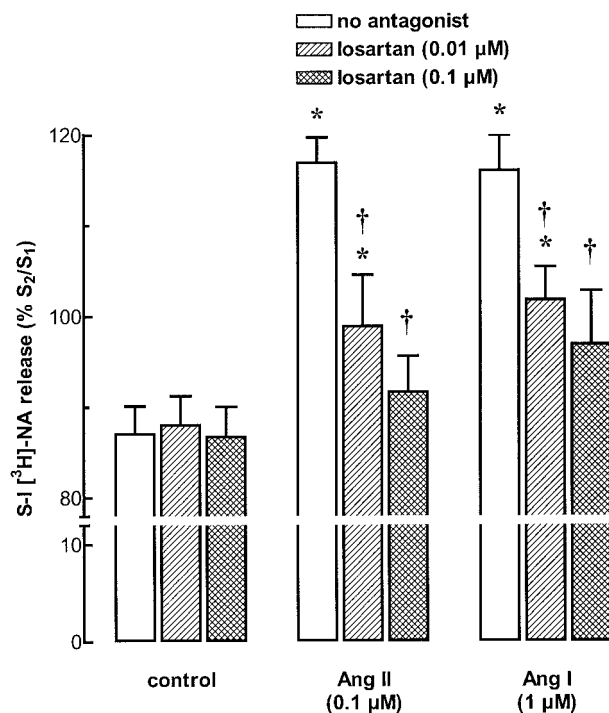


Figure 4 Effects of Ang II and Ang I in the absence and presence of losartan on the stimulation-induced (S-I) release of [^3H]NA from the rat prostate. The intrinsic sympathetic nerves of the prostate were subjected to two 60 s periods of electrical field stimulation at 5 Hz, given 30 min apart. Ang II (0.1 μM), Ang I (1 μM) or losartan (0.01 and 0.1 μM) was added to the PSS superfusing the prostate preparations 15 min before the second period of stimulation and then remained present throughout. The stimulation-induced efflux for the second period of stimulation was expressed as a percentage of the value for the first period (% S_2/S_1). Each column represents the mean \pm S.E.M. of four to six prostate preparations. *Significant difference from corresponding control ($P < 0.05$, ANOVA, Dunnett's test); †significant effect of losartan ($P < 0.05$, ANOVA, SNK test).

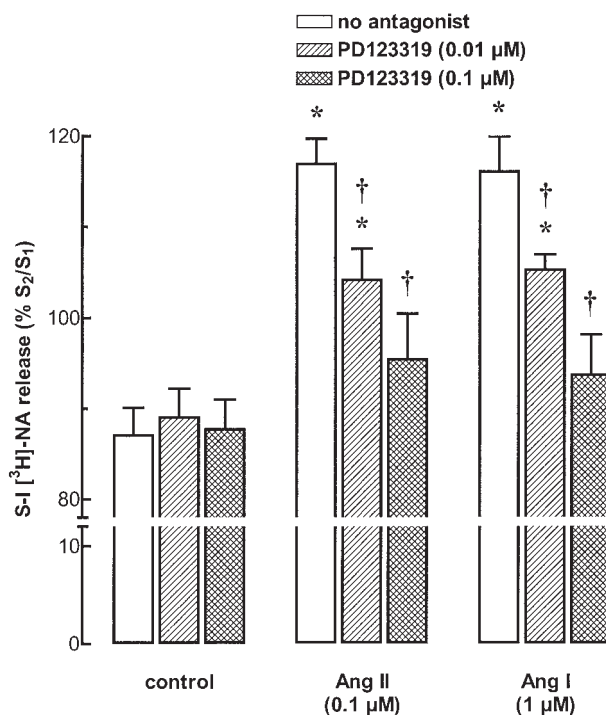


Figure 5 Effects of Ang II and Ang I in the absence and presence of PD123319 on the stimulation-induced (S-I) release of [^3H]NA from the rat prostate. The intrinsic sympathetic nerves of the prostate were subjected to two 60 s periods of electrical field stimulation at 5 Hz, given 30 min apart. Ang II (0.1 μM), Ang I (1 μM) or PD123319 (0.01 and 0.1 μM) was added to the PSS superfusing the prostate preparations 15 min before the second period of stimulation and then remained present throughout. The stimulation-induced efflux for the second period of stimulation was expressed as a percentage of the value for the first period (% S_2/S_1). Each column represents the mean \pm S.E.M. of four to six prostate preparations. *Significant difference from corresponding control ($P < 0.05$, ANOVA, Dunnett's test); †significant effect of PD123319 ($P < 0.05$, ANOVA, SNK test).

of stimulation, captopril (3 μM) markedly inhibited the facilitation of stimulation-induced efflux by Ang I (Fig. 7; $P < 0.05$).

Effects of bradykinin in the absence and presence of Hoe140 or captopril

The potential effects of bradykinin on transmitter NA release in the prostate were also investigated. Bradykinin (1 μM), introduced 15 min before the second period of stimulation, produced a significant increase in the stimulation-induced efflux, approximately 35% above control (Fig. 7; $P < 0.05$). The selective B_2 receptor antagonist Hoe140 was used to characterise the bradykinin receptor subtype involved in mediating the effects of bradykinin on stimulation-induced efflux. When introduced alone 15 min before the second period of stimulation, Hoe140 had no effect on the stimulation-induced efflux. However, when introduced in

combination with bradykinin (1 μM) 15 min before the second period of stimulation, Hoe140 (0.3 and 1 μM) significantly inhibited the facilitatory effect of bradykinin (1 μM) on the stimulation-induced efflux (Fig. 7; $P < 0.05$).

