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Urinary responses to acute moxonidine are inhibited by natriuretic peptide receptor antagonist

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1 We have previously shown that acute intravenous injections of moxonidine and clonidine increase plasma atrial natriuretic peptide (ANP), a vasodilator, diuretic and natriuretic hormone. We hypothesized that moxonidine stimulates the release of ANP, which would act on its renal receptors to cause diuresis and natriuresis, and these effects may be altered in hypertension.

2 Moxonidine (0, 10, 50, 100 or 150 μg in 300 μl saline) and clonidine (0, 1, 5 or 10 μg in 300 μl saline) injected intravenously in conscious normally hydrated normotensive Sprague–Dawley rats (SD, $\sim$200 g) and 12–14-week-old Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) dose-dependently stimulated diuresis, natriuresis, kaliuresis and cGMP excretion, with these effects being more pronounced during the first hour post-injection. The actions of 5 μg clonidine and 50 μg moxonidine were inhibited by yohimbine, an α_2-adrenoceptor antagonist, and efaroxan, an imidazoline I_1-receptor antagonist.

3 Moxonidine (100 μg) stimulated ($P<0.01$) diuresis in SHR (0.21 ± 0.04 vs 1.16 ± 0.06 ml h^{-1} 100 g^{-1}), SD (0.42 ± 0.06 vs 1.56 ± 0.19 ml h^{-1} 100 g^{-1}) and WKY (0.12 ± 0.04 vs 1.44 ± 0.21 ml h^{-1} 100 g^{-1}). Moxonidine-stimulated urine output was lower in SHR than in SD and WKY. Moxonidine-stimulated sodium and potassium excretions were lower in SHR than in SD, but not WKY, demonstrating an influence of strain but not of pressure. Pretreatment with the natriuretic peptide antagonist anantin (5 or 10 μg) resulted in dose-dependent inhibition of moxonidine-stimulated urinary actions. Anantin (10 μg) inhibited ($P<0.01$) urine output to 0.38 ± 0.06, 0.12 ± 0.01, and 0.16 ± 0.04 ml h^{-1} 100 g^{-1} in SD, WKY, and SHR, respectively. Moxonidine increased ($P<0.01$) plasma ANP in SD (417 ± 58 vs 1021 ± 112 pg ml^{-1}) and WKY (309 ± 59 vs 1433 ± 187 pg ml^{-1}), and in SHR (853 ± 96 vs 1879 ± 229 pg ml^{-1}).

4 These results demonstrate that natriuretic peptides mediate the urinary actions of moxonidine through natriuretic peptide receptors.
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Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; I_1-receptors, imidazoline I_1-receptors; NPR-A, natriuretic peptide receptor-A; SD, Sprague–Dawley; SHR, spontaneously hypertensive rats; WKY, Wistar–Kyoto

Introduction

Centrally acting antihypertensive compounds, such as clonidine, moxonidine, and rilmenidine, reduce blood pressure by acting, albeit with different affinities, on α_2-adrenoceptors and imidazoline I_1-receptors (I_1-receptors), resulting in sympathoinhibition. In addition, these drugs may directly act on the kidneys to stimulate diuresis and natriuresis, thus contributing to short- and long-term control of blood pressure (Ziegler *et al*., 1996; Ernsberger, 2000).

Several groups have investigated the mechanisms involved in the renal responses to acute injections of these centrally acting compounds. Smyth & Penner (1998) have shown that intracerebroventricular administration of moxonidine produces significant increases in urine and sodium excretion that are totally blocked by intravenous prazosin, implicating inhibition of renal nerve activity and subsequent α_1-adrenoceptor stimulation in these effects. Further studies have shown that, independent of the renal nerves and vasopressin, moxonidine may exert direct effects on the kidney to cause diuresis and natriuresis (Allan *et al*., 1993; Smyth & Penner, 1998), by acting on its receptors in the proximal tubules (Bidet *et al*., 1990; Limon *et al*., 1992; Li & Smyth, 1993a; Bohmann *et al*., 1994; Greven & von Bronewski-Schwarzer, 2001) to inhibit the Na^+–H^+ exchanger (Schlatter *et al*., 1997).

Comparing two imidazoline compounds with different affinities for I_1-receptors vs α_2-adrenoceptors, Hohage *et al*. (1997b) reported that intravenous moxonidine, which binds to I_1-receptors with greater affinity than clonidine, transiently increased fractional fluid and sodium excretion in anesthetized rats, whereas equal concentrations of clonidine resulted in a sustained increase in fractional fluid excretion. The effects

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were inhibited by selective antagonists, leading to the conclusion that the drugs acted on two different receptors (Hohage *et al*., 1997b).

