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The role of the peripheral sympathetic nervous system in the natriuresis following central administration of an I_1 imidazoline agonist, moxonidine

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1 Central administration of the I_1-imidazoline receptor agonist moxonidine increases sodium excretion without alteration of blood pressure. In the present study we determined whether this natriuretic action was mediated through a decrease in activity of the sympathetic nervous system, as has been reported for the antihypertensive action of this compound. Interruption of the sympathetic nervous system was achieved with prazosin (α_1-adrenoceptor antagonist) and renal denervation.

2 In pentobarbitone-anaesthetized Sprague-Dawley rats, intracerebroventricular (i.c.v.) injection of moxonidine alone increased urine volume and sodium excretion. Prazosin (0.15 mg kg^{-1}, i.v.) alone decreased urine flow rate and sodium excretion as compared to the vehicle controls. In the presence of prazosin, i.c.v. injection of moxonidine failed to increase sodium excretion or urine volume as compared to animals which received the prazosin alone.

3 The administration of moxonidine (i.c.v.) to sham renal-denervated animals caused an increase in urine flow rate, urine sodium excretion, osmolar clearance and free water clearance. The increase in sodium excretion and osmolar clearance were completely attenuated in renal denervated rats, however, urine flow rate was still increased and this was secondary to the increase in free water clearance which remained intact.

4 These results indicate the importance of an intact sympathetic nervous system in the renal response to i.c.v. moxonidine. Moreover, the differential antagonism of these interventions on solute and water excretion indicate that they may be mediated at two separate sites and/or receptors following i.c.v. moxonidine.

Keywords: Imidazoline receptor; sympathetic nervous system; intracerebroventricular; osmolar clearance; free water clearance; denervation; α_1 -adrenoceptor; natriuresis; central nervous system

Introduction

The imidazoline receptor has been described recently and located in several different tissues by radioligand binding studies (Parini *et al.*, 1989; Brown *et al.*, 1990). In the central nervous system the imidazoline receptor has been found in high concentrations in the rostral ventrolateral medulla (Bricca *et al.*, 1989; Gomez *et al.*, 1991). Specific imidazoline receptor agonists have been identified: moxonidine shows a higher affinity for the imidazoline receptor than the α_2-adrenoceptor in the central nervous system (Ernsberger *et al.*, 1992). Central administration of compounds with high affinity for the imidazoline receptor has been demonstrated to result in changes in blood pressure (Ernsberger *et al.*, 1988; 1990). Michel & Insel (1989) suggested that there were different imidazoline receptor sites and subsequently two sub-types were proposed, the I_1-imidazoline receptor which had a higher affinity for clonidine, and the I_2-imidazoline receptor which had a higher affinity for idazoxan (Michel & Ernsberger, 1992; Ernsberger, 1992).

The intrarenal administration of moxonidine, a selective I_1-imidazoline receptor agonist, increases urine flow rate with a concomitant increase in sodium excretion (Allan *et al.*, 1993). This natriuresis was antagonized by idazoxan, an imidazoline receptor antagonist, but was not affected by rauwolscine, an α_2-adrenoceptor antagonist. This suggested a role for the imidazoline receptor in renal sodium handling. We also found that the administration of moxonidine into the lateral cerebral ventricle induced an increase in urine volume by increasing sodium and free water excretion (Penner & Smyth, 1994). In these studies, the observed changes in urine flow rate and sodium excretion were attenuated by central administration of idazoxan. The mechanism by which the intrarenal infusion of moxonidine increased urine flow rate was considered to involve a direct stimulation of imidazoline receptors in the kidney. The mechanism by which the i.c.v. injection of moxonidine was able to increase urine flow rate was less clear.

A number of different mechanisms could potentially be important in the effects observed following i.c.v. moxonidine. The central nervous system has been shown to play a role in the modulation of renal sodium excretion (Kopp & DiBona, 1992). The previously reported increase in renal salt and water excretion following i.c.v. moxonidine may conceivably have been mediated by a change in the activity of the sympathetic nervous system. Studies have demonstrated that the decrease in blood pressure observed following the central administration of imidazoline agonists involved a decrease in activity of the sympathetic nervous system (Tibirica *et al.*, 1989; 1991; 1992). In the present study, to evaluate the role of the sympathetic nervous system in this response, the input of the sympathetic nervous system to the kidney was removed by pharmacological and surgical intervention. One set of experiments was performed using intravenous prazosin to antagonize selectively peripheral α_1-adrenoceptors, while a second series of experiments utilized renal denervation. The response to i.c.v. moxonidine was determined in the presence and absence of these interventions.