The ACE inhibitor captopril was used again to determine whether exogenously applied bradykinin is subject to degradation by ACE in the prostate. As shown in Fig. 7, in the presence of captopril (3 μM), introduced 15 min before the second period of stimulation, bradykinin further increased the stimulation-induced efflux, to approximately 50% above control and about 15% greater than that achieved with bradykinin alone ($P < 0.05$). Interestingly, when introduced in combination with captopril (3 μM) 15 min before the second period of stimulation, Hoe140 (1 μM) inhibited the increase in stimulation-induced efflux produced by captopril (3 μM) alone (Fig. 7; $P < 0.05$).

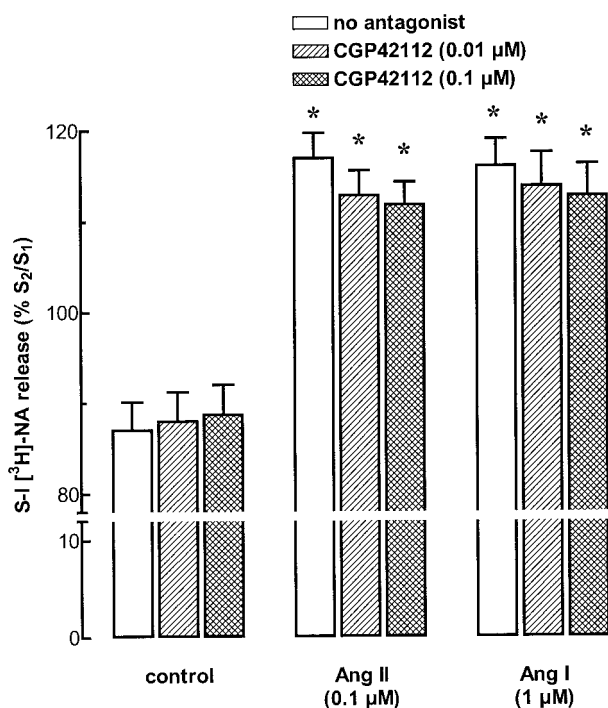


Figure 6 Effects of Ang II and Ang I in the absence and presence of CGP42112 on the stimulation-induced (S-I) release of [³H]NA from the rat prostate. The intrinsic sympathetic nerves of the prostate were subjected to two 60 s periods of electrical field stimulation at 5 Hz, given 30 min apart. Ang II (0.1 μM), Ang I (1 μM) or CGP42112 (0.01 and 0.1 μM) was added to the PSS superfusing the prostate preparations 15 min before the second period of stimulation and then remained present throughout. The stimulation-induced efflux for the second period of stimulation was expressed as a percentage of the value for the first period (S_2/S_1). Each column represents the mean \pm S.E.M. of four to six prostate preparations. *Significant difference from corresponding control ($P < 0.05$, ANOVA, Dunnett's test).

Effects of drugs on resting effluxes Addition of all the various drugs tested had little or no effect on the mean values of resting efflux ($\% R_2/R_1$) (data not shown).

Competition displacement of [¹²⁵I]-Ang II binding from the cloned rat AT_{1a} , AT_{1b} or AT_2 receptor in CHO-K1 cells

In order to characterise further the receptor subtype mediating the effects of Ang II on NA release, binding studies were also performed in CHO cells expressing each of the cloned rat AT_{1a} , AT_{1b} and AT_2 receptor. Figure 8 shows competition displacement curves of [¹²⁵I]-Ang II binding to the cloned rat AT_{1a} , AT_{1b} and AT_2 receptor expressed in CHO-K1 cells (three experiments performed in triplicate). The AT_{1a} receptor displayed a K_D of 1.8 ± 0.3 nM for Ang II and a receptor density (B_{max}) of 1066 ± 356 fmol/mg protein. The K_D and B_{max} for the AT_{1b} receptor were 4.9 ± 1.4 and 571 ± 160 fmol/mg protein respectively, whereas those for the AT_2 receptor were

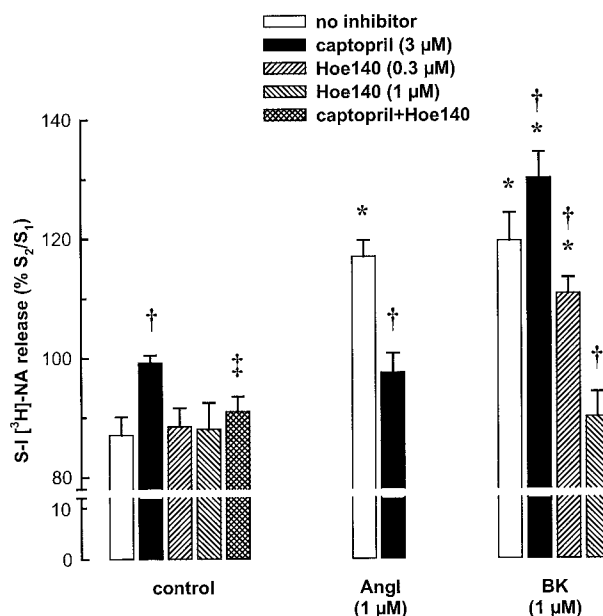


Figure 7 Effects of Ang I and bradykinin (BK) in the absence and presence of captopril or Hoe140 on the stimulation-induced (S-I) release of [³H]NA from the rat prostate. The intrinsic sympathetic nerves of the prostate were subjected to two 60 s periods of electrical field stimulation at 5 Hz, given 30 min apart. Ang I (1 μM), bradykinin (1 μM), captopril (3 μM) or Hoe140 (0.3 and 1 μM) was added to the PSS superfusing the prostate preparations 15 min before the second period of stimulation and then remained present throughout. The stimulation-induced efflux for the second period of stimulation was expressed as a percentage of the value for the first period ($\% S_2/S_1$). Each column represents the mean \pm S.E.M. of four to six prostate preparations. *Significant difference from corresponding control ($P < 0.05$, ANOVA, Dunnett's test); †significant effect of captopril or HOE140 ($P < 0.05$, ANOVA, SNK test); ‡significant difference between captopril plus Hoe140 and captopril alone ($P < 0.05$, ANOVA, SNK test).