In contrast, Hohage *et al*. (1997a) demonstrated, in spontaneously hypertensive rats (SHR), that moxonidine, but not equal concentrations of clonidine, stimulated diuresis and natriuresis, and that the effects were long-lasting, suggesting altered response in hypertension. However, the influence of hypertension was not investigated, as the study did not include normotensive controls. Li *et al*. (1994) reported that the renal effects of intravenous moxonidine were attenuated in 1 kidney-1 clip (1K1C) hypertensive rats compared to sham controls, and attributed the attenuation to downregulation of renal imidazoline receptors in this model (Li & Smyth, 1993a).

In earlier studies, we have demonstrated that acute intravenous injections of various doses of clonidine and moxonidine in normotensive Sprague–Dawley (SD) rats evoked dose-dependent diuresis and natriuresis, and contrary to the finding of Hohage *et al*. (1997a, b), clonidine was at least 10 times more potent that moxonidine. The renal effects were inhibited by yohimbine as well as by efaroxan, a selective imidazoline I_1-receptor antagonist (Mukaddam-Daher & Gutkowska, 2000). We also reported preliminary results (Mukaddam-Daher & Gutkowska, 1999) from SHR and Wistar–Kyoto (WKY) rats, showing that moxonidine significantly stimulated diuresis and natriuresis and that the effects were totally inhibited by efaroxan and partially by yohimbine, indicating that the effects of moxonidine were primarily mediated by imidazoline receptors. Other experiments revealed that the efaroxan dose used in that study (500 μg per rat) was too high to correctly draw a conclusion on the receptor type mediating these effects. Our investigations, however, revealed that acute treatment with clonidine and moxonidine was associated with a dose-dependent increase of plasma atrial natriuretic peptide (ANP) and urinary excretion of cGMP, an index of natriuretic peptide activity. ANP is a potent vasodilator, diuretic and natriuretic hormone, primarily of cardiac origin. Accordingly, we proposed a new mechanism of action of moxonidine, namely, the involvement of natriuretic peptides in these effects. Direct proof of this hypothesis remained to be performed.

Owing to controversial reports on the mechanisms and receptor type(s) involved in the renal effects of imidazoline compounds, and regulation in hypertension, the present study was designed to examine renal responses to acute intravenous moxonidine and clonidine in conscious hypertensive rats (SHR) compared to two normotensive models, SD and WKY rats. The direct involvement of natriuretic peptides in these effects was demonstrated by using anantin, the first microbially produced competitive peptide antagonist of natriuretic peptides. At doses that do not evoke agonistic effects, anantin dose-dependently inhibits ANP-induced intracellular cGMP accumulation in bovine aorta smooth muscle cells (Weber *et al*., 1991; Wyss *et al*., 1991).

Methods

Female SHR (12–14 weeks old) and age-matched normotensive WKY, as well as normotensive SD rats ($\sim$200 g) purchased from Charles River (St-Constant, Quebec, Canada), were housed in a temperature- and light-controlled room with free access to food and water. The two normotensive control groups were used to investigate the selective effect of blood pressure without the confounding influence of genetic background. Experiments were approved by the Animal Care Committee of the CHUM, according to the Canadian Council on Animal Care guidelines.

All experiments were started in the morning (around 08:00 h). One dose of moxonidine (0, 1, 10, 50, 100 or 150 μg) or the reference drug clonidine (0, 1, 5, or 10 μg) was injected into the tail vein in different groups. The injection procedure took about 60 s. Then, the rats were placed individually in Nalgene plastic metabolic cages (Braintree Scientific, Inc., Braintree, MA, U.S.A.) without food and water. Spontaneously voided urine was collected every hour, over four consecutive hours, for the measurement of urine volume and electrolyte excretion. In other experiments, rats were injected with 50 μg moxonidine or 5 μg clonidine after 10-min pretreatment with efaroxan (250 or 25 μg) or yohimbine (50 or 25 μg) in 300 μl saline.

The contribution of natriuretic peptides to the urinary effects of 100 μg moxonidine was investigated in separate groups of rats, after 10-min pretreatment with anantin (5 or 10 μg per rat). The anantin doses were chosen in preliminary experