J. Physiol. (1987), **393**, *pp.* 425–436
With 1 text-figure
Printed in Great Britain

THE ROLE OF ANGIOTENSIN II IN THE RENAL RESPONSES TO SOMATIC NERVE STIMULATION IN THE RAT

BY RAJASH K. HANDA AND EDWARD J. JOHNS
From the Department of Physiology, The Medical School, Birmingham B15 2TJ

(*Received 8 January 1987*)

SUMMARY

1. Electrical stimulation of the brachial nerves at 3 Hz (15 V, 0·2 ms), in sodium pentobarbitone-anaesthetized rats whose renal arterial pressure was held constant, elicited a 26% increase in systemic blood pressure, a 15% rise in heart rate, an 11% reduction in renal blood flow, did not alter glomerular filtration rate and significantly reduced absolute and fractional sodium excretions and urine flow by 44, 49 and 31%, respectively.

2. In a separate group of rats, brachial nerve stimulation at 3 Hz increased plasma renin activity approximately 2-fold, while in animals in which the brachial nerves were not stimulated plasma renin activity did not change.

3. Following inhibition of the renin–angiotensin system with captopril or sar-1-ile-8-angiotensin II, brachial nerve stimulation resulted in similar increases in systemic blood pressure and heart rate as in the animals with an intact renin–angiotensin system but, in captopril-infused rats, did not change renal haemodynamics or urine flow while absolute and fractional sodium excretions were reduced by 20 and 25%, respectively. In sar-1-ile-8-angiotensin II-infused animals, similar nerve stimulation decreased renal blood flow by 12%, glomerular filtration rate by 7% and absolute and fractional sodium excretions and urine flow by 25, 18 and 18%, respectively. These decreases in sodium and water output were significantly smaller than those observed in animals with an intact renin–angiotensin system.

4. Stimulation of the brachial nerves increased post-ganglionic efferent renal nerve activity by 20% and the magnitude of this response was unaffected following inhibition of the renin–angiotensin system.

5. The results show that low rates of brachial nerve stimulation in the rat can increase efferent renal nerve activity and result in an antinatriuresis and antidiuresis which is dependent on the presence of angiotensin II, and appears to be due to an action of angiotensin II at the level of the kidney.

INTRODUCTION

The autonomic nervous system constitutes an important mechanism for the physiological regulation of renal function and enables the kidney to respond rapidly and efficiently to changing requirements in the regulation of cardiovascular and body fluid homeostasis. Increased activity within the renal nerves can lead to renal

vasoconstriction, increased tubular sodium and water reabsorption and increased renin secretion. The nerve-mediated increase in sodium reabsorption and renin secretion can occur without alteration in either renal blood flow or glomerular filtration and has been taken to represent a direct action of those nerves ending on tubular epithelial cells (DiBona, 1982) and the renin-containing cells of the juxtaglomerular apparatus (Keeton & Campbell, 1981).

It is known that activation of sensory receptors of the muscle and skin, as may occur during exercise or as a consequence of trauma and pain, can reflexly increase sympathetic discharge to the kidney in the cat (Coote & Perez-Gonzalez, 1970) and dog (Whitwam, Fussey & Kidd, 1979), and alter renal vascular function in these species (Johansson, 1962; Thames & Abboud, 1979; Holdaas, Kopp & DiBona, 1985). Moreover, it has been recently demonstrated that activation of these somatic afferents can also lead to renal nerve-mediated increases in renin secretion in the dog (Holdaas & DiBona, 1984; Zambraski, Tucker, Lakas, Grassl & Scanes, 1984) and neurally induced antinatriuresis in the rat (Handa & Johns, 1983), which were independent of renal haemodynamic changes. It would, therefore, seem that under conditions of minimal alterations in renal haemodynamics, changes in renin release and tubular sodium reabsorption may be the major pathways by which the renal nerves exert their action when stimulated reflexly.

Angiotensin II within the circulation can have a number of direct and indirect actions whereby it can influence the rate of sodium excretion. This can occur as angiotensin II directly stimulating sodium reabsorption across the tubules (Harris & Navar, 1985) or indirectly by reductions in renal blood flow and glomerular filtration or by stimulating aldosterone release (Keeton & Campbell, 1981). There is increasing evidence that angiotensin II is able to facilitate adrenergic transmission (Zimmerman, 1981) and the question arises as to whether this could occur within the kidney. Recently we have shown that supression of angiotensin II production by captopril decreased the ability of electrical stimulation of the renal nerves to cause an antinatriuresis and antidiuresis which was compatible with angiotensin II being required at the neuro-effector junctions (Handa & Johns, 1985).

The aim of the present study was to determine whether the antinatriuresis and antidiuresis resulting from reflex activation of the renal nerves was dependent on angiotensin II. Reflex activation of the renal nerves was achieved by electrically stimulating the somatic afferent nerves of the brachial plexus and the renin–angiotensin system was interrupted using two different pharmacological approaches: firstly, inhibition of angiotensin-converting enzyme with captopril; and secondly, antagonism of angiotensin II receptors with sar-1-ile-8-angiotensin II.