3.3 ± 0.7 nM and 1254 ± 301 fmol/mg protein. Displacement of [¹²⁵I]-Ang II by losartan was observed for the AT_{1a} and AT_{1b} receptors, but not the AT_2 receptor, whereas PD123319 and CGP 42112 displaced bound [¹²⁵I]-Ang II from the AT_2 receptor, but not the AT_{1a} or AT_{1b} receptor.

Discussion

Recent evidence from our laboratory suggests that the local RAS is activated in BPH. It is well documented that Ang II, the bioactive product of the RAS, facilitates sympathetic transmission in many cardiovascular organs by enhancing the release of the chemical transmitter NA from sympathetic nerve terminals. However, nothing is known about the interaction of the RAS with sympathetic neurotransmission in the prostate, which may possibly be implicated in the pathophysiology of BPH. Therefore, the

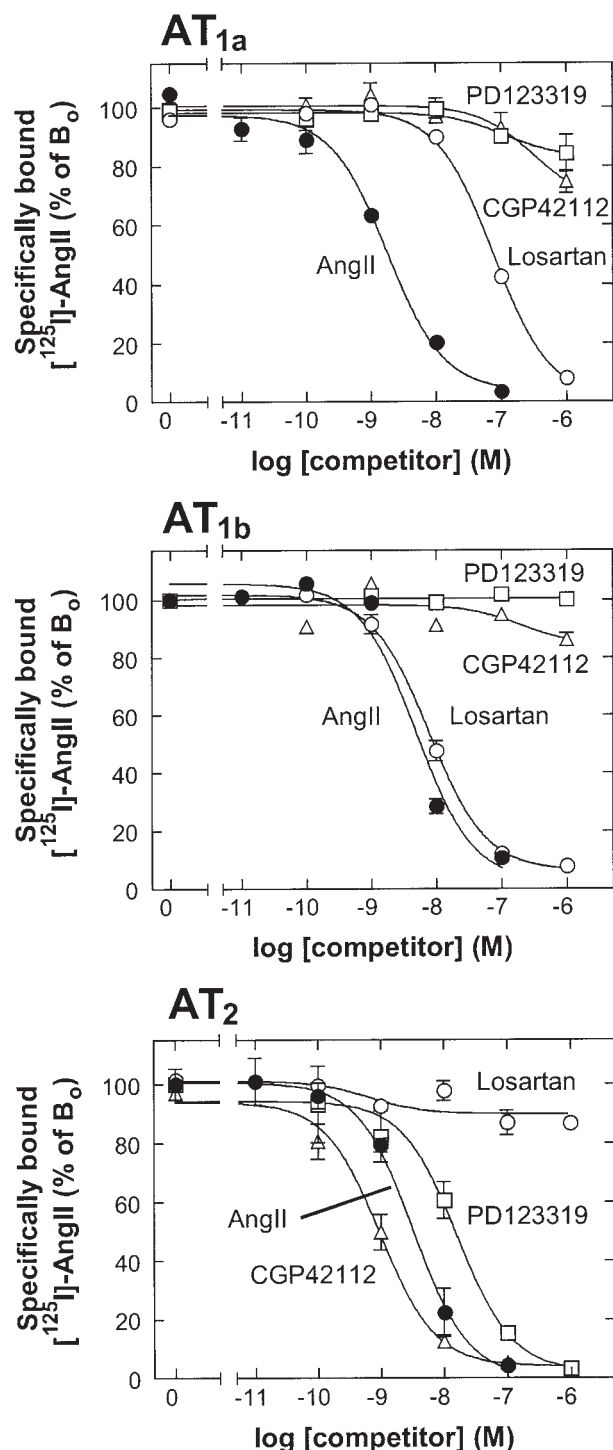


Figure 8 Competition displacement of specific [^{125}I]-Ang II binding by unlabelled Ang II, losartan, PD123319 and CGP42112 (1×10^{-11} – 1×10^{-6} M) in CHO-K1 cells expressing the cloned rat AT_{1a}, AT_{1b}, or AT₂ receptor. Data are expressed as a percentage of initial specific binding (B_0) in the absence of any drugs (% of B_0). Each point represents the mean \pm S.E.M. of three experiments performed in triplicate.

present study was undertaken to determine whether exogenous and locally generated Ang II enhances transmitter NA release in the rat prostate, in an effort to ascribe a functional role of the local RAS in modulating sympathetic activity in the prostate.

Radiotracer techniques have been used widely and extensively to monitor transmitter NA release in many sympathetically innervated tissues. The noradrenergic transmitter stores of the rat prostate were labelled with [^3H]NA, as described previously (Fabiani & Story 1994, 1996). Electrical field stimulation evoked an increase in the efflux of radioactivity from the radiolabelled prostate preparations. The neuronal Na^+ channel blocker tetrodotoxin, the neuronal N-type Ca^{2+} channel blocker ω -conotoxin, and the removal of extracellular Ca^{2+} each abolished the stimulation-induced efflux of radioactivity. This confirms that the stimulation-induced efflux is caused by the neuronal exocytotic release of [^3H]NA by a mechanism dependent on the influx of Ca^{2+} through N-type Ca^{2+} channels. It is well established that sympathetic nerve terminals are endowed with α_2 -adrenoceptors that subserve auto-inhibition of transmitter NA release (Starke 1987, Rand *et al.* 1990, Fuder & Muscholl 1996). In the present study, the α_2 -adrenoceptor antagonist idazoxan markedly enhanced stimulation-induced [^3H]NA release in the rat prostate, which is consistent with blockade of prejunctional inhibitory α_2 -adrenoceptors on sympathetic nerve terminals, and similar to that observed in other tissues, such as rat atria (Loiacono *et al.* 1985, Story *et al.* 1985), human atria (Abadie *et al.* 1996), rat kidney (Rump *et al.* 1990), rat mesenteric artery (Fabiani & Story 1996) and rabbit ear artery (Loiacono *et al.* 1985). These initial findings suggest that the stimulation-induced efflux from the radiolabelled rat prostate is caused by the evoked exocytotic release of [^3H]NA from sympathetic nerves and is subject to auto-inhibition mediated by prejunctional α_2 -adrenoceptors. Thus the stimulation-induced efflux may be taken as an index of transmitter NA release from sympathetic nerves of the rat prostate.

It is generally accepted that one of the most important actions of Ang II on sympathetic neurotransmission is the prejunctional facilitation of transmitter NA release (Story & Ziogas 1987, Saxena 1992). In the present study, Ang II, over a concentration range of 0.001–1 μM , enhanced the stimulation-induced efflux of [^3H]NA in a concentration-dependent manner, with a maximal effect at 0.1 μM , of approximately 35% above control. The facilitatory effect of Ang II on stimulation-induced [^3H]NA release in the rat prostate is consistent with findings in other tissues such as the rat caudal artery (Cox *et al.* 1995, 1996a,b), rat and human kidney (Rump *et al.* 1990, 1995), guinea-pig (Brasch *et al.* 1993) and human atria (Rump *et al.* 1994, Abadie *et al.* 1996).

Many tissues, including blood vessels, heart, kidney, adrenals and brain, are capable of generating Ang II locally

(Campbell 1987, Dzau 1988, Johnston 1992). In the present study, the precursor molecule Ang I was therefore used to determine whether locally generated Ang II could modulate transmitter NA release in the prostate. Similar to Ang II, the inactive precursor Ang I also enhanced the [³H]NA release in a concentration-dependent fashion but was approximately 10-fold less potent than Ang II. The facilitatory effect of Ang I on [³H]NA release in the rat prostate was markedly inhibited by the ACE inhibitor captopril, indicating that the effects of Ang I are due to local conversion to Ang II via an ACE-dependent pathway. This finding suggests that a tissue-based RAS is indeed present and functionally active in the rat prostate and capable of generating Ang II locally. Facilitation of [³H]NA release by Ang I in the rat prostate is consistent with other studies in guinea-pig atria (Ziogas *et al.* 1984, Brasch *et al.* 1993), rat kidney (Böke & Malik 1983, Rump *et al.* 1990), human kidney (Rump *et al.* 1995), and rat vena cava (Ziogas & Story 1991), which was reportedly caused by locally generated Ang II.

We also endeavoured to characterise the Ang II receptor subtype(s) mediating the actions of Ang II on sympathetic transmission in the rat prostate. Previous studies suggest that, in a number of tissues from different species such as guinea-pig atria (Brasch *et al.* 1993), rat atria (Gironacci *et al.* 1994), human atria (Rump *et al.* 1994), rat trachea (Boicos *et al.* 1996), human kidney (Rump *et al.* 1995) and various mouse tissues (Cox *et al.* 1999), the facilitatory effect of Ang II on transmitter NA release is mediated by AT₁ receptors, as they were inhibited by losartan and unaffected by PD123319. However, in the present study, the increase in [³H]NA release by both Ang II and Ang I in the rat prostate was significantly inhibited, in a concentration-dependent manner, by the selective AT₁ receptor antagonist losartan in addition to the AT₂ receptor antagonist PD 123319, whereas the other AT₂ receptor antagonist, CGP42112, had no effect. These findings suggest that the receptor mediating the effects of exogenous and locally generated Ang II on NA release in the rat prostate involves a receptor that is sensitive to both losartan and PD123319. Similar findings have also been reported by Cox *et al.* (1995, 1996a,b) who showed that the facilitation of [³H]NA release by Ang II in the rat caudal artery was also inhibited by both losartan and PD123319.

The other AT₂ receptor antagonist, CGP42112, did not alter the facilitatory effect of Ang II and Ang I on [³H]NA release, which suggests that AT₂ receptors may not be involved. Cloning studies have revealed the existence of at least two subtypes of the AT₁ receptor in rodents (rats and mice), but not higher species or humans, which have been designated AT_{1a} and AT_{1b} (Iwai & Inagami 1992, Yoshida *et al.* 1992). Interestingly, it has been reported in rat mesangial cells that two distinct AT₁ receptor binding sites exist, which are coupled to activation of phospholipase C-mediated intracellular Ca²⁺ mobilisation and inhibition

of adenylate cyclase, and sensitive to inhibition by losartan and PD123319 but not CGP42112 (Ernsberger *et al.* 1992, Zhou *et al.* 1993, Madhun *et al.* 1993). The AT₁ binding site that displayed a greater affinity for losartan was denoted AT_{1A}, and the other binding site, which displayed a greater affinity for PD123319, was denoted AT_{1B} (here, subscript A and B denote the pharmacologically characterised receptors, as opposed to the cloned receptors denoted by subscript a and b).

To delineate further the Ang II receptor subtype involved in mediating the effects of Ang II on transmitter NA release in the rat prostate, Ang II binding studies were performed in CHO cells expressing each of the cloned rat Ang II receptors (AT_{1a}, AT_{1b} and AT₂) to establish their sensitivity to inhibition by losartan, PD123319 and CGP42112. As expected, losartan inhibited Ang II binding in a concentration-dependent manner in CHO cells transfected with either the AT_{1a} or AT_{1b} receptor, whereas PD123319 and CGP42112 had little or no effect. Conversely, PD123319 and CGP42112 inhibited Ang II binding in CHO cells expressing the AT₂ receptor and losartan had little or no effect. As the functional receptor mediating the effects of Ang II on NA release in the rat prostate was sensitive to inhibition by both losartan and PD123319 but not CGP42112, this receptor subtype does not appear to correspond to the cloned AT_{1a}, AT_{1b} or AT₂ as determined by binding studies in CHO cells. We therefore propose that the functional receptor mediating the effects of Ang II on sympathetic transmission in the rat prostate may be similar to the functional AT_{1B} receptor as described above (Ernsberger *et al.* 1992, Zhou *et al.* 1993, Madhun *et al.* 1993, Cox *et al.* 1995, 1996a,b), which is pharmacologically distinct from the cloned rat AT_{1b} receptor.

We have observed in other studies the overwhelming presence of typical AT₁ receptors in both the human (Dinh *et al.* 2001a,b) and rat prostate (unpublished observations). Given that several isoforms of the AT₁ receptor exist in the rat but not in the human, coupled with the apparent absence of AT₂ receptors in the rat prostate, the functional AT receptor mediating the effects of Ang II on NA release in the rat prostate would appear to represent a novel subtype of the AT₁ receptor that is uniquely sensitive to both losartan and PD123319 but not to CGP42112. This novel prejunctional AT receptor mediating the effects of Ang II on NA release in the rat prostate represents only a fraction of the total AT receptor pool in the rat prostate. Furthermore, given that the human prostate expresses almost exclusively AT₁ receptors but not AT₂ receptors, and that humans are not known to express any isoforms of the AT₁ receptor, it seems highly likely that the potential effects of Ang II on NA release in the human prostate would also be mediated by the AT₁ receptor, which is still consistent with the findings in the rat prostate. Thus the characterisation of this functional AT_{1B} receptor subtype mediating the effects of Ang II

on NA release in the rat prostate represents a unique phenomenon in the rat and might not be pertinent in the human prostate, as humans are not known to contain any isoforms of the AT₁ receptor. Unfortunately, because of the unavailability of fresh and functionally viable human prostate tissues, it is not possible also to conduct NA release studies in the human prostate in order to compare directly the effects of Ang II on NA release in the rat prostate, and the receptor subtype involved, with that in the human prostate. Nonetheless, the findings of the present study provide direct evidence that Ang II facilitates NA release in the rat prostate, which may have direct implications for the pathophysiology of BPH in humans (see below).

Very recently, it was reported that AT₁ and bradykinin B₂ receptors form stable heterodimers in vascular smooth muscle cells and that this association dramatically affects the signalling and regulation of the AT₁ receptor (AbdAlla *et al.* 2000). Whether AT₁ and B₂ receptors dimerise and cross-modulate in the prostate remains to be determined, but such a possibility may also be responsible for the changes in AT₁ receptor pharmacology and functionality observed.

ACE has broad substrate specificity which, in addition to converting Ang I to Ang II, also degrades bradykinin to inactive peptide fragments (Fabiani *et al.* 2000). In the present study, captopril itself produced a significant increase in [³H]NA release. The facilitatory effect of captopril on transmitter NA release in the rat prostate may potentially be the result of accumulation of bradykinin as a consequence of impaired bradykinin degradation after ACE inhibition. Indeed, the observed increase in [³H]NA release in the rat prostate was inhibited by the bradykinin B₂ antagonist Hoe140, suggesting that this facilitatory effect of captopril was mediated by bradykinin. Bradykinin has been shown to enhance transmitter NA release in a variety of tissues, including rat kidney (Böke & Malik 1983) and human kidney (Rump *et al.* 1995), rat and mouse vas deferens (Llona *et al.* 1991), rat atria (Chulak *et al.* 1995) and human atria (Rump *et al.* 1997), and rat hypothalamus (Tsuda *et al.* 1993). Consistent with these observations, bradykinin enhanced [³H]NA release in the rat prostate to an extent similar to that produced by Ang II or Ang I, suggesting that bradykinin enhances sympathetic transmission in the prostate. Moreover, the effect of bradykinin on [³H]NA release was further enhanced after ACE inhibition with captopril, suggesting that exogenous bradykinin is subject to degradation by prostatic ACE. This is consistent with the findings of other studies in which the facilitatory effect of bradykinin on NA release was revealed after ACE inhibition with captopril in the human kidney and atria (Rump *et al.* 1995, 1997). These effects of bradykinin on [³H]NA release in the rat prostate were significantly inhibited by Hoe140, suggesting that facilitation of transmitter NA release by bradykinin in the rat prostate is mediated by B₂ receptors. This is also in

accordance with other studies in which bradykinin was found to increase NA release in the rat heart (Kurz *et al.* 1997) and mouse heart (Chulak *et al.* 1998), pithed rat and PC12 cells (Dendorfer & Dominiak 1995, Dendorfer *et al.* 1996) via B₂ receptors – an effect that was markedly antagonised by Hoe140.

These findings suggest that ACE may have an important neuromodulatory role on sympathetic transmission in the prostate by regulating the synthesis of Ang II and the metabolism of bradykinin, both of which have demonstrable effects on transmitter NA release in the rat prostate. Therefore, although ACE inhibition may suppress local Ang II-mediated effects on sympathetic transmission in the prostate, this may potentially be compensated by the effects mediated by bradykinin accumulation. Further to this, bradykinin has been shown to induce contraction of the canine prostate, which can be potentiated further by ACE inhibition (Steidle *et al.* 1990). Thus, if the RAS has an obligatory role in the pathophysiology of BPH, then suppression of the RAS with AT₁ receptor blockers, rather than ACE inhibition, may offer potential benefits by virtue of the fact that the former do not interfere with bradykinin metabolism.