Methods

Experimental preparation

Male Sprague-Dawley rats were obtained from the University of Manitoba (Charles River Breeding stock) and cared for according to regional animal care standards. Animals were

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housed at 23°C with 12 h light/12 h dark cycle. Denervation or sham-denervation of the renal sympathetic nerves to the left kidney was carried out during ether anaesthesia (see below) in animals weighing 180–220 g as previously described (Fortin & Sundaresan, 1989; Greenberg *et al.*, 1993). Three days later, the right kidney was removed under ether anaesthesia as previously described (Penner & Smyth, 1994). Three to four days later, cannulae were implanted into the lateral cerebroventricles (see below). The individual experiments were performed three to four days following the implantation of the lateral cerebroventricular cannula.

Intracerebroventricular cannula

The method described previously (Penner *et al.*, 1990) was used. Briefly, the rats were anaesthetized by an intraperitoneal injection of pentobarbitone (50 mg kg^{-1}). The cannula was a modified 23 gauge needle, with a solid obturator threaded through the lumen and extending 0.5 mm beyond the tip of the needle. The head of the rat was placed in a stereotaxic apparatus (model 900, David Kopf, Tujunga, CA, USA). The coordinates for implantation into the lateral cerebral ventricle in relation to the skull were 0.3 mm posterior to the bregma, 1.4 mm lateral to the mid-line and 3.5 mm below the surface of the skull. Three stainless steel jewelry screws, in an assemblage of acrylic cement, were used to anchor the cannula to the skull. At the time of the injection, on the day of the experiment, the obturator was removed and a 31 gauge injector tube was inserted which extended 1.0 mm below the end of the guide tube (23 gauge needle), entering the ventricle. The injector tube was then connected to a 10 μl Hamilton syringe for administration of drug or vehicle. Verification of the cannula location in the cerebral ventricle was done at the end of the experiment by injection of dye through the cannula and postmortem brain sections.

Renal denervations

Denervation was performed in a similar manner to the protocol followed by Greenberg *et al.* (1993). The kidney was exposed through a flank incision. Utilizing a dissecting microscope the renal artery, renal vein and pedicle were stripped from surrounding tissue. The renal artery and vein were then painted with a 10% solution of phenol in 95% ethyl alcohol. The sham renal-denervation animals had the same surgical procedure performed without the stripping and phenol application. The adequacy of denervation was determined by measuring the catecholamine content per gram of kidney tissue using a Catecholamine Research Assay System (Amersham Canada Ltd., Oakville, Ontario, Canada) 12 days after sham-denervation and denervation.

Experimental protocol

On the day of the experiment the rats were anaesthetized with pentobarbitone (BDH Chemicals Ltd., Poole, England, 50 mg kg^{-1}). The animals were placed on a heating blanket which was thermostatically controlled to maintain body temperature at 37.5°C. A tracheotomy was performed and the animals were allowed to breath spontaneously. The carotid artery was cannulated for measurement of blood pressure, heart rate and obtaining a blood sample at the end of the experiment. The jugular vein was cannulated for the administration of drugs. Blood pressure (mmHg) and heart rate (beats min^{-1}) were monitored with a Statham pressure transducer (model P23Dc) connected to a Grass polygraph model V. The remaining kidney was exposed through a flank incision and the ureter was cannulated with PE-50 for collection of urine. A modest diuresis was maintained throughout the experiment by administration of saline (0.9% NaCl, 97 μl min^{-1}). Following a 45 min stabilization period, three consecutive urine collections of 30 min each were obtained in pre-weighed tubes and urine volumes determined gravimetrically. Moxonidine or saline vehicle was administered i.c.v. in a volume of 5 μl over 1 min with a 10 μl Hamilton syringe immediately following the first urine collection. At the completion of the experiment a blood sample was obtained and the plasma frozen for later analysis. Creatinine levels in the urine and plasma were measured with a Beckman Creatinine 2 Analyzer; sodium was measured with a Beckman Klina Flame Photometer; and osmolality was assessed with a Precision System Micro Osmometer. Creatinine clearance (ml min^{-1}), urine sodium excretion (μmol min^{-1}), osmolar clearance (μl min^{-1}) and free water clearance (μl min^{-1}) were calculated with standard formulae. The specific experimental protocols are given below.