METHODS

Male Sprague–Dawley rats, in the weight range 340–400 g, were anaesthetized by an intraperitoneal injection of sodium pentobarbitone (0·24 mmol/kg) and maintained with periodic supplementary doses administered intravenously. A tracheotomy was performed, a catheter inserted into the right common carotid artery for arterial blood sampling and systemic blood pressure recording (Statham P23Dc pressure transducer attached to a Grass model 5 or model 7 Polygraph), and the left jugular vein cannulated to allow the immediate intravenous infusion of saline (150 mmol NaCl) at a rate of 6 ml/h which was continued at this rate throughout the experiment. Heart rate was measured by either counting the number of arterial pulse pressure

waves during a 6 s period and then corrected to beats/min, or continuously recorded by amplifying the arterial pressure waveform to trigger a tachograph unit on the model 7 Grass polygraph.

The left kidney was exposed by a ventral mid-line incision and its ureter cannulated for the collection of urine. Renal arterial pressure was monitored (Statham P23Dc pressure transducer) by means of a cannula introduced into the aorta, through the femoral artery, with its tip 2–3 mm below the left renal artery. A silk thread was passed around the aorta, rostral to both renal arteries, and attached to a screw device such that renal arterial pressure could be regulated at a constant level during the experimental periods.

Somatic nerve stimulation. The nerves contained within the right brachial region were isolated, crushed and the central ends stimulated using bipolar silver wire electrodes connected to a Grass model 8 stimulator which delivered pulses at 15 V, 0·2 ms duration and at a frequency of 3 Hz for 25 min.

Renal function measurements. On completion of surgery a 1 ml primer solution of saline containing inulin (20 mg/ml) was given I.V. followed by a constant infusion of inulin (8 mg/ml) in saline at a rate of 6 ml/h. Renal function measurements were begun 2 h later.

The experimental protocol consisted of five clearance periods: two control 30 min clearance periods followed by a 20 min clearance period during which the brachial nerves were electrically stimulated and a further two 30 min recovery clearance periods. To clear pre-formed urine from the dead space of the ureteral cannula, at least 5 min were allowed from the beginning and cessation of nerve stimulation before urine collection was begun for the subsequent clearance period.

Arterial blood samples (0·35 ml) were collected into cooled syringes, previously rinsed with EDTA (160 mmol/l), at the beginning and end of each clearance period. The blood samples were immediately centrifuged, the plasma stored (−17 °C) until assayed and the remaining red blood cells resuspended in saline and infused back into the animal after removal of the subsequent blood sample. Preliminary experiments showed that this blood sampling procedure had no consistent effect on the haematocrit of the animals. Plasma and urine samples were deproteinized (Somogyi, 1930) and the inulin content assayed (Bojesen, 1952). Glomerular filtration rate was calculated as the clearance of inulin and renal blood flow was measured using a non-cannulating flow probe (Carolina EP 100 series) placed around the renal artery and attached to an electromagnetic flowmeter (Carolina FM 501). Plasma and urinary sodium concentrations were measured using a Beckman flame photometer.

Inhibitors of the renin–angiotensin system. The angiotensin-converting enzyme inhibitor, captopril, or the angiotensin II receptor antagonist, sar-1-ile-8-angiotensin II, was dissolved in saline containing inulin (8 mg/ml) and infused into the animal at a rate of 0·38 or 0·086 mmol kg^{-1} h^{-1} I.V., respectively. One hour after completion of surgery, the systemic pressor and renal vasoconstrictor effects of I.V. bolus doses of 77 pmol angiotensin I and 97 pmol angiotensin II were measured. The infusion was then changed to one containing either captopril or sar-1-ile-8-angiotensin II and their effectiveness to suppress the renin–angiotensin system was assessed 40 min later and at the end of the experiment by their respective abilities to inhibit the vascular responses to further doses of angiotensin I and angiotensin II.

Renin studies. Separate groups of rats were used for the plasma renin activity studies. These animals underwent the same surgical and infusion procedures as those used in the renal function studies. Two hours after completion of surgery the first arterial blood sample (0·7 ml) was removed for basal plasma renin activity levels. The brachial nerves were then placed on the electrodes and either stimulated at 15 V, 0·2 ms and at a rate of 3 Hz or sham-stimulated, that is the stimulator was not switched on at the appropriate time. Renal arterial pressure was maintained at a constant level throughout the period of brachial nerve stimulation. Twenty-five minutes later a second arterial blood sample was removed for plasma renin activity measurement.

Blood for plasma renin activity was collected into cooled syringes, previously rinsed with EDTA. It was immediately centrifuged at 4 °C and the plasma stored (−17 °C) until assayed. On return of the resuspended red blood cells to the animal, a 10 min equilibration period elapsed before continuing the experimental protocol.

The plasma was incubated at 37 °C for 1 h in the presence of enzymatic inhibitors allowing the generation of angiotensin I which was then estimated by radioimmunoassay using commercial kits (C.I.S., U.K. Ltd). Plasma renin activity was expressed as pmol angiotensin I generated per millilitre per hour (pmol ml^{-1} h^{-1}).

Efferent renal nerve activity measurements. The effect of brachial nerve stimulation on efferent

renal nerve activity was examined in a separate group of rats which had undergone similar surgical preparation to those in the renal function studies. Efferent renal nerve activity was recorded using fine silver-wire electrodes placed on the central end of the renal nerves which had been crushed close to the kidney before they entered the coeliac ganglion. All nerves and exposed tissues were protected from drying and cooling by a deep pool of paraffin at 37 °C contained within the skin edges.