The findings of the present study provide direct evidence that Ang II enhances NA release from sympathetic nerves of the rat prostate. These data establish a novel functional role for the RAS in the modulation of sympathetic transmission in the prostate, which may have important implications for the understanding of the pathophysiology of BPH. Increased local sympathetic activity is a characteristic feature of BPH and represents a target for drug treatment with α_1 -adrenoceptor blockers. Recent findings from our laboratory suggest that the local RAS is activated in BPH. Specifically, we have shown that the expression of ACE mRNA and protein (Nassis *et al.* 2000, 2001) and Ang II peptide (Dinh *et al.* 2001a) is significantly increased in BPH compared with the normal prostate. Furthermore, we have demonstrated that AT₁ receptors predominate in the human prostate and are down-regulated in BPH, which may be due to receptor internalisation as a result of receptor hyper-stimulation by increased tissue concentrations of Ang II (Dinh *et al.* 2001a,b). It is possible, therefore, that hyperactivity of the local RAS resulting in increased tissue concentrations of Ang II may represent an important factor in the pathophysiology of BPH by enhancing local sympathetic activity in the prostate. Facilitation of NA release from sympathetic nerves by Ang II would consequently result in hyper-stimulation of α_1 -adrenoceptors, causing contraction of prostatic smooth muscle and urethral compression, with subsequent resistance to urinary outflow.

In conclusion, exogenous and locally generated Ang II facilitates the release of NA from sympathetic nerves of the rat prostate by a prejunctional mechanism. The receptor subtype mediating the effects of Ang II on sympathetic transmission in the rat prostate is unclear, but may involve

a novel functional Ang II receptor distinct from the cloned AT_{1a}, AT_{1b} or AT₂. These novel data provide direct evidence in support of a functional role for the local RAS in modulating sympathetic activity in the prostate, which may have important implications for the pathophysiology of BPH.

Acknowledgements

We gratefully acknowledge the financial support of the National Health & Medical Research Council of Australia, Commonwealth Department of Veteran's Affairs, Sir Edward Dunlop Medical Research Foundation, Clive & Vera Ramaciotti Medical Research Foundation and Austin Hospital Medical Research Foundation.

References

- Abadie C, Foucart S, Page P & Nadeau R 1996 Modulation of noradrenaline release from isolated human atrial appendages. *Journal of the Autonomic Nervous System* **61** 269–276.
- AbdAlla S, Lother H & Quitterer U 2000 AT₁-receptor heterodimers show enhanced G-protein activation and altered receptor sequestration. *Nature* **407** 94–98.
- Boicos K, Cox SL, Fabiani ME & Story DF 1996 AT₁ receptors subserved enhancement of noradrenergic transmission by angiotensin II in the rat trachea. *Proceedings of the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists* **3** 112.
- Böke T & Malik KU 1983 Enhancement by locally generated angiotensin II of release of the adrenergic transmitter in the isolated rat kidney. *Journal of Pharmacology and Experimental Therapeutics* **226** 900–907.
- Brasch H, Sieroslowski L & Dominiak P 1993 Angiotensin II increases norepinephrine release from atria by acting on angiotensin subtype 1 receptors. *Hypertension* **22** 699–704.
- Campbell DJ 1987 Circulating and tissue angiotensin systems. *Journal of Clinical Investigation* **79** 1–6.
- Chulak C, Couture R & Foucart S 1995 Modulatory effect of bradykinin on the release of noradrenaline from rat isolated atria. *British Journal of Pharmacology* **115** 330–334.
- Chulak C, Couture R & Foucart S 1998 Modulatory effect of bradykinin on noradrenaline release in isolated atria from normal and B₂ knockout transgenic mice. *European Journal of Pharmacology* **346** 167–174.
- Chung O, Kuhl H, Stoll M & Unger T 1998 Physiological and pharmacological implications of AT₁ versus AT₂ receptors. *Kidney International* **67** (Suppl) S95–S99.
- Cox SL, Ben A, Story DF & Ziogas J 1995 Evidence for the involvement of different receptor subtypes in the pre- and postjunctional actions of angiotensin II at rat sympathetic neuroeffector sites. *British Journal of Pharmacology* **114** 1057–1063.