Intracerebroventricular moxonidine with or without intravenous prazosin: Prazosin (0.15 mg kg^{-1}) or saline vehicle was administered intravenously at the beginning of the stabilization period in a volume of 0.15 ml over 30 s (Penner & Smyth, 1988). The i.c.v. injections (moxonidine or saline vehicle) were given immediately after the first 30 min urine collection and before the subsequent two 30 min urine collection periods. Both prazosin and moxonidine were dissolved in 0.9% NaCl for administration. There were four groups of animals with 6 animals per group: vehicle control group (5 μl of 0.9% NaCl given i.c.v. over 1 min and 0.15 ml of 0.9% NaCl given i.v.); i.c.v. moxonidine group (1 nmol moxonidine in 5 μl given i.c.v. over 1 min and 0.15 ml of 0.9% NaCl given i.v.); prazosin group (5μl of 0.9% NaCl given i.c.v. over 1 min and 0.15 mg kg^{-1} of prazosin in 0.15 ml given i.v.) and the moxonidine and prazosin group (1 nmol moxonidine in 5 μl given i.c.v. over 1 min and 0.15 mg kg^{-1} of prazosin in 0.15 ml given i.v.).

Intracerebroventricular moxonidine with or without renal denervation: Two series of experiments were completed, one in sham-denervated rats, the other in renal denervated rats. In sham-denervated rats, the effects of i.c.v. administration of vehicle (saline) or moxonidine at doses of 0.3 nmol and 1 nmol on the renal function were determined. In animals which had undergone renal denervation, the above series of three experiments were repeated. Each group of rats contained 6 animals.

Statistical analysis

The data are presented as the mean ± standard error of the mean (s.e.mean). Each group contained 6 animals. Where appropriate, repeated measures of analysis of variance (ANOVA) or non paired *t* test (catecholamine content of sham versus denervated kidneys) were utilised to assess the data. Data in which significant differences were found with the ANOVA had the interactions analyzed by a Fisher's least squares difference multiple comparison test (Winer, 1971). A P value of $\leqslant 0.05$ was considered significant.

Drugs

Moxonidine was supplied by Beiersdorf, AG, Hamburg, Germany. Prazosin was purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A.

Results

Intracerebroventricular moxonidine with or without intravenous prazosin

For all groups studied (vehicle control, i.c.v. moxonidine, i.v. prazosin, i.c.v. moxonidine and i.v. prazosin) neither creatinine clearance nor the heart rate were altered when the first baseline collection was compared with the two collection periods following i.c.v. administration of the moxonidine or vehicle (Table 1). The baseline and subsequent heart rate values for the group which received both i.c.v. moxonidine and i.v. pra-

zosin were higher than the control group (Table 1). In the prazosin group the blood pressure was lower than the vehicle control group during all collection periods. In the animals given both moxonidine and prazosin the baseline blood pressure was lower than the corresponding vehicle control group. The blood pressure during the second and third collection period was elevated compared to the baseline value in this group, but similar to the respective vehicle control (Table 1).

Moxonidine (1 nmol) administered i.c.v. resulted in an increase in the urine flow rate (periods 2 and 3, Figure 1a) and urine sodium excretion (period 3, Figure 1b) as compared to the vehicle control group and the respective baseline. Intravenous administration of prazosin decreased urine flow rate and sodium excretion as compared to the vehicle control group (Figure 1a, b). Moxonidine administered i.c.v. in the presence of i.v. prazosin failed to increase urine flow and sodium excretion rate higher than that observed with the prazosin alone group (Figure 1a, b). The moxonidine group when compared to the vehicle control group increased both osmolar clearance in the third collection and free water clearance in the second collection (Table 2). After i.v. prazosin treatment, moxonidine failed to alter osmolar or free water clearance from the respective control period (Table 2).

Dose-response to intracerebroventricular moxonidine with and without renal denervation

The noradrenaline content in the kidneys obtained from the sham-denervated group ($n=8$) was 124.2 ± 3.1 pg g^{-1} kidney wet wt. while the level found in the denervated kidneys ($n=9$) was 2.0 ± 0.5 pg g^{-1} kidney wet wt. ($P<0.01$). Three groups of animals underwent sham renal denervation, control (i.c.v. saline, $n=7$), 0.3 nmol i.c.v. moxonidine ($n=7$) and 1 nmol i.c.v. moxonidine ($n=6$). No differences in blood pressure, heart rate or creatinine clearance between these groups were found (data not shown). A similar 3 groups of animals ($n=7$ in each group) underwent renal denervation and did not show a change in blood pressure or creatinine clearance following i.c.v. moxonidine at 0.3 or 1 nmol when compared to the vehicle group (data not shown). In the group in which 1 nmol of moxonidine was administered i.c.v. the heart rates were increased during the second (423 ± 13 beats min^{-1}) and third collection period (417 ± 11 beats min^{-1}) compared to the other groups (control, 394 ± 14, 380 ± 15; moxonidine 0.3 nmol, 391 ± 13, 389 ± 12 beats min^{-1}) but not from its respective baseline value (406 ± 13 beats min^{-1}). Following i.c.v. administration of 0.3 or 1 nmol of moxonidine in sham-denervated animals, an increase in the urine flow rate with a concomitant increase in the urine sodium excretion was found (Figure 2a, b). In the renal denervated rats, urine flow rate was

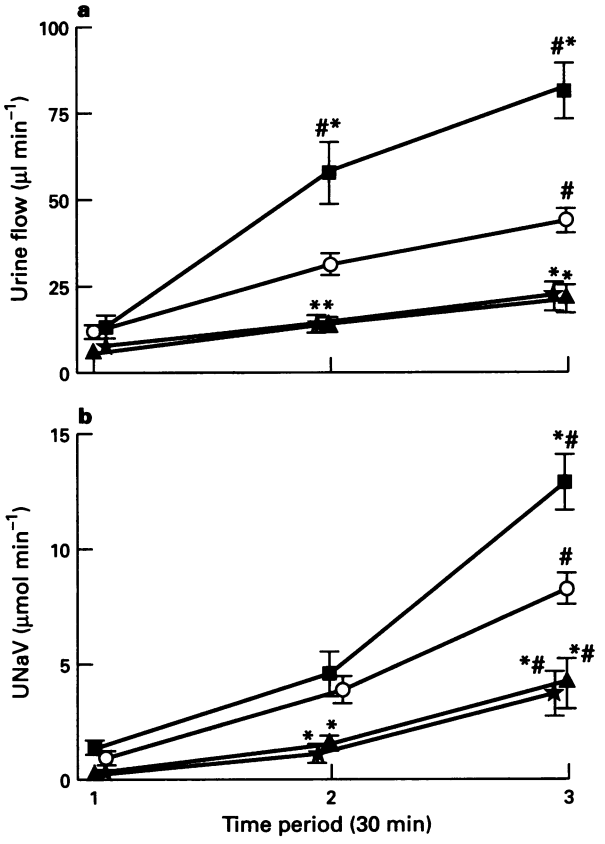

Figure 1 Renal changes seen after i.c.v. saline and i.v. saline (○); i.c.v. moxonidine (1 nmol) and i.v. saline (■); i.c.v. saline and i.v. prazosin (0.15 mg) (▲) and i.c.v. moxonidine (1 nmol) and i.v. prazosin (0.15 mg) (★) on urine flow rate in $\mu l\,min^{-1}$ (a) and sodium excretion in $\mu mol\,min^{-1}$ (b) (6–7 per group). The first time period represents a pretreatment 30 min control urine collection. Time periods 2 and 3 represent 30 min urine collections following intervention. The data are presented as the mean ± s.e.mean. $*P<0.05$ between the vehicle and intervention groups; $\#P<0.05$ between the baseline collection and the subsequent collections.