On completing surgery, the animal was immobilized by the skeletal muscle relaxant, gallamine triethiodide (11–22 μmol/kg I.V.), and artificially respired at a rate of 60/min (B. Braun Melsungen AG, Type 874 092). One hour later the brachial nerves were stimulated at 15 V, 0·2 ms and at a rate of 3 Hz for 25 min, during which 1 min recordings of renal nerve activity were taken every fifth minute. The I.V. infusion of saline was either continued or changed to one containing captopril at a concentration such that it was delivered into the animals at a rate of 0·38 mmol kg^{-1} h^{-1}. Two hours later the brachial nerves were again stimulated and further efferent renal nerve activity recorded.

Efferent renal nerve activity recordings were fed into a pre-amplifier (Medelec AA6 Mk III), adjusted for low-frequency cuts between 16 and 50 Hz, high-frequency cuts between 1·6 and 3·2 kHz and with the gain varied between 5 and 20 μV/cm. The resultant signal was then recorded on a multichannel tape-recorder (Instrumentation Cassette Recorder, Data Acquisition Ltd) for later analysis. After passing through a window discriminator to remove baseline noise, the residual signal was quantified by an electronic integrator. The mean value of efferent renal nerve activity over the 1 min selected periods, during brachial nerve stimulation, were expressed as a mean percentage increase from basal levels immediately prior to the onset of nerve stimulation.

Statistics. All values were expressed as means $\pm$ S.E.M. A mean value of the two clearances before and two clearances after the period of brachial nerve stimulation was compared to the value obtained during nerve stimulation. The absolute and percentage changes quoted in the text represent the mean changes recorded in individual animals. Paired and unpaired Student's t tests were used for analysis of significance within and across groups, respectively. Differences were considered significant at the 5% level.

RESULTS

Renal function measurements

The effect of brachial nerve stimulation at 3 Hz on cardiovascular and renal function in saline-infused animals is presented in Table 1. Systemic blood pressure and heart rate were increased significantly by 26% ($P < 0·001$) and 15% ($P < 0·005$), respectively, and while renal arterial pressure was maintained at control levels, there was a significant 11% ($P < 0·02$) reduction in renal blood flow, no change in glomerular filtration rate and significant decreases in absolute and fractional sodium excretions and urine flow of 44% ($P < 0·001$), 49% ($P < 0·001$) and 31% ($P < 0·005$), respectively. All variables measured returned to values which were not significantly different from control levels during the recovery period.

The effects of similar rates of brachial nerve stimulation in captopril-infused animals are shown in Table 2. Prior to captopril administration, bolus injection of 77 pmol angiotensin I transiently increased systemic blood pressure by 27 ± 3 mmHg and decreased renal blood flow by $9·4 \pm 2·2$ ml min^{-1} kg^{-1}. These vasoconstrictor actions of angiotensin I were completely abolished 40 min after the start of the captopril infusion and a similar degree of inhibition was obtained at the end of the experiment. The control values of cardiovascular and excretory function in the group of animals given captopril were similar to those recorded in saline-infused animals; however, renal blood flow in captopril-infused animals was significantly ($P < 0·02$) greater. Brachial nerve stimulation resulted in a significant rise in systemic blood pressure and heart rate of 18% ($P < 0·01$) and 16% ($P < 0·01$), respectively, which

were responses similar in magnitude to those obtained in saline-infused animals. With renal arterial pressure controlled at pre-stimulation levels, brachial nerve stimulation did not change renal blood flow, glomerular filtration rate and urine flow, but significantly reduced absolute and fractional sodium excretions by 20% ($P < 0.02$) and 25% ($P < 0.001$), respectively. The magnitude of these renal blood

TABLE 1. Effect of low-frequency brachial nerve stimulation on cardiovascular and renal function ($n = 7$).

	Control	Experimental	Recovery
Systemic blood pressure (mmHg)	126·0 ± 3·0	161·4 ± 5·8****	130 ± 2·3
Heart rate (beats/min)	372 ± 18	427 ± 11***	—
Renal arterial pressure (mmHg)	119·9 ± 3·7	119·8 ± 3·7	122·4 ± 3·8
Renal blood flow (ml min^{-1} kg^{-1})	20·8 ± 1·7	17·5 ± 1·3*	18·8 ± 1·7
Glomerular filtration rate (ml min^{-1} kg^{-1})	4·8 ± 0·6	5·0 ± 0·4	4·8 ± 0·3
Urine flow (μl min^{-1} kg^{-1})	44·7 ± 5·2	31·7 ± 3·4***	48·3 ± 6·6
Absolute sodium excretion (μmol min^{-1} kg^{-1})	10·5 ± 1·7	6·3 ± 0·9****	12·2 ± 1·9
Fractional sodium excretion (%)	1·52 ± 0·33	0·82 ± 0·13****	1·61 ± 0·17

Mean values ± S.E.M. are shown. n = number of animals. The P values represent a comparison between the mean of the control and recovery values with that obtained during the brachial nerve stimulation period (experimental): * $P < 0.02$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.001$.

TABLE 2. Effect of low rates of brachial nerve stimulation on cardiovascular and renal function in captopril-infused (0·38 mmol kg^{-1} h^{-1}) animals ($n = 6$)

	Control	Experimental	Recovery
Systemic blood pressure (mmHg)	121·4 ± 3·1	142·7 ± 4·5**	121·1 ± 2·4
Heart rate (beats/min; $n = 4$)	396 ± 22	459 ± 19**	—
Renal arterial pressure (mmHg)	120·4 ± 2·2	119·3 ± 2·0	119·1 ± 2·2
Renal blood flow (ml min^{-1} kg^{-1})	27·7 ± 1·8	27·7 ± 2·2	28·7 ± 3·0
Glomerular filtration rate (ml min^{-1} kg^{-1})	5·0 ± 0·3	5·4 ± 0·4	5·2 ± 0·4
Urine flow (μl min^{-1} kg^{-1})	52·1 ± 7·2	49·7 ± 6·9	54·7 ± 8·7
Absolute sodium excretion (μmol min^{-1} kg^{-1})	12·1 ± 1·6	10·5 ± 1·8*	13·9 ± 2·4
Fractional sodium excretion (%)	1·47 ± 0·17	1·18 ± 0·16****	1·65 ± 0·23

Key as in Table 1.

flow and excretory responses in the captopril-infused animals were all significantly ($P < 0.05$, $P < 0.02$, $P < 0.05$, $P < 0.005$, respectively) less than those obtained during brachial nerve stimulation in animals with an intact renin–angiotensin system.

The results obtained from the third group of animals infused with sar-1-ile-8-angiotensin II are shown in Table 3. Administration of the bolus dose of angiotensin II caused a transient increase in systemic blood pressure of 59 ± 2 mmHg and a decrease in renal blood flow of 10·4 ± 1·4 ml min^{-1} kg^{-1}. The vasoconstrictor actions of further doses of angiotensin II were totally abolished 40 min after the start of the sar-1-ile-8-angiotensin II infusion and at the end of the experiment. In these animals, control values of systemic blood pressure and heart rate were significantly ($P < 0.01$, $P < 0.001$, respectively) less than corresponding values in saline-infused animals, while the resting heart rate was significantly ($P < 0.001$) less than in captopril-infused animals. Control levels of renal blood flow and glomerular filtration rate were smaller, but not such as to reach statistical significance, than those in saline-infused animals and significantly ($P < 0.005$, $P < 0.025$, respectively) less than in animals

infused with captopril. However, control values of renal arterial pressure and renal excretion variables were similar in all three groups. Activation of the brachial nerves in these animals given sar-1-ile-8-angiotensin II increased systemic blood pressure by 35% ($P < 0.001$) and heart rate by 27% ($P < 0.005$), which were responses similar in magnitude to those obtained in saline-infused and captopril-infused animals. Brachial nerve stimulation also led to a reduction in renal blood flow and glomerular filtration rate of 12% ($P < 0.005$) and 7% ($P < 0.01$), respectively, while renal

TABLE 3. Effect of low rates of brachial nerve stimulation on cardiovascular and kidney function in sar-1-ile-8-angiotensin II-infused animals ($n = 7$)

	Control	Experimental	Recovery
Systemic blood pressure (mmHg)	112·8 ± 4·0	151·4 ± 7·9****	112·8 ± 5·0
Heart rate (beats/min)	246 ± 17	312 ± 23***	—
Renal arterial pressure (mmHg)	117·4 ± 4·2	114·8 ± 3·8	115·4 ± 4·6
Renal blood flow (ml min^{-1} kg^{-1})	15·2 ± 2·4	13·8 ± 2·3***	16·1 ± 2·4
Glomerular filtration rate (ml min^{-1} kg^{-1})	3·9 ± 0·4	3·5 ± 0·4**	3·6 ± 0·4
Urine flow (μl min^{-1} kg^{-1})	42·6 ± 5·0	33·8 ± 5·6**	37·8 ± 6·1
Absolute sodium excretion (μmol min^{-1} kg^{-1})	10·5 ± 2·1	7·5 ± 1·4**	9·7 ± 2·0
Fractional sodium excretion (%)	1·59 ± 0·24	1·28 ± 0·21**	1·55 ± 0·30

Key as in Table 1.

arterial pressure was unchanged. This reduction in renal blood flow was similar to that obtained in saline-infused rats but significantly ($P < 0.05$) greater than the unchanged renal blood flow observed in captopril-infused rats, whereas the decrease in glomerular filtration rate was significantly ($P < 0.005$) different from the unchanged glomerular filtration rate seen in both saline-infused and captopril-infused animals. During the period of brachial nerve stimulation, absolute and fractional sodium excretions and urine flow were significantly reduced by 25, 18 and 18% (all $P < 0.01$), respectively, which were responses similar in magnitude to those obtained in captopril-infused animals and significantly ($P < 0.05$, $P < 0.005$, $P < 0.025$, respectively) less than those observed in saline-infused animals. In all groups of rats, plasma sodium concentrations were stable and no statistical differences could be detected either within or between experimental groups. A comparison of the percentage changes in renal function in all three groups, during brachial nerve stimulation, is shown in Fig. 1.

