Br. J. Pharmacol. (1993), **108**, 870–875

Renal imidazoline preferring sites and solute excretion in the rat

*D.R. Allan, *†S.B. Penner & [1]*†D.D. Smyth

Departments of *Internal Medicine and †Pharmacology & Therapeutics, University of Manitoba, 770 Bannatyne Ave., Winnipeg, Manitoba, Canada R3E 0W3

1 Moxonidine has been found to have an approximately 600 fold greater affinity for I_1 imidazoline preferring sites as compared to α_2-adrenoceptors in the rat kidney. The effects of an intrarenal infusion of moxonidine in an anaesthetized rat preparation were investigated and contrasted with the effects previously reported for α_2-adrenoceptor stimulation.

2 An intrarenal infusion of moxonidine (1, 3 and 10 nmol kg^{-1} min^{-1}) produced an increase in urine flow rate and sodium excretion. Moxonidine increased urine volume through an increase in osmolar clearance rather than an increase in free water clearance as previously reported for α_2-adrenoceptor stimulation.

3 The effects of moxonidine also appeared to be unique from the effects of α_2-adrenoceptor stimulation. An imidazoline preferring site specific blocking dose of idazoxan (0.3 mg kg^{-1}), but not an α_2-adrenoceptor specific blocking dose of rauwolscine (0.3 mg kg^{-1}) attenuated the renal effects of moxonidine (3 nmol kg^{-1} min^{-1}). Moreover, unlike α_2-adrenoceptor agonists, the effects of moxonidine were not altered by prior treatment with a V_2 vasopressin receptor antagonist.

4 These results indicate differences between stimulation of α_2-adrenoceptors and I_1 imidazoline preferring sites in the rat kidney and suggest a direct physiological function of renal imidazoline preferring sites.

Keywords: Moxonidine; clonidine; vasopressin (V2) antagonist; natriuresis; α_2-adrenoceptor

Introduction

Several studies (Strandhoy *et al.*, 1982; Blandford & Smyth, 1988; Gesek & Strandhoy, 1990) have suggested a heterogeneous response to α_2-adrenoceptor stimulation in the kidney. Studies in our laboratory have found a dose-selective dissociation of water and solute clearance for α_2-adrenoceptor stimulation in the anaesthetized rat (Blandford & Smyth, 1988). However, recent studies in the rat indicate that this dissociation, may in fact represent two distinct α_2-adrenoceptor subtypes and/or receptors (Smyth *et al.*, 1992). A similar discrepancy had been previously reported for α_2-adrenoceptors in the central nervous system. The antihypertensive effect of clonidine which had initially been thought to function through the α_2-adrenoceptor, was found to be mediated through stimulation of an imidazoline preferring site (IPS) (Bousquet *et al.*, 1984; Ernsberger *et al.*, 1988a). Thus, it appeared that many effects which were previously attributed to the α_2-adrenoceptor may have in fact been mediated through another receptor/site, the imidazoline preferring site.

Radioligand binding studies have also indicated a class of receptors which are distinct from α_2-adrenoceptors. Studies to characterize α_2-adrenoceptors, with the radiolabelled antagonists rauwolscine and idazoxan, indicated that in fact two distinct populations of receptors may be involved (Michel *et al.*, 1989; 1990). Similarly, clonidine was found to label two distinct populations of receptors (Ernsberger *et al.*, 1987; 1988b). Subsequent studies have demonstrated that these non-adrenoceptor binding sites have a low affinity for catecholamines and a greater affinity for imidazolines (Bricca *et al.*, 1989; Ernsberger *et al.*, 1990). The apparent heterogeneity of these imidazoline sites has resulted in the proposal of two subtypes, I_1 and I_2 (Ernsberger, 1992; Reis *et al.*, 1992). The I_1 subtype appears to predominate in the rat kidney (Ernsberger *et al.*, 1992).

Moxonidine was previously reported to act as a central α_2-adrenoceptor agonist in the treatment of hypertension (Planitz, 1984). However, recent studies have found that moxonidine has a very high affinity for I_1-imidazoline sites in the central nervous system with an approximately 70 fold selectivity for I_1 imidazoline sites as compared to α_2-adrenoceptors (Ernsberger *et al.*, 1992). Moreover, radioligand binding studies in the rat renal medulla have shown that moxonidine has a 600 fold higher affinity for the I_1-imidazoline preferring site (IPS) than for the α_{2B}-adrenoceptor (Ernsberger *et al.*, 1992). Conceivably, more specific stimulation of these imidazoline preferring sites may elicit different physiological responses from those previously found for α_2-adrenoceptor agonists. We therefore determined the effects of an intrarenal infusion of moxonidine on water and solute excretion in the intact anaesthetized rat.

Methods

The standard procedures have previously been reported by our laboratory (Blandford & Smyth, 1988; 1990). Briefly, male Sprague-Dawley rats (200–225 g) were obtained from The University of Manitoba (Charles River Breeding Stock) and cared for as per regional animal care standards protocol. They were fed a standard Purina rat chow diet, with free access to tap water, in cages at 22°C with a 12 h light/dark cycle.