- Cox SL, Story DF & Ziogas J 1996a Multiple prejunctional actions of angiotensin II on noradrenergic transmission in the caudal artery of the rat. *British Journal of Pharmacology* **119** 976–984.
- Cox SL, Story DF & Ziogas J 1996b Angiotensin II receptors involved in the enhancement of noradrenergic transmission in the caudal artery of the spontaneously hypertensive rat. *British Journal of Pharmacology* **119** 965–975.
- Cox SL, Trendelenburg AU & Starke K 1999 Prejunctional angiotensin receptors involved in the facilitation of noradrenaline release in mouse tissues. *British Journal of Pharmacology* **127** 1256–1262.
- Csikos T, Chung O & Unger T 1998 Receptors and their classification: focus on angiotensin II and the AT₂ receptor. *Journal of Human Hypertension* **12** 311–318.
- Dendorfer A & Dominiak P 1995 Characterization of bradykinin receptors mediating catecholamine release in PC12 cells. *Naunyn-Schmiedeberg's Archives of Pharmacology* **351** 274–281.
- Dendorfer A, Hauser W, Falias D & Dominiak P 1996 Bradykinin increases catecholamine release via B₂ receptors. *Pflügers Archives* **432** (Suppl 3) R99–R106.
- Dinh DT, Frauman AG, Somers GR, Ohishi M, Zhuo J, Casley DJ, Johnston CI & Fabiani ME 2001a Evidence for activation of the renin–angiotensin system in the human prostate: increased angiotensin II and reduced AT₁ receptor expression in benign prostatic hyperplasia. *Journal of Pathology* (In Press).
- Dinh DT, Frauman AG, Sourial M, Casley DJ, Johnston CI & Fabiani ME 2001b Identification, distribution and expression of angiotensin II receptors in the normal human prostate and benign prostatic hyperplasia. *Endocrinology* **142** 1349–1356.
- Dzau VJ 1988 Circulating versus local renin–angiotensin system in cardiovascular homeostasis. *Circulation* **7** 14–13.
- Ernsberger P, Zhou J, Damon TH & Douglas JG 1992 Angiotensin II receptor subtypes in cultured rat renal mesangial cells. *American Journal of Physiology* **263** F411–F416.
- Fabiani ME 1999 Angiotensin receptor subtypes: novel targets for cardiovascular therapy. *Drug News and Perspectives* **12** 207–215.
- Fabiani ME & Story DF 1994 Prejunctional effects of cromakalim, nicorandil and pinacidil on noradrenergic transmission in rat isolated mesenteric artery. *Journal of Autonomic Pharmacology* **14** 87–98.
- Fabiani ME & Story DF 1996 Inhibition of sympathetic noradrenergic transmission by guanabenz and guanethidine in rat isolated mesenteric artery: involvement of neuronal potassium channels. *Pharmacological Research* **33** 171–180.
- Fabiani ME, Dinh DT, Nassis L & Johnston CI 2000 Angiotensin-converting enzyme: basic properties, distribution, and functional role. In *Hypertension: A Companion to Brenner and Rector's The Kidney*, ch 9, pp 90–100. Eds S Oparil & MA Weber. Philadelphia: WB Saunders Co.
- Forray C, Bard JA, Wetzel JM, Chiu G, Shapiro E, Tang R, Lepor H, Hartig PR, Weinshank RL, Brancheck TA & Gluchowski C 1994 The α₁-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1c}-subtype. *Molecular Pharmacology* **45** 703–708.
- Fuder H & Muscholl E 1996 Heteroreceptor-mediated modulation of noradrenaline and acetylcholine release from peripheral nerves. *Reviews of Physiology Biochemistry and Pharmacology* **12** 327–380.
- Furuya S, Kumamoto Y, Yokoyama E, Tsukamoto T, Izumi T & Abiko Y 1982 Alpha-adrenergic activity and urethral pressure in prostatic zone in benign prostatic hypertrophy. *Journal of Urology* **128** 836–839.
- Garraway WM, Collins GN & Lee RJ 1991 High prevalence of benign prostatic hypertrophy in the community. *Lancet* **338** 469–471.
- Gironacci MM, Adler-Graschinsky E, Pena C & Enero MA 1994 Effects of angiotensin II and angiotensin-(1–7) on the release of [³H] norepinephrine from rat atria. *Hypertension* **24** 457–460.
- Griendling KK, Lassegue B & Alexander RW 1996 Angiotensin receptors and their therapeutic implications. *Annual Review of Pharmacology and Toxicology* **36** 281–306.
- Isaacs JT & Coffey DS 1989 Etiology and disease process of benign prostatic hyperplasia. *Prostate* **2** (Suppl) 33–50.
- Iwai N & Inagami T 1992 Identification of two subtypes in the rat type I angiotensin II receptor. *FEBS Letters* **298** 257–260.
- Johnston CI 1990 Biochemistry and pharmacology of the renin–angiotensin system. *Drugs* **39** (Suppl 1) 21–31.
- Johnston CI 1992 Franz Volhard Lecture. Renin–angiotensin system: a dual tissue and hormonal system for cardiovascular control. *Journal of Hypertension* **10** (Suppl 7) S13–S26.

- Kirby RS 1989 Alpha-adrenoceptor inhibitors in the treatment of benign prostatic hyperplasia. *American Journal of Medicine* **87** (Suppl 2A): 26S–30S.
- Kurz T, Tolg R & Richardt G 1997 Bradykinin B₂-receptor-mediated stimulation of exocytotic noradrenaline release from cardiac sympathetic neurons. *Journal of Molecular and Cellular Cardiology* **29** 2561–2569.
- Loiaccono RE, Rand MJ & Story DF 1985 Interaction between the inhibitory action of acetylcholine and the alpha-adrenoceptor autoinhibitory feedback system on release of [³H]-noradrenaline from rat atria and rabbit ear artery. *British Journal of Pharmacology* **84** 697–705.
- Llona I, Galleguillos X, Belmar J & Huidobro-Toro JP 1991 Bradykinin modulates the release of noradrenaline from vas deferens nerve terminals. *Life Sciences* **48** 2585–2592.
- McNeal J 1990 Pathology of benign prostatic hyperplasia: insight into etiology. *Urologic Clinics of North America* **17** 477–486.
- McVary KT, Razzaq A, Lee C, Venegas MF, Rademaker A & McKenna KE 1994 Growth of the rat prostate gland is facilitated by the autonomic nervous system. *Biology of Reproduction* **51** 99–107.
- Madhuz ZT, Ernsberger P, Ke FC, Zhou J, Hopfer U & Douglas JG 1993 Signal transduction mediated by angiotensin II receptor subtypes expressed in rat renal mesangial cells. *Regulatory Peptides* **44** 149–157.
- Madsen FA & Bruskwewitz RC 1995 Benign prostatic hyperplasia: pathophysiology and pharmacological treatment. *Current Opinion in Nephrology and Hypertension* **4** 455–459.
- Marshall I, Burt RP & Chapple CR 1995 Noradrenaline contractions of human prostate mediated by α_{1A} (α_{1C})-adrenoceptor subtype. *British Journal of Pharmacology* **115** 781–786.
- Maruenda J, Bhatnagar V & Lowenthal DT 1999 Hypertension in the elderly with coexisting benign prostatic hyperplasia. *Urology* **53** (Suppl 3a) 7–12.
- Nassis L, Frauman AG, Johnston CI & Fabiani ME 2000 Localisation and expression of angiotensin-converting enzyme protein and mRNA in the human prostate. *Journal of the American Society of Nephrology* **11** 425A–426A.
- Nassis L, Frauman AG, Ohishi M, Zhuo J, Casley DJ, Johnston CI & Fabiani ME 2001 Localisation of angiotensin-converting enzyme in the human prostate: Pathological expression in benign prostatic hyperplasia. *Journal of Pathology* (In Press).
- Nicholls MG, Richards AM & Agarwal M 1998 The importance of the renin-angiotensin system in cardiovascular disease. *Journal of Human Hypertension* **12** 295–299.
- Peach MJ 1977 Renin-angiotensin system: biochemistry and mechanisms of action. *Physiological Reviews* **57** 313–370.
- Purdy RE & Weber MA 1988 Angiotensin II amplification of adrenergic vasoconstriction: role of receptor reserve. *Circulation Research* **63** 748–757.
- Rand MJ, Tung LH, Louis WJ & Story DF 1986 Cardiac alpha-adrenoceptors: postjunctional and prejunctional. *Journal of Molecular and Cellular Cardiology* **18** (Suppl 5) 17–32.
- Rand MJ, Majewski H & Story DF 1990 Modulation of neurotransmission. In *Cardiovascular Pharmacology*, pp 229–292. Ed. M Antonaccio. New York: Raven Press.
- Rump LC, Schuster MJ, Wilde K & Schollmeyer P 1990 Modulation of noradrenaline release from rat cortical kidney slices: effects of angiotensin I and II. *British Journal of Clinical Pharmacology* **30** (Suppl 1) 168S–170S.
- Rump LC, Schwertfeger E, Schaible U, Fraedrich G & Schollmeyer P 1994 β_2 -Adrenergic receptor and angiotensin II receptor modulation of sympathetic neurotransmission in human atria. *Circulation Research* **74** 434–440.
- Rump LC, Bohmann C, Schaible U, Schultze-Seemann W & Schollmeyer PJ 1995 β -Adrenergic, angiotensin II, and bradykinin receptors enhance neurotransmission in human kidney. *Hypertension* **26** 445–451.
- Rump LC, Berlit T, Schwertfeger E, Beyersdorf F, Schollmeyer P & Bohmann C 1997 Angiotensin converting enzyme inhibition unmasks the sympathofacilitatory effect of bradykinin in human right atrium. *Journal of Hypertension* **15** 1263–1270.
- Saxena PR 1992 Interaction between the renin-angiotensin-aldosterone and sympathetic nervous systems. *Journal of Cardiovascular Pharmacology* **19** (Suppl 6) S80–S88.
- Starke K 1987 Presynaptic alpha-autoreceptors. *Reviews of Physiology Biochemistry and Pharmacology* **107** 73–146.
- Steidle CP, Cohen ML & Neubauer BL 1990 Bradykinin-induced contractions of canine prostate and bladder: effect of angiotensin converting enzyme inhibition. *Journal of Urology* **144** 390–392.
- Story DF & Ziogas J 1987 Interaction of angiotensin II with noradrenergic transmission. *Trends in Pharmacological Sciences* **8** 269–271.
- Story DF, Standford-Starr CA & Rand MJ 1985 Evidence for the involvement of α_1 -adrenoceptors in negative feedback regulation of noradrenergic transmitter release in rat atria. *Clinical Science* **68** (Suppl 10) 111S–115S.
- Swillens S 1992 How to estimate the total receptor concentration when the specific radioactivity of the ligand is unknown. *Trends in Pharmacological Sciences* **13** 430–434.
- Thekkumkara TJ, Du J, Dostal DE, Motel TJ, Thomas WG & Baker KM 1995 Stable expression of a functional rat angiotensin II (AT_{1A}) receptor in CHO-K1 cells: rapid desensitization by angiotensin II. *Molecular and Cellular Biochemistry* **146** 79–89.
- Thomas WG, Motel TJ, Kule CE, Karoor V & Baker KM 1998 Phosphorylation of the angiotensin II (AT_{1A}) receptor carboxyl terminus: a role in receptor endocytosis. *Molecular Endocrinology* **12** 1513–1524.
- Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JA & Smith RD 1993 Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacological Reviews* **45** 205–251.
- Tsuda K, Tsuda S, Goldstein M, Nishio I & Masuyama Y 1993 Effects of bradykinin on [³H]-norepinephrine release in rat hypothalamus. *Clinical and Experimental Pharmacology and Physiology* **20** 787–791.
- Unger T, Chung O, Csikos T, Culman J, Gallinat S, Gohlke P, Hohle S, Meffert S, Stoll M, Stroth U & Zhu YZ 1996 Angiotensin receptors. *Journal of Hypertension* **14** (Suppl 5) S95–S103.
- Van Sande ME, Scharpé SL, Neels HM & Van Camp KO 1985 Distribution of angiotensin converting enzyme in human tissues. *Clinica Chimica Acta* **147** 255–260.
- Yokoyama M, Hiwada K, Kokubu T, Takaha M & Takeuchi M 1980 Angiotensin-converting enzyme in human prostate. *Clinica Chimica Acta* **100** 253–258.
- Yoshida H, Kakuchi J, Guo DF, Furuta H, Iwai N, van der Meer-de Jong R, Inagami T & Ichikawa I 1992 Analysis of the evolution of angiotensin II type 1 receptor gene in mammals (mouse, rat, bovine and human). *Biochemical and Biophysical Research Communications* **186** 1042–1049.
- Zhou J, Ernsberger P & Douglas JG 1993 A novel angiotensin receptor subtype in rat mesangium. Coupling to adenylyl cyclase. *Hypertension* **21** 1035–1038.
- Ziogas J & Story DF 1991 Angiotensin II generation in the rat vena cava: stimulation of local synthesis by beta-adrenoceptor activation. *Naunyn-Schmiedeberg's Archives of Pharmacology* **343** 31–36.
- Ziogas J, Story DF & Rand MJ 1984 Effects of locally generated angiotensin II on noradrenergic transmission in guinea-pig isolated atria. *European Journal of Pharmacology* **106** 11–18.

Received 23 February 2001

Accepted 5 July 2001