Renin responses

In a group of six animals subjected to the same surgical and experimental procedures, the basal level of plasma renin activity was 2·86 ± 0·99 pmol ml^{-1} h^{-1}. The electrodes were placed on the brachial nerves and 25 min later plasma renin activity was unchanged at 2·59 ± 0·62 pmol ml^{-1} h^{-1}. In a further group of seven animals, plasma renin activity was 2·88 ± 0·70 pmol ml^{-1} h^{-1} before and after 25 min of brachial nerve stimulation at 3 Hz was 7·03 ± 0·52 pmol ml^{-1} h^{-1}, a significant ($P < 0.001$) twofold increase.

Efferent renal nerve activity responses

Brachial nerve stimulation resulted in a 19·5 ± 1·2% ($P < 0.001$) increase in efferent renal nerve activity in four animals infused with saline throughout the

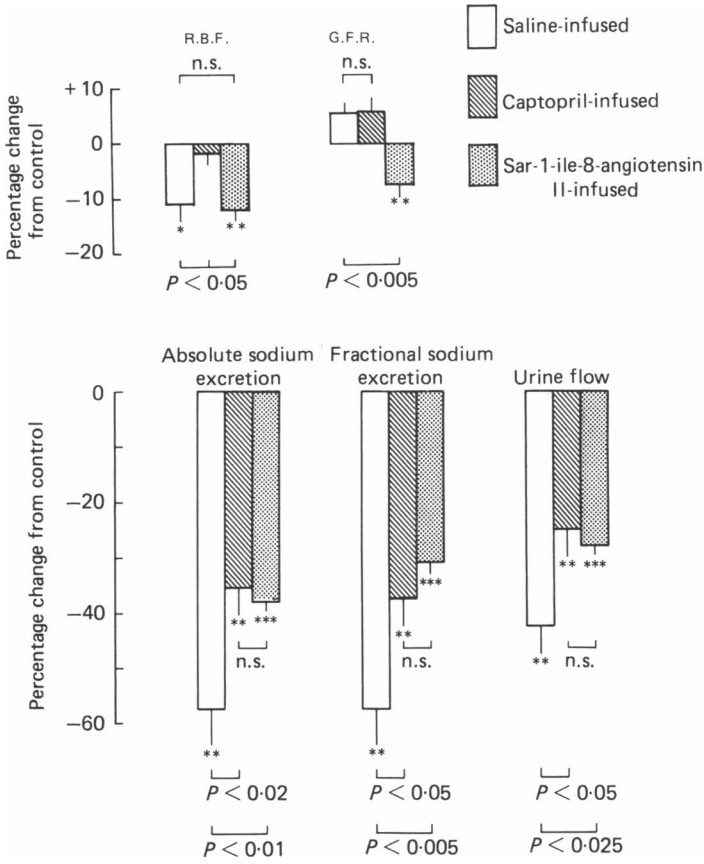

Fig. 1. A comparison of the responses, presented as percentage changes, which take place during low rates of brachial nerve stimulation at 15 V, 0·2 ms and 3 Hz in renal blood flow (R.B.F.), glomerular filtration rate (G.F.R.), absolute sodium excretion, fractional sodium excretion and urine flow. Open columns, control (seven animals); hatched columns, captopril infused at 0·38 mmol kg^{-1} h^{-1}, I.V. (six animals); stippled columns, sar-1-ile-8-angiotensin II infused at 0·086 mmol kg^{-1} h^{-1}, I.V. (seven animals). $*P < 0·05$; $**P < 0·01$; $***P < 0·001$ and indicate differences from control values. P values in the Figure represent comparisons between groups. N.S., not significant.

experiment. In these same animals, a second period of brachial nerve stimulation caused an increase in efferent renal nerve activity of $27·0 \pm 1·2\%$ ($P < 0·001$) which was significantly ($P < 0·01$) greater than that obtained during the first period of nerve stimulation. Brachial nerve stimulation during saline infusion, in a separate group of four animals, resulted in an $18·6 \pm 2·4\%$ ($P < 0·005$) increase in renal nerve activity and during subsequent infusion with captopril the rise was $22·4 \pm 2·4\%$ ($P < 0·005$), which was significantly ($P < 0·05$) greater than the 18·6% increase. The apparent potentiation of efferent renal nerve activity during the second period of brachial nerve stimulation was similar in both groups of animals.

DISCUSSION

It was the aim of this study to examine whether the antinatriuresis and antidiuresis of reflex stimulation of the renal nerves, caused by activation of somatic afferent sensory fibres, required the presence of an intact renin–angiotensin system. The results demonstrated that in the rat the low rates of brachial nerve stimulation which reflexly increased efferent renal nerve activity and decreased sodium excretion were dependent on angiotensin II.

Both systemic blood pressure and heart rate were significantly increased by brachial nerve stimulation and such effects of somatic nerve activation have been reported in a number of species (Sato & Schmidt, 1973), including the rat (Shyu, Andersson & Thoren, 1984), and have been shown to be due to activation of somatic group III and IV afferent fibres (Coote & Perez-Gonzalez, 1970; Tibes, 1977). The associated modification in renal function at constant renal arterial pressure was a perceptible renal vasoconstriction with no change in glomerular filtration and a marked increase in sodium and water retention.