The right kidney was removed via a flank incision under ether anaesthesia 7 to 14 days prior to experimentation. Rats were anaesthetized with pentobarbitone (Nembutal, BDH Chemicals, Poole, England, 50 mg kg^{-1}, i.p.). Anaesthesia was supplemented with additional doses of 5 mg kg^{-1} as required. The animal was placed on a Harvard Animal Blanket Control Unit with a rectal thermometer probe set for 37.5°C. A tracheotomy was performed leaving the rat to breathe spontaneously through PE-240 tubing. The left

[1] Author for correspondence.

carotid artery was cannulated with PE-60 tubing and connected to a Statham pressure transducer (Model P23Dc) and Grass Model 5 Polygraph for the monitoring of blood pressure and heart rate. The left jugular vein was cannulated with PE-160 tubing for the infusion of normal saline at 97 $\mu l\ min^{-1}$. A left flank incision was performed and the remaining kidney exposed. The ureter was catheterized for the timed collection of urine into pre-weighed vials. Urine volume was determined gravimetrically. A 31 gauge stainless steel needle was inserted into the renal artery for the infusion of study drug or vehicle with a Harvard sage pump. Following surgery the rat was allowed to stabilize for 45 min before the collection of urine. A 15 min baseline control urine collection was obtained to ensure the surgical procedure had not altered renal function. An intrarenal infusion (3.4 $\mu l\ min^{-1}$) or moxonidine (1, 3 or 10 $nmol\ kg^{-1}\ min^{-1}$) or vehicle (saline) was started and maintained for the duration of the experiment during which 4 consecutive 15 min urine collections were obtained.

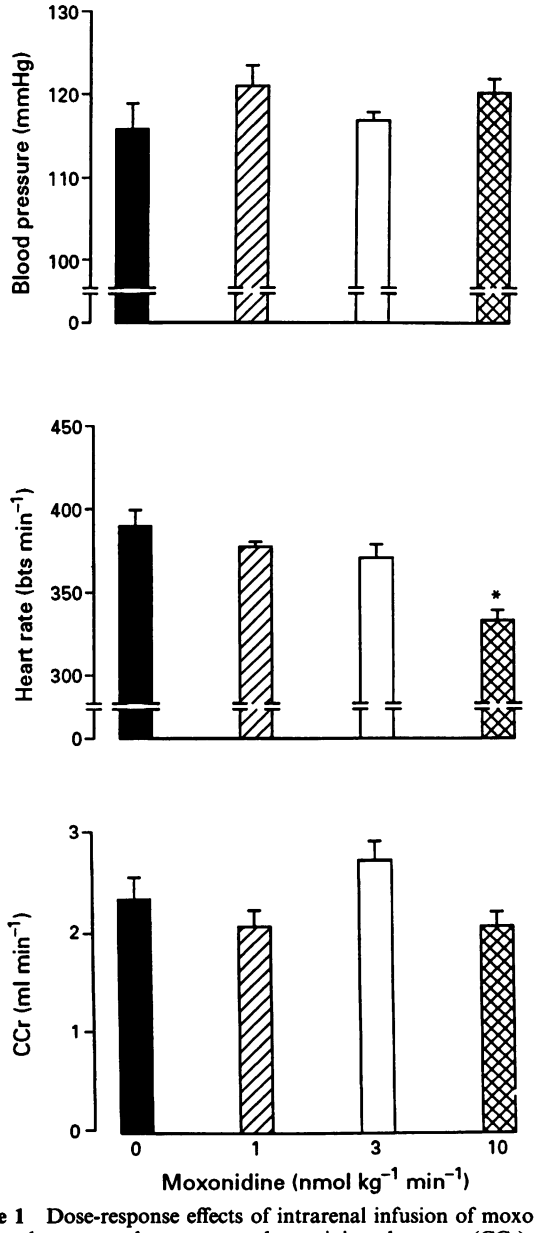

Figure 1 Dose-response effects of intrarenal infusion of moxonidine on blood pressure, heart rate and creatinine clearance (CCr) in the rat. Each group represents the mean ± s.e.mean (vertical bars) of at least 6 experiments. Moxonidine at 0 $nmol\ kg^{-1}\ min^{-1}$ represents vehicle (saline) infusion. $*P<0.05$ versus control vehicle.

V_2-vasopressin receptor antagonist

The role of vasopressin in the renal response to moxonidine was studied by administration of the specific V_2-vasopressin receptor antagonist, [d$(CH_2)_5$, D-Ile^2, Ile^4]-AVP. At the start of the second urine collection, the V_2 antagonist was administered as a slow intravenous bolus. A dose of 30 $nmol\ kg^{-1}$ was used as this dose had previously been demonstrated to block the renal effects of an α_2-adrenoceptor agonist, clonidine (Blandford & Smyth, 1990). Rats were randomly assigned to one of four study groups. Group 1 received an intrarenal infusion of saline (vehicle) at 3.4 $\mu l\ min^{-1}$. Group 2 received an intrarenal infusion of moxonidine at 3 $nmol\ kg^{-1}\ min^{-1}$. Group 3 received intrarenal saline with an intravenous administration of the specific V_2 antagonist. Group 4 received both the intrarenal moxonidine at 3 $nmol\ kg^{-1}\ min^{-1}$ following the intravenous dose of the V_2 antagonist.