Table 1 Effect of experimental interventions on haemodynamic parameters

	Time period	*Blood pressure* (mmHg)	*Creatinine clearance* (ml min^{-1})	*Heart rate* (beats min^{-1})
Vehicle control	1	122±3	2.1±0.2	389±10
	2	125±3	1.9±0.3	387±8
	3	124±4	1.7±0.1	384±10
Moxonidine	1	117±3	2.1±0.1	406±10
	2	120±3	2.1±0.1	391±12
	3	124±3	2.2±0.1	417±14*
Prazosin	1	107±5*	2.1±0.2	413±8*
	2	114±4*	2.0±0.2	410±9*
	3	114±5*	1.9±0.2	397±8
Moxonidine and prazosin	1	111±4*	2.2±0.2	426±11*
	2	121±5#	2.1±0.1	423±12*
	3	126±5#	2.1±0.1	414±13*

The four groups were as follows: vehicle control, i.c.v. saline (0.9% NaCl, 5 μl administered over 1 min) and i.v. saline (0.9% NaCl, 0.15 ml given over 30 s); moxonidine given i.c.v. (1 nmol) and i.v. saline; prazosin administered i.v. (0.15 mg given over 30 s) and i.c.v. saline; i.c.v. moxonidine (1 nmol) and i.v. prazosin (0.15 mg). The parameters given in the table are; blood pressure in mmHg, creatinine clearance in ml min^{-1} and heart rate in beats min^{-1}. Each group contained 6–7 rats. The first time period represents a pretreatment 30 min baseline urine collection. Time periods 2 and 3 represent subsequent 30 min urine collections following intervention. The data are presented as the mean ± s.e.mean. $*P<0.05$ between the vehicle and intervention groups; $\#P<0.05$ between the baseline collection and the subsequent collections.

again increased following both doses of moxonidine; however, unlike the sham-operated animals, urine sodium excretion was not increased as compared to the vehicle-treated animals (Figure 2c, d).

Table 2 Effect of experimental intervention on solute and water excretion

	Time period	*Osmolar clearance* ($\mu l\ min^{-1}$)	*Free water clearance* ($\mu l\ min^{-1}$)
Vehicle control	1	50±3	-39±3
	2	77±6	-46±4
	3	103±5	-59±3
Moxonidine	1	53±6	-40±5
	2	82±8	-24±6*
	3	129±7*	-48±8
Prazosin	1	35±4	-30±3
	2	62±6	-48±4
	3	81±10	-61±6
Moxonidine and prazosin	1	40±4	-33±4
	2	62±5	-48±3
	3	85±7	-63±3

The four groups were as follows: vehicle control, i.c.v. saline (0.9% NaCl, 5 µl administered over 1 min) and i.v.saline (0.9% NaCl, 0.15 ml given over 30 s); moxonidine given i.c.v (1 nmol) and i.v. saline; prazosin administered i.v. (0.15 mg given over 30 s) and i.c.v. saline; i.c.v. moxonidine (1 nmol) and i.v. prazosin (0.15 mg) on osmolar clearance in $\mu l\ min^{-1}$ and free water clearance in $\mu l\ min^{-1}$ (6–7 per group). The first time period represents a pretreatment 30 min control urine collection. Time periods 2 and 3 represent 30 min urine collections following interventions. The data presented as the mean ± s.e.mean. $^*P<0.05$ between the vehicle and intervention groups; $\#P<0.05$ between the baseline collection and the subsequent collections.

The osmolar clearance was also increased after administration of i.c.v. moxonidine at 0.3 and 1 nmol in both post treatment collections (Figure 3a). Only the second urine collection of the 0.3 nmol dose of i.c.v. moxonidine resulted in an increase in free water clearance (Figure 3b). Consistent with this lack of effect on sodium excretion in the renal denervated rats, i.c.v. moxonidine at both doses tested also failed to increase osmolar clearance when compared to the control group (Figure 3c). The increase in urine flow rate in the denervated rats following both doses of i.c.v. moxonidine was related to the increase in free water clearance remaining intact (Figure 3d). Thus, the renal denervation blocked the ability of the i.c.v. moxonidine to increase osmolar clearance but not free water clearance.