Since activation of the brachial nerves was shown to elicit an increase in efferent renal nerve activity and the associated antinatriuresis and antidiuresis can be virtually abolished by prior renal denervation (Handa & Johns, 1983), it is reasonable to suggest that the decrease in sodium and water excretion, observed in the present study, is predominantly mediated by an increase in activity within the renal nerves. Morphological studies have demonstrated an extensive adrenergic innervation of the renal tubular epithelial cells in the rat (Barajas, Powers & Wang, 1984) while functional studies have shown that increased efferent renal nerve activity, induced reflexly (Zambraski, DiBona & Kaloyanides, 1976; DiBona & Johns, 1980; Anderson, Henrich, Gross & Dillingham, 1982) or by direct electrical stimulation (Bello-Reuss, Trevino & Gottschalk, 1976; DiBona & Sawin, 1982), and at levels which did not alter renal haemodynamics, caused increased sodium reabsorption at both proximal tubules (Bello-Reuss *et al.* 1976; Pelayo, Ziegler, Jose & Blantz, 1983) and thick ascending limb of the loop of Henle (DiBona & Sawin, 1982; Bencsath, Szenasi & Takacs, 1985). It would seem that at the low levels of brachial nerve stimulation this particular action of the renal nerves was primarily responsible for inducing the antinatriuresis and antidiuresis.

Measurement of plasma renin activity clearly showed that stimulation of the brachial nerves at low rates also caused a large rise in the circulating levels of renin, reflecting increased secretion from the kidney. Previous studies have shown that activation of somatic nerves can induce renal nerve-mediated renin release in the dog (Holdaas & DiBona, 1984; Zambraski *et al.* 1984). A similar mechanism most probably operates in the rat, since brachial nerve stimulation can reflexly increase renal nerve activity (present study) and ganglionic blockade significantly attenuates the rise in plasma renin levels in response to exercise and pain in the rat (Bozovic & Castenfors, 1967). This was most probably due to the renal sympathetic nerves directly causing renin release from the renin-containing cells of the juxtaglomerular apparatus (DiBona, 1982). The question then arose whether angiotensin II contributed to the modification in kidney function during somatic nerve activation, particularly on sodium and water output.

Infusion of the angiotensin-converting enzyme inhibitor, captopril, or the angiotensin II receptor antagonist, sar-1-ile-8-angiotensin II, at rates that did not alter systemic blood pressure, were shown to effectively suppress the renin–angiotensin system as evidenced by the abolition of vasoconstrictor responses to angiotensin I and angiotensin II, respectively. The rise in systemic blood pressure and heart rate following brachial nerve stimulation were not significantly affected by inhibition of the renin–angiotensin system showing that these responses were not dependent on angiotensin II.

Control values of renal haemodynamic and excretion function of animals infused with captopril or sar-1-ile-8-angiotensin II were similar to those in saline-infused animals, except for a significantly elevated renal blood flow in the captopril-infused group, which has consistently been observed at this infused dose of the converting enzyme inhibitor (Handa & Johns, 1985). The small reduction in renal blood flow during brachial nerve stimulation was not present in the captopril-infused animals, whereas in animals infused with a lower dose rate of sar-1-ile-8-angiotensin II, both renal blood flow and glomerular filtration decreased. The cause of these small differences in renal haemodynamic responses to brachial nerve stimulation following inhibition of the renin–angiotensin system is unclear.

The major effect of inhibiting the renin–angiotensin system was to attenuate the large falls in sodium and water output during brachial nerve stimulation. Although captopril may have promoted an increased accumulation of bradykinin, a compound with natriuretic properties, it is known that angiotensin II receptor antagonists do not interfere with bradykinin catabolism, and since the magnitude of attenuation of the renal excretory responses to afferent nerve stimulation were similar for captopril and sar-1-ile-8-angiotensin II-infused animals, bradykinin was unlikely to have been involved. It is known that adrenergic stimulation promotes sodium and fluid reabsorption in the rat by activating tubular α-adrenoceptors (Johns & Manitius, 1986). However, the doses of captopril and sar-1-ile-8-angiotensin II used in this study do not manifest α-adrenergic antagonistic properties (Handa, 1985). The fact that the magnitude of attenuation of the renal excretory response was similar in both captopril-infused and sar-1-ile-8-angiotensin II-infused animals, in spite of the slightly differing haemodynamic changes, supports the view that the modification in renal excretory function was due to inhibiting the actions of endogenous angiotensin II.

Circulating angiotensin II can act on structures within the central nervous system to increase sympathetic discharge (Ganong, Rudolph & Zimmerman, 1979). In order to examine whether angiotensin II was involved centrally in mediating the brachial nerve-induced reflex, renal nerve activity was recorded when the brachial nerves were stimulated. The animals in this study were given gallamine and artificially respired which meant that they were not directly comparable to the animals given pentobarbitone in the functional studies. Nevertheless, it was clearly shown from the neurophysiological studies that the reflex increase in efferent renal nerve activity during brachial nerve stimulation was not reduced when the saline infusate was replaced with one containing captopril. Indeed, there was a small potentiation of the response with time. Thus, the observed actions of renin–angiotensin system inhibitors to attenuate renal sodium and water retention, appeared unrelated to a possible

central mechanism which altered the reflex increase in efferent renal nerve activity, but most likely resided at an action of angiotensin II at the level of the kidney.

It may be argued that the small changes in renal haemodynamics which occurred during brachial nerve stimulation in the drug-infused animals could have been responsible for the attenuated antinatriuresis. However, this is unlikely as although in the captopril-treated animals renal blood flow did not change during reflex activation of the renal nerves, in the presence of sar-1-ile-8-angiotensin II there was a reduction in blood flow yet the attenuation of the antinatriuresis and antidiuresis was of the same magnitude. This pattern of response indicated a minimal contribution of these small changes in renal blood flow to the tubular responses. Alternatively, the attenuated neurally induced increase in sodium and fluid retention could, in part, reflect the removal of a direct stimulatory action of angiotensin II on tubular epithelial cells to increase sodium reabsorption (Harris & Navar, 1985). This possibility could contribute to our results, but there is some evidence that it does not as two recent studies have shown that the adrenergically mediated increase in tubular sodium reabsorption could be completely blocked with the α_1-adrenoceptor antagonist, prazosin, without changing the magnitude of the rise in plasma renin (Osborn, Holdaas, Thames & DiBona, 1983; Hesse & Johns, 1985), and presumably if angiotensin was having a tubular action its effect should still have been observed.

It is now recognized that angiotensin II can modify the effectiveness of the sympathetic nervous system by facilitating the release of noradrenaline from nerve endings via a presynaptic mechanism (Zimmerman, 1978, 1981). Such an action of angiotensin II has been shown to occur at the neuro-effector junctions of the rat renal vasculature (Böke & Malik, 1983). In a recent study (Handa & Johns, 1985) we found that direct electrical activation of the renal nerves, at different frequencies, resulted in an antinatriuresis and antidiuresis which could be attenuated by captopril and this was taken to indicate that at the tubular level angiotensin II was a necessary requirement for the full expression of the actions of the renal nerves. The results of the present study would also be compatible with the view that angiotensin II may interact synergistically with the renal nerves, during reflex activation, to decrease sodium excretion by facilitating adrenergic transmission at the neuro-tubular epithelial junction. Several studies in the dog support this contention, as it was shown that captopril or angiotensin II receptor antagonists attenuated the renal nerve-mediated antinatriuresis during hypercapnic acidosis (Anderson *et al.* 1982) or shock avoidance (Koepke & Obrist, 1983), without changing renal haemodynamic responses. Together these studies strongly suggest that angiotensin II can facilitate neurotransmission at the neuro-tubular epithelial junction comparable to that exhibited at the neuro-vascular junctions of the sympathetic nervous system.

This study has shown that activation of somatic nerves in the rat brachial plexus can increase efferent renal nerve activity and results in a marked increase in tubular sodium and water reabsorption and plasma renin levels, with minimal actions on renal haemodynamics. Blockade of the renin–angiotensin system with captopril or sar-1-ile-8-angiotensin II attenuates the increase in sodium and water retention during afferent nerve activation and is due to an angiotensin II-dependent mechanism which does not involve a central site of action, but most probably acts

at the level of the kidney by facilitating neurotransmission at the neuro-tubular epithelial junction. Thus the full expression of reflexly increased renal nerve activity, via somatic nerve stimulation, on tubular sodium and water reabsorption, is dependent upon an intact renin–angiotensin system.

We are most grateful to Professor J. H. Coote for his advice and the use of laboratory facilities in the neurophysiological studies. The financial assistance of E. R. Squibb is gratefully acknowledged, R.K.H. was in receipt of an SERC scholarship.

REFERENCES

ANDERSON, R. J., HENRICH, W. L., GROSS, P. A. & DILLINGHAM, M. A. (1982). Role of renal nerves, angiotensin II and prostaglandins in the antinatriuretic response to acute hypercapnic acidosis in the dog. *Circulation Research* **50**, 294–300.

BARAJAS, L., POWERS, K. & WANG, P. (1984). Innervation of the renal cortical tubules: a quantitative study. *American Journal of Physiology* **247**, F50–60.

BELLO-REUSS, E., TREVINO, D. L. & GOTTSCHALK, C. W. (1976). Effect of renal sympathetic nerve stimulation on proximal water and sodium reabsorption. *Journal of Clinical Investigation* **57**, 1104–1107.

BENCSATH, P., SZENASI, G. & TAKACS, L. (1985). Water and electrolyte transport in Henlé's loop and distal tubule after renal sympathectomy in the rat. *American Journal of Physiology* **249**, F308–314.

BOJESEN, E. (1952). A method for determination of inulin in plasma and urine. *Acta medica scandinavica* **142**, suppl. 266, 275–282.

BÖKE, T. & MALIK, K. U. (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.

BOZOVIC, L. & CASTENFORS, J. (1967). Effect of ganglionic blocking on plasma renin activity in exercising and pain-stressed rats. *Acta physiologica scandinavica* **70**, 290–292.

COOTE, J. H. & PEREZ-GONZALEZ, J. F. (1970). The response of some sympathetic neurones to volleys in various afferent nerves. *Journal of Physiology* **208**, 261–278.

DIBONA, G. F. (1982). The functions of the renal nerves. *Reviews of Physiology, Biochemistry and Pharmacology* **94**, 75–181.