Specific α_2-adrenoceptor and IPS antagonists

To determine the specificity of moxonidine, 3 $nmol\ kg^{-1}\ min^{-1}$ for the IPS over the α_2-adrenoceptor, the specific imidazoline site antagonist, idazoxan, or the specific α_2-adrenoceptor antagonist, rauwolscine, were given in a dose of 0.3 $mg\ kg^{-1}$ as a slow i.v. bolus at 30 min into the stabilization period. In the presence of these antagonists, the renal responses to moxonidine and clonidine (0.37 $nmol\ kg^{-1}\ min^{-1}$) were determined.

At the end of the experiment a sample of blood was collected through the carotid line. The plasma was frozen. Methylene blue was injected through the renal arterial line to confirm proper positioning of the needle. Urine and plasma

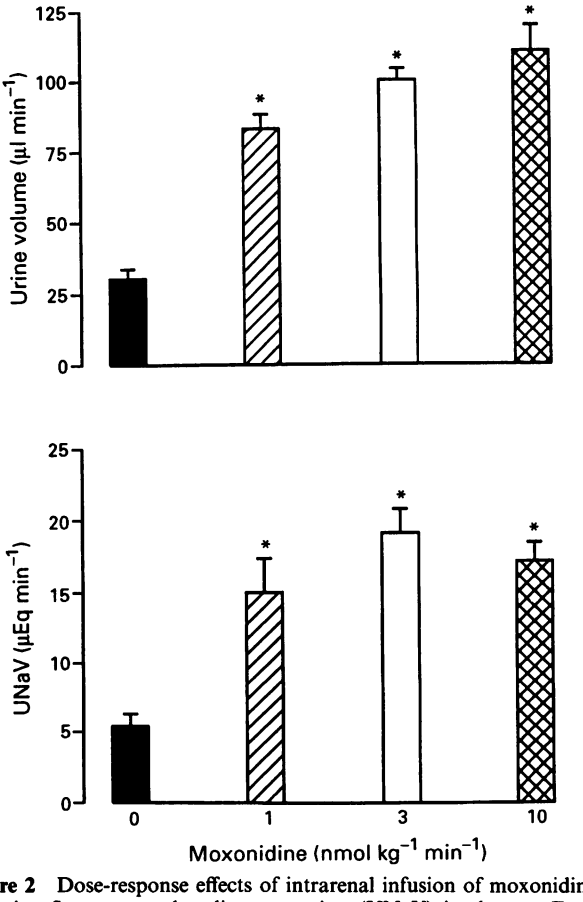

Figure 2 Dose-response effects of intrarenal infusion of moxonidine on urine flow rate and sodium excretion (UNaV) in the rat. Each group represents the mean ± s.e.mean (vertical bars) of at least 6 experiments. $*P<0.05$ versus control vehicle.

creatinine were measured with a Beckman Creatinine 2 Analyzer. Urine and plasma osmolality was measured with a Precision Systems Micro Osmometer. Sodium and potassium levels in urine and plasma were measured by a Beckman Klina Flame Photometer.

Statistical analysis

Each experimental group consisted of 6 rats. Data are presented as the mean ± standard error (s.e.) of the mean. Data were analyzed by repeated measures of analysis of variance. Significant interactions were further analyzed by a one way ANOVA Fisher's least squares difference multiple comparison test (Winer, 1971). A P value of <0.05 was deemed significant and is denoted by a * in the figures. For the purpose of presentation, data from the fifth collection period are shown since this is representative of differences observed between groups.

Drugs

Clonidine (Sigma Chemical Company, St. Louis, MO, U.S.A.); [d(CH_2)$_5$, D-Ile^2, Ile^4]-AVP (Penninsula Laboratories, Inc., Belmont, CA, U.S.A.); rauwolscine (Atomergic Chemicals Corp., Plainview, NY, U.S.A.); idazoxan (Research Biochemicals, Inc., Natick, MA, U.S.A.) and moxonidine (Supplied by Beiersdorf, AG, Hamburg, Germany) were used.

Results

The first urine collection for each experiment served as a preparation control (data not shown). This allowed evaluation of baseline renal function for each experiment and the determination of altered values secondary to the surgical procedure. In all groups studied, the measured parameters did not differ between groups during this first collection period prior to any experimental intervention. The fifth collection period is representative of the differences observed between groups and consequently these data are presented in detail.

In the present study, moxonidine failed to alter blood pressure and creatinine clearance at all three doses investigated (Figure 1). The highest infusion rate investigated, however, did produce a small but significant decrease in heart rate. Moxonidine increased urine flow rate and sodium excretion but not potassium excretion (data not shown) at all three doses investigated (Figure 2). The above increase in urine flow rate was secondary to an increase in solute excretion as reflected by an increase in osmolar clearance without a change in free water clearance (Figure 3).