Discussion

The administration of an I_1-imidazoline receptor agonist, moxonidine, into the lateral cerebral ventricle resulted in an increase in sodium excretion at low doses and an increase in free water clearance at higher doses which was blocked by central administration of idazoxan, an imidazoline receptor antagonist (Penner & Smyth, 1994). Rilmenidine given i.c.v. also resulted in an increase in both solute and water excretion (Penner & Smyth, 1992; 1995). These findings suggest that the renal changes were, at least in part, secondary to the stimulation of the central imidazoline receptor. The mechanism(s) responsible for the increase in urine flow rate and sodium excretion following i.c.v. administration of I_1-imidazoline receptor agonists has not been defined. Since the activation of the peripheral α_1-adrenoceptor (Penner & Smyth, 1988) and changes in the central sympathetic nervous system (Kopp &

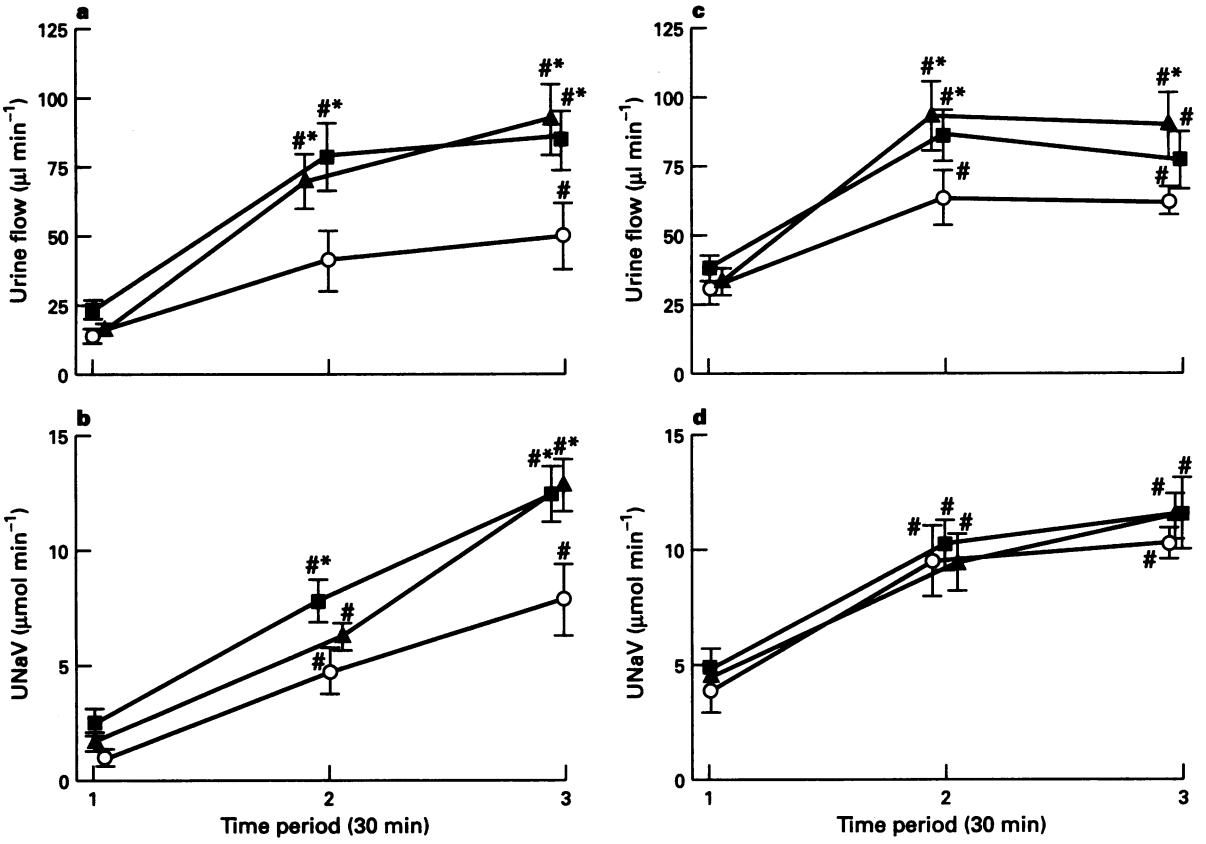

Figure 2 The effects of i.c.v. saline (○) ($n=7$); i.c.v. moxonidine (0.3 nmol) (■) ($n=7$); i.c.v. moxonidine (1 nmol) (▲) ($n=6$) on urine flow rate in $\mu l\ min^{-1}$ (a) and urine sodium excretion in $\mu mol\ min^{-1}$ (b) in sham-denervated animals; urine flow rate in $\mu l\ min^{-1}$ (c) and urine sodium excretion in $\mu mol\ min^{-1}$ (d) in denervated animals (7 per group). The time periods are as described in Figure 1. The data are presented as the mean ± s.e.mean. $^*P<0.05$ between the vehicle and intervention groups; $\#P<0.05$ between the baseline collection and the subsequent collections.

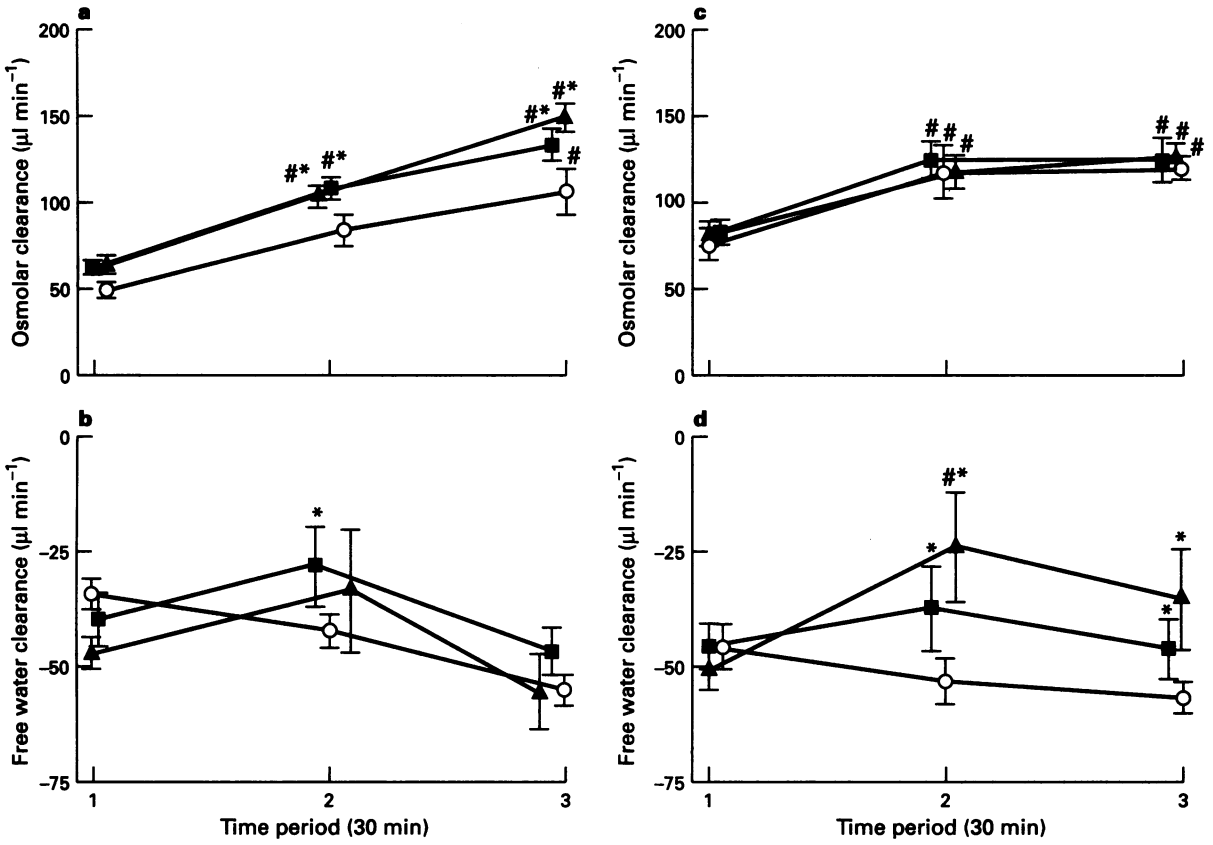

Figure 3 The effects of i.c.v. saline (○) ($n=7$); i.c.v. moxonidine (0.3 nmol) (■) ($n=7$); i.c.v. moxonidine (1 nmol) (▲) ($n=6$) on osmolar clearance in $\mu l\,min^{-1}$ (a) and free water clearance in $\mu l\,min^{-1}$ (b) in sham-denervated animals; osmolar clearance in $\mu l\,min^{-1}$ (c) and free water clearance in $\mu l\,min^{-1}$ (d) in denervated animals (7 per group). The time periods are as described in Figure 1. The data are presented as the mean ± s.e.mean. *$P<0.05$ between the vehicle and intervention groups; #$P<0.05$ between the baseline collection and the subsequent collections.

DiBona, 1992) have been shown to modulate sodium excretion, the possibility of the imidazoline receptor interacting with the activity of the peripheral sympathetic nervous system was considered. Kline & Cechetto (1993) had previously shown that intact renal nerves were necessary for the natriuresis observed following peripheral rilmenidine administration. In the current studies, the administration of intravenous prazosin completely blocked the ability of i.c.v. moxonidine to increase osmolar clearance and free water excretion. In the renal denervated rats, the catecholamine levels were reduced almost to zero, demonstrating the effectiveness of the procedure. Following denervation there was again a complete blockade of the ability of i.c.v. moxonidine to increase osmolar clearance. However, in these denervated rats, urine flow rate was still increased following i.c.v. moxonidine, indicating that the increase in free water clearance remained intact. Thus, the attenuation of the response of increased osmolar clearance and free water clearance following i.v. prazosin but attenuation of only osmolar clearance following renal denervation would be consistent with different mechanisms being responsible for altering renal solute clearance and free water clearance following central administration of an I_1 -imidazoline receptor agonist.

Clonidine and structurally related compounds have been shown to lower blood pressure through stimulation of imidazoline receptors located centrally (Feldman *et al.*, 1990; Tibirica *et al.*, 1991; Gomez *et al.*, 1991). Clonidine, through what now appears to be imidazoline receptors located in the ventrolateral medulla, has been found to lower blood pressure in a number of different species (Gillis *et al.*, 1985; Sinha *et al.*, 1985; McAuley *et al.*, 1988; Feldman *et al.*, 1990). This blood pressure lowering has been attributed to a suppression of catecholaminergic neurones located centrally which in turn would lower peripheral sympathetic nerve activity (Tibirica *et al.*, 1989; 1992). The central nervous system has also been shown to modulate renal sodium excretion through changes in renal nerve activity, irrespective of changes in blood pressure (Koepke *et al.*, 1987). The present results with central administration of moxonidine would be consistent with the central imidazoline receptor also playing a role in the modulation of solute and water handling by the kidney, secondary to changes in activity of the sympathetic nervous system.

Previous studies from our laboratory have demonstrated a dissociation between water and sodium handling by the kidney when various α_2-adrenoceptor or imidazoline receptor agonists were given directly into the renal artery (Smyth *et al.*, 1992). The identification by radioligand binding studies of imidazoline receptors in the kidney (Bidet *et al.*, 1990) supported the possibility that at least part of the effect seen following direct administration into the kidney may be through stimulation of a local imidazoline receptor. Additional studies, using the selective I_1 imidazoline receptor agonist moxonidine, infused directly into the renal artery, demonstrated a dose-related natriuresis (Allan *et al.*, 1993). The specific imidazoline receptor antagonist, idazoxan, inhibited the response but rauwolscine (specific α_2-adrenoceptor antagonist) was without effect. This was consistent with the increase in sodium excretion being secondary to activation of renal imidazoline receptors. At present, it is not clear whether centrally and peripherally I_1 imidazoline receptors function independently of each other to alter sodium and water excretion. However, with moxonidine, peripheral administration increased urine flow rate secondary to an increase in osmolar clearance, while central administration increased both osmolar clearance and free water clearance. The current study supports the concept that the central I_1-imidazoline receptor modulates renal sodium excretion which at least in part is mediated by the peripheral α_1-adrenoceptor. The mechanism for the dissociation between the water and sodium changes observed following

i.c.v. moxonidine is not clear. These observed differences may be due to central interaction with vasopressin, different central areas of activation or different receptors. Future studies will be needed to clarify these issues.

In conclusion, interruption of the sympathetic nervous system either pharmacologically (prazosin) or surgically (renal denervation) attenuated the ability of i.c.v. moxonidine to increase urine flow rate. These results strongly indicate the importance of the sympathetic nervous system in the mediation of this natriuretic response. However, the ability of prazosin, but not renal denervation, to block the increase in free water clearance indicates that i.c.v. moxonidine may be increasing free water clearance and osmolar clearance at two distinct sites and/or receptors in the central nervous system.

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