DIBONA, G. F. & JOHNS, E. J. (1980). A study of the role of the renal nerves in the renal responses to 60° head-up tilt in the anaesthetized dog. *Journal of Physiology* **299**, 117–126.

DIBONA, G. F. & SAWIN, L. L. (1982). Effect of renal nerve stimulation on NaCl and H_2O transport in Henle's loop of the rat. *American Journal of Physiology* **243**, F576–580.

GANONG, W. F., RUDOLPH, C. D. & ZIMMERMAN, H. (1979). Neuroendocrine components in the regulation of blood pressure and renin secretion. *Hypertension* **1**, 207–218.

HANDA, R. K. (1985). *Nervous System and the Kidney*. Ph.D. Thesis, University of Birmingham.

HANDA, R. K. & JOHNS, E. J. (1983). The effect of somatic afferent nerve stimulation on renal function in the rat. *Journal of Physiology* **349**, 62*P*.

HANDA, R. K. & JOHNS, E. J. (1985). Interaction of the renin–angiotensin system and the renal nerves in the regulation of rat kidney function. *Journal of Physiology* **369**, 311–321.

HARRIS, P. J. & NAVAR, L. G. (1985). Tubular transport responses to angiotensin. *American Journal of Physiology* **248**, F621–630.

HESSE, I. F. A. & JOHNS, E. J. (1985). The role of α-adrenoceptors in the regulation of tubular sodium reabsorption and renin secretion in the rabbit. *British Journal of Pharmacology* **84**, 715–724.

HOLDAAS, H. & DIBONA, G. F. (1984). Stimulatory and inhibitory reflexes from somatic receptors: effects on renin release. *American Journal of Physiology* **246**, R1005–1010.

HOLDAAS, H., KOPP, U. C. & DIBONA, G. F. (1985). Modulation of reflex renal vasoconstriction by increased endogenous renal prostaglandin synthesis. *Journal of Pharmacology and Experimental Therapeutics* **232**, 725–731.

JOHANSSON, B. (1962). Circulatory responses to stimulation of somatic afferents. *Acta physiologica scandinavica* **57**, suppl. 198, 1–91.

JOHNS, E. J. & MANITIUS, J. (1986). An investigation into the α-adrenoceptor mediating renal nerve-induced calcium reabsorption by the rat kidney. *British Journal of Pharmacology* **89**, 91–97.

KEETON, T. K. & CAMPBELL, W. B. (1981). The physiologic alteration of renin release. *Pharmacological Reviews* **32**, 82–227.

KOEPKE, J. P. & OBRIST, P. A. (1983). Angiotensin II in the renal excretory response to behavioural stress in conscious dogs. *American Journal of Physiology* **245**, R259–264.

OSBORN, J. L., HOLDAAS, H., THAMES, M. D. & DIBONA, G. F. (1983). Renal adrenoceptor mediation of antinatriuretic and renin secretion responses to low frequency renal nerve stimulation in the dog. *Circulation Research* **53**, 298–305.

PELAYO, J. C., ZIEGLER, M. G., JOSE, P. A. & BLANTZ, R. C. (1983). Renal denervation in the rat: analysis of glomerular and proximal tubular function. *American Journal of Physiology* **244**, F70–77.

SATO, A. & SCHMIDT, R. F. (1973). Somatosympathetic reflexes: afferent fibers, central pathways, discharge characteristics. *Physiological Reviews* **53**, 916–947.

SHYU, B. C., ANDERSSON, S. A. & THOREN, P. (1984). Circulatory depression following low frequency stimulation of the sciatic nerve in anaesthetized rats. *Acta physiologica scandinavica* **121**, 97–102.

SOMOGYI, M. (1930). A method for the preparation of blood filtrates for the determination of sugar. *Journal of Biological Chemistry* **86**, 655–663.

THAMES, M. D. & ABBOUD, F. M. (1979). Interaction of somatic and cardiopulmonary receptors in the control of renal circulation. *American Journal of Physiology* **237**, H560–565.

TIBES, U. (1977). Reflex inputs to the cardiovascular and respiratory centres from dynamically working canine muscles: some evidence for involvement of Group III or IV nerve fibres. *Circulation Research* **41**, 332–341.

WHITWAM, J. G., FUSSEY, I. V. & KIDD, C. (1979). Responses in sympathetic nerves of the dog evoked by stimulation of somatic nerves. *Brain Research* **165**, 219–233.

ZAMBRASKI, E. J., TUCKER, M. S., LAKAS, C. S., GRASSL, S. M. & SCANES, C. G. (1984). Mechanism of renin release in exercising dog. *American Journal of Physiology* **246**, E71–76.

ZAMBRASKI, E. J., DIBONA, G. F. & KALOYANIDES, G. J. (1976). Effect of sympathetic blocking agents on the antinatriuresis of reflex renal nerve stimulation. *Journal of Pharmacology and Experimental Therapeutics* **198**, 464–472.

ZIMMERMAN, B. G. (1978). Actions of angiotensin on adrenergic nerve endings. *Federation Proceedings* **37**, 199–202.

ZIMMERMAN, B. G. (1981). Adrenergic facilitation by angiotensin: does it serve a physiological function? *Clinical Science* **60**, 343–348.