The increase in osmolar clearance and not free water clearance reported above suggested that moxonidine, unlike α_2-adrenoceptor agonists, was mediating these effects independent of vasopressin. The significance of vasopressin in the above response to moxonidine was determined by the pretreatment of rats with a specific V_2 vasopressin receptor antagonist (Figure 4). As shown above in Figure 3, moxonidine alone increased urine volume primarily through an increase in solute (sodium) excretion. The V_2 antagonist alone increased urine volume by increasing free water clearance. When moxonidine was infused in the presence of the V_2 antagonist, a further increase in urine flow rate but not sodium excretion was observed. No significant differences in blood pressure, heart rate, creatinine clearance or potassium excretion were observed (data not shown).

A third series of experiments involved the use of the imidazoline antagonist, idazoxan, and the α_2-adrenoceptor antagonist, rauwolscine. As shown above, moxonidine increased urine flow rate and urine sodium excretion. Idazoxan (IPS antagonist) but not rauwolscine (α_2-adrenoceptor antagonist) significantly attenuated the renal effects of moxonidine (Figure 5). Conversely the same dose of rauwolscine, which failed to block the action of moxonidine, blocked the effects of clonidine (Figure 6). Consistent with two sites of action, idazoxan pretreatment at a dose that blocked the effects of moxonidine failed to alter the effect of clonidine.

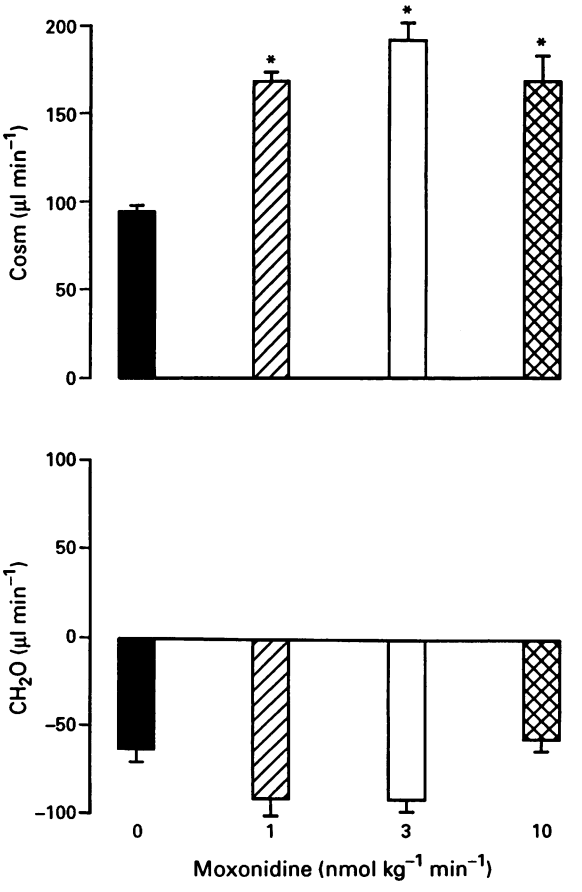

Figure 3 Dose-response effects of intrarenal infusion of moxonidine on osmolar clearance (Cosm) and free water clearance (CH_2O) in the rat. Each group represents the mean ± s.e.mean (vertical bars) of at least 6 experiments. *$P<0.05$ versus control vehicle.

Discussion

Both moxonidine and clonidine contain an imidazolidine ring structure and a phenylephrine catecholamine structure (Planitz, 1984). Despite this similarity in structure, recent radioligand binding studies suggest that moxonidine has a much greater affinity for the IPS than clonidine (Ernsberger *et al.*, 1992). The significance of the IPS was initially reported by Bousquet *et al.* (1984). These studies divided the effects of agonists, following microinjection into the nucleus reticularis lateralis, based on structure rather than previously reported specificity for α_2-adrenoceptors. They found that α_2-adrenoceptor agonists with a basic phenylethylamine structure, such as noradrenaline or α-methylnoradrenaline failed to lower the blood pressure. However, when agonists with an imidazoline moiety were injected into this same area, the blood pressure was found to drop significantly. These studies questioned whether the centrally mediated blood pressure lowering effects of many purported α_2-adrenoceptor agonists was in fact mediated at α_2-adrenoceptors.

The majority of functional studies have concentrated on the hypotensive effects of compounds with imidazoline structures as mediated through the central nervous system. However, imidazoline preferring sites have also been identified in the kidney (Michel & Insel, 1989; Parini *et al.*, 1989; Bidet *et*

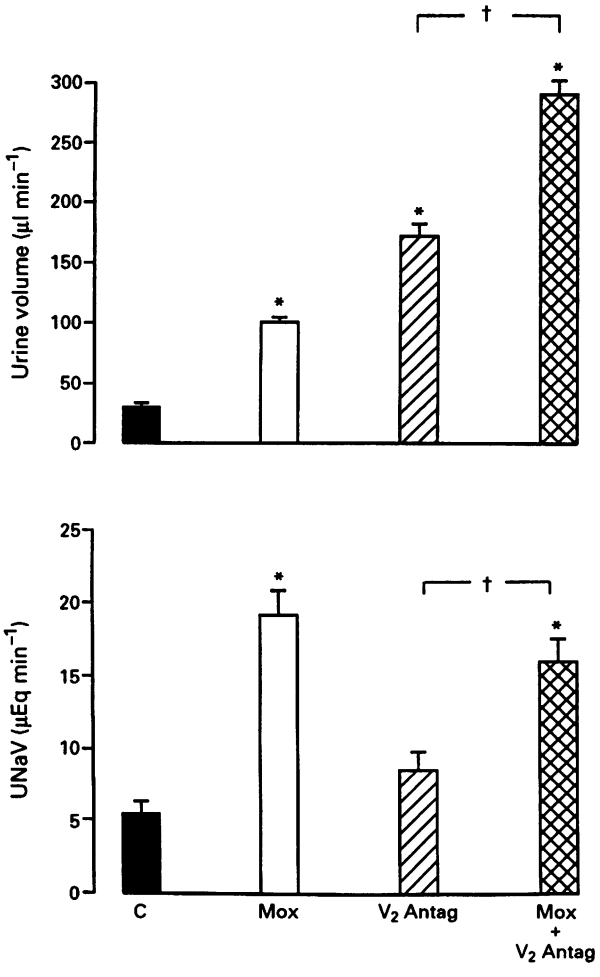

Figure 4 Effects of moxonidine in the presence and absence of a specific V_2 antagonist on urine volume and sodium excretion (UNaV) in the rat. C, control; Mox, moxonidine (3 nmol kg^{-1} min^{-1}); V_2 Antag, V_2 vasopressin receptor antagonist (30 nmol kg^{-1}); Mox + V_2 Antag, moxonidine following V_2 antagonist pretreatment. Each group represents the mean ± s.e.mean (vertical bars) of at least 6 experiments. $^*P<0.05$ versus control; $\dagger P<0.05$ between groups.

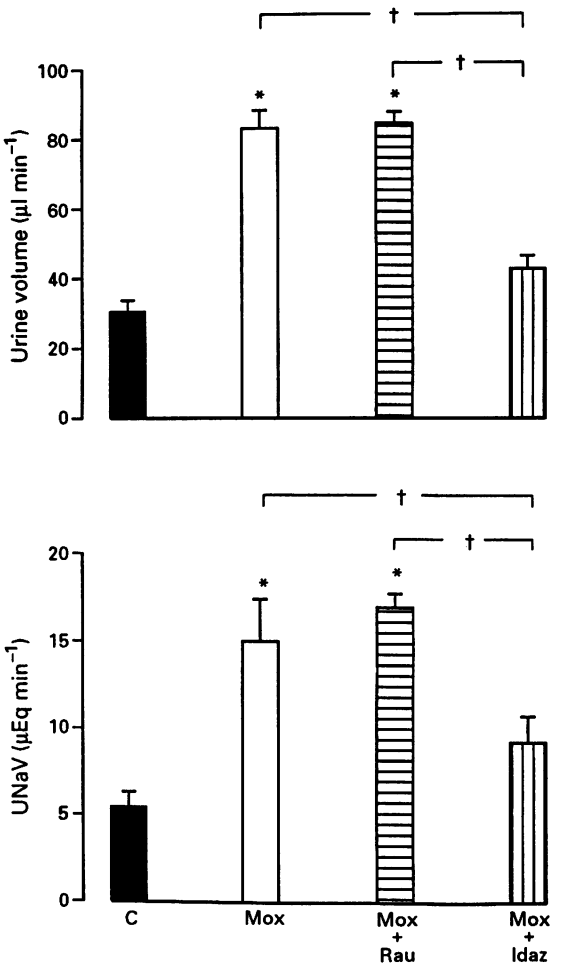

Figure 5 Effects of an intrarenal infusion of moxonidine in the absence and presence of a specific α_2-adrenoceptor antagonist (rauwolscine) and a specific IPS antagonist (idazoxan). C, vehicle control; Mox, moxonidine (3 nmol kg^{-1} min^{-1}); Mox + Rau, moxonidine (3 nmol kg^{-1} min^{-1}) + rauwolscine (0.3 mg kg^{-1}); Mox + Idaz, moxonidine (3 nmol kg^{-1} min^{-1}) + idazoxan (0.3 mg kg^{-1}). Each group represents the mean ± s.e.mean (vertical bars) of at least 6 experiments. $^*P<0.05$ versus control; $\dagger P<0.05$ between groups.

al., 1990). Studies with isolated renal tubular cells found that $^{22}Na^+$ entry could be blocked by idazoxan. A concurrent cellular alkalinization suggested that the IPS was associated with the $Na^+ - H^+$ antiporter. The effect of stimulating these sites on solute and water excretion in the intact animal had not been determined even though these sites had been identified in the kidney biochemically.

The above studies indicated that many effects previously attributed to activation of the α_2-adrenoceptor may in fact involve another site. Studies which have centred on the renal effects of purported α_2-adrenoceptor agonists have also indicated that more than one site and/or receptor may be involved. Strandhoy *et al.* (1982) first showed that a systemic intravenous infusion of clonidine in the dog produced a greater increase in urine flow rate and sodium excretion than a direct intrarenal infusion. Studies in our laboratory have focussed on the ability of a number of α_2-adrenoceptor agonists to increase urine flow rate based on changes in water (free water clearance) and/or solute (osmolar clearance) excretion. Results of a number of studies have been consistent with the effects of α_2-adrenoceptor agonists being mediated at more than one site and/or receptor. For example, low intrarenal infusion rates of clonidine increase free water clearance, while higher infusion rates also increase osmolar clearance (Blandford & Smyth, 1988). Although intrarenal infusion of clonidine produced an increase in urine flow rate secondary to an increase in free water clearance, intravenous infusions were found to increase osmolar clearance (Blandford & Smyth, 1989). Indomethacin pretreatment also had disparate effects, with an enhancement of the osmolar effects and an attenuation of the free water effects of clonidine (Blandford & Smyth, 1991). Thus, as with central administration of α_2-adrenoceptor agonists, the effects in the periphery also indicated other sites may be involved.

A recent study in our laboratory compared the ability of three purported α_2-adrenoceptor agonists to increase water (free water) and solute (osmolar) excretion (Smyth *et al.*, 1992). Clonidine and 2,6 dimethyl clonidine, two purported α_2-adrenoceptor agonists (Fondacaro *et al.*, 1989), displayed similar potencies in the ability to increase urine flow rate. However, the effects of clonidine were mediated primarily by an increase in free water clearance and the effects of 2,6 dimethyl clonidine were mediated primarily by an increase in osmolar clearance. Moreover, pretreatment with a specific V_2 vasopressin-specific antagonist completely attenuated the renal actions of clonidine but not of 2,6 dimethyl clonidine. These findings were consistent with the existence of two subtypes of α_2-adrenoceptors and/or two unique receptors. Although not previously documented, 2,6, dimethyl clonidine has been found to possess a high affinity for IPSs (Dr Paul Ernsberger, personal communication). Consequently, in the

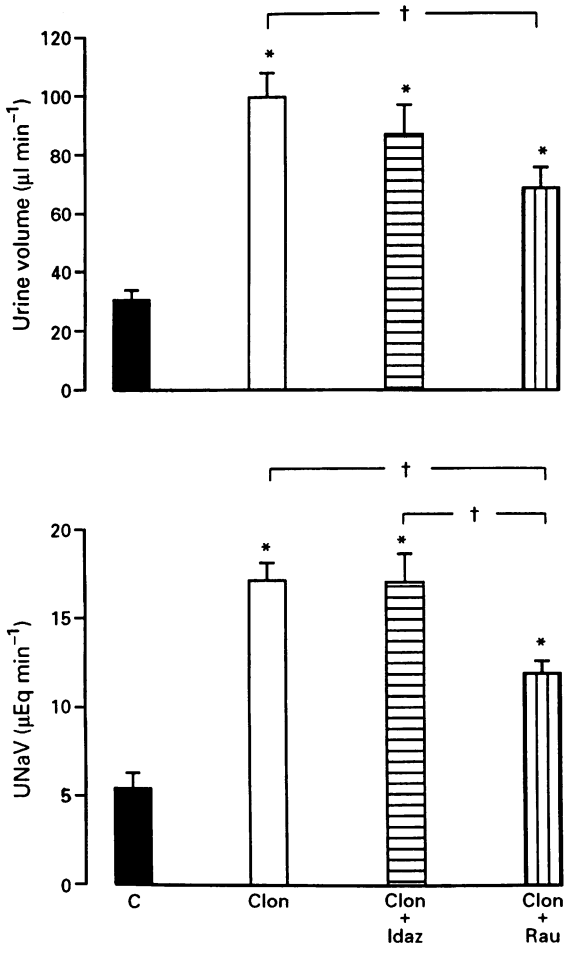

Figure 6 Effects of an intrarenal infusion of clonidine in the absence and presence of a specific α_2-adrenoceptor antagonist (rauwolscine) and a specific IPS antagonist (idazoxan). C, vehicle control; Clon, clonidine (0.37 nmol kg^{-1} min^{-1}); Clon + Idaz, clonidine (0.37 nmol kg^{-1} min^{-1}) + idazoxan (0.3 mg kg^{-1}); Clon + Rau, clonidine (0.37 nmol kg^{-1} min^{-1}) + rauwolscine (0.3 mg kg^{-1}). Each group represents the mean ± s.e.mean (vertical bars) of at least 6 experiments. *$P<0.05$ versus control; †$P<0.05$ between groups.

present study, we investigated the effects of a specific IPS agonist, moxonidine, on renal function.

The present studies provide three separate lines of evidence that stimulation of the IPS represents a distinct site from the α_2-adrenoceptor. Moxonidine produced a marked enhancement of sodium excretion and osmolar clearance at all 3 doses tested with no change in free water clearance. Studies with α_2-adrenoceptor agonists have shown an increase in urine flow rate associated with an increase in free water clearance (Stanton *et al.*, 1987; Blandford & Smyth, 1988). Only a very modest increase in osmolar clearance was reported following α_2-adrenoceptor stimulation. These different effects on free water clearance suggested that stimulation of α_2-adrenoceptors but not the IPS may be dependent on the renal effects of vasopressin. Previous studies in our laboratory demonstrated that pretreatment with a vasopressin V_2 receptor antagonist to block the effects of vasopressin completely attenuated the effect of α_2-adrenoceptor stimulation with low dose clonidine (Blandford & Smyth, 1990). These results were consistent with previous studies which had reported that the effects of renal α_2-adrenoceptor stimulation in the rat, both *in vivo* and *in vitro*, were dependent on the presence of vasopressin (Krothapalli *et al.*, 1983; Smyth *et al.*, 1985; Pettinger *et al.*, 1987). In contrast, administration of moxonidine in the presence of a V_2 antagonist increased urine flow rate further than that observed with the V_2 antagonist alone. The failure of the V_2 antagonist to attenuate the response to moxonidine is consistent with this site functioning independently of vasopressin.

Another line of evidence which suggests that the α_2-adrenoceptor and the IPS represent distinct sites and subserve distinct functions involved the use of idazoxan and rauwolscine at doses that were specific for the imidazoline preferring site and the α_2-adrenoceptor respectively. A number of studies had found that idazoxan bound with a high affinity to non-adrenoceptor sites and that these sites were different from α_2-adrenoceptors (Michel *et al.*, 1989; 1990). We therefore used idazoxan to antagonize these non-adrenoceptor sites, which were conceivably the sites at which the effects of moxonidine were mediated. Idazoxan but not rauwolscine blocked the enhancement of urine volume, sodium excretion and osmolar clearance caused by moxonidine. These studies in themselves did not support the specificity of the antagonists. However rauwolscine, at the same dose that failed to block the effects of moxonidine, attenuated the effects of clonidine. Similarly, idazoxan, at doses that blocked moxonidine, failed to alter the effects of clonidine. The selective blockade of these to agonists by rauwolscine and idazoxan provides support for the actions being mediated at two distinct sites.

These three lines of evidence provide strong support that stimulation of the I_1-imidazoline preferring site with moxonidine increases solute excretion at a site distinct from the α_2-adrenoceptor. As distinct from α_2-adrenoceptors, it appears that I_1-imidazoline preferring sites increase urine flow rate secondarily to an increase in solute excretion and this effect is independent of the renal action of vasopressin.

D.D.S. is the recipient of a Scientist Award from the Medical Research Council of Canada. D.R.A. is the recipient of a Clinical Fellowship from the Medical Research Council of Canada. This work was supported by the Medical Research Council of Canada. The authors wish to express their gratitude to Catherine Lydon-Hassen for her expert technical assistance.

References

BIDET, M., POUJEOL, P. & PARINI, A. (1990). Effect of imidazolines on Na^+ transport and intracellular pH in renal proximal tubule cells. *Biochim. Biophys. Acta*, **1024**, 173–178.

BLANDFORD, D.E. & SMYTH, D.D. (1988). Dose selective dissociation of water and solute excretion after alpha-2 adrenoceptor stimulation. *J. Pharmacol. Exp. Ther.*, **247**, 1181–1186.

BLANDFORD, D.E. & SMYTH, D.D. (1989). Enhanced natriuretic potency of intravenous clonidine: exta renal site of action? *Eur. J. Pharmacol.*, **174**, 181–188.

BLANDFORD, D.E. & SMYTH, D.D. (1990). Role of vasopressin in response to intrarenal infusions of alpha-2 adrenoceptor agonists. *J. Pharmacol. Exp. Ther.*, **255**, 264–270.

BLANDFORD, D.E. & SMYTH, D.D. (1991). Potentiation of the effects of renal α_2-adrenoceptor stimulation by indomethacin. *Can. J. Physiol. Pharmacol.*, **69**, 1196–1203.

BOUSQUET, P., FELDMAN, J. & SCHARTZ, J. (1984). Central cardiovascular effects of the alpha adrenergic drugs: differences between catecholamines and imidazolines. *J. Pharmacol. Exp. Ther.*, **230**, 232–236.

BRICCA, G., DONTENWILL, M., MOLINES, A., FELDMAN, J., BELCOURT, A. & BOUSQUET, P. (1989). The imidazoline preferring receptor: binding studies in bovine, rat and human brainstem. *Eur. J. Pharmacol.*, **162**, 1–9.

ERNSBERGER, P. (1992). Heterogeniety of imidazoline binding sites: proposed I1 and I2 subtypes. *Fundam. Clin. Pharmacol.*, **6** (suppl 1), 55s, (abstract).

ERNSBERGER, P., GIULIANO, R., WILLETTE, R.N., GRANATA, A.R. & REIS, D.J. (1988a). Hypotensive action of clonidine analogues correlates with binding affinity at imidazole and not alpha-2-adrenergic receptors in the rostral ventrolateral medulla. *J. Hypertens.*, **6** (suppl 4), S554–S557.

ERNSBERGER, P., GIULIANO, R., WILLETTE, R.N. & REIS, D.J. (1990). Role of imidazoline receptors in the vasodepressor response to clonidine analogs in the rostral ventrolateral medulla. *J. Pharmacol. Exp. Ther.*, **253,** 408–418.

ERNSBERGER, P., MEELY, M.P., MANN, J.J. & REIS, D.J. (1987). Clonidine binds to imidazoline binding sites as well as α_2-adrenoceptors in the ventrolateral medulla. *Eur. J. Pharmacol.*, **134,** 1–13.

ERNSBERGER, P., MEELY, M.P. & REIS, D.J. (1988B). An endogenous substance with clonidine-like properties: selective binding to imidazole sites in the ventrolateral medulla. *Brain Res.*, **441,** 309–318.

ERNSBERGER, P., WESTBROOKS, K.L., CHRISTEN, M.O. & SCHAFER, S.G. (1992). A second generation of centrally acting antihypertensive agents act on putative I_1-imidazoline receptors. *J. Cardiovasc. Pharmacol.*, **20** (suppl 4), (in press).

FONDACARO, J.D., MCCAFFERTY, G.P., KOLPAK, D.C. & SMITH, P.L. (1989). Antidiarrheal activity of alpha-2 adrenoceptor agonist SK&F 35886. *J. Pharmacol. Exp. Ther.*, **249,** 221–228.

GESEK, F.A. & STRANDHOY, J.W. (1990). Dual interactions between alpha-2 adrenoceptor agonists and the proximal Na-H exchanger. *Am. J. Physiol.*, **258,** F636–F642.

KROTHOPALLI, R.K., DUFFY, W.B., SENEKJIAN, H.O. & SUKI, W.N. (1983). Modulation of the hydroosmotic effect of vasopressin on the rabbit cortical collecting tubule by adrenergic agents. *J. Clin. Invest.*, **72,** 287–294.

MICHEL, M.C. & INSEL, P.A. (1989). Are there multiple imidazoline binding sites. *Trends Pharmacol. Sci.*, **10,** 342–344.

MICHEL, M.C., BRODDE, O.-E., SCHNEPEL, B., BEHRENDT, J., TSCHADA, R., MOTULSKY, H.J. & INSEL, P.A. (1989). [^{3}H]idazoxan and some other alpha-2 adrenergic drugs also bind with a high affinity to a non-adrenergic site. *Mol. Pharmacol.*, **35,** 324–330.

MICHEL, M.C., REGAN, J.W., GERHARDT, M.A., NEUBIG, R.R., INSEL, P.A. & MOTULSKY, H.J. (1990). Nonadrenergic [^{3}H]idazoxan binding sites are physically distinct from alpha-2 adrenergic receptors. *Mol. Pharmacol.*, **37,** 65–68.

PARINI, A., COUPRY, I., GRAHAM, R.M., UZIELLI, I., ATLAS, D. & LANIER, S.M. (1989). Characterization of an imidazoline/guanidinium receptive site distinct from the alpha-2 adrenergic receptor. *J. Biol. Chem.*, **264,** 11874–11878.

PETTINGER, W.A., UMEMURA, S., SMYTH, D.D. & JEFFRIES, W.B. (1987). Renal alpha-2 adrenoceptors and the adenylate cyclase-cAMP system: Biochemical and physiological interactions. *Am. J. Physiol.*, **252,** F199–F208.

PLANITZ, V. (1984). Crossover comparison of moxonidine and clonidine in mild to moderate hypertension. *Eur. J. Clin. Pharmacol.*, **27,** 147–152.

REIS, D.J., REGUNATHAN, S., WANG, H., FEINSTEIN, D.L. & MEELEY, M.P. (1992). Imidazoline receptors in the nervous system. *Fundam. Clin. Pharmacol.*, **6** (suppl 1), 23s–29s.

SMYTH, D.D., LI, P., BLANDFORD, D.E. & PENNER, S.B. (1992). Opposite rank order of potency for alpha-2 adrenoceptor agonists on water and solute excretion in the rat: Two sites and/or receptors. *J. Pharmacol. Exp. Ther.*, **261,** 1080–1086.

SMYTH, D.D., UMEMURA, S. & PETTINGER, W.A. (1985). Renal nerve stimulation causes alpha-1 adrenoceptor mediated sodium retention but not alpha-2 adrenoceptor antagonism of vasopressin. *Circ. Res.*, **57,** 304–311.

STRANDHOY, J.W., MORRIS, M. & BUCKALEW, JR., V. (1982). Renal effects on the antihypertensive, guanabenz in the dog. *J. Pharmacol. Exp. Ther.*, **221,** 347–352.

STANTON, B., PUGLISI, E. & GELLAI, M. (1987). Localization of alpha-2 adrenoceptor-mediated increase in renal Na^+, K^+, and water excretion. *Am. J. Physiol.*, **252,** F1016–1021.

WINER, B.J. (1971). *Statistical Principles in Experimental Design*, 2nd ed. New York: McGraw-Hill.

(Received August 12, 1992
Revised October 30, 1992
Accepted November 4, 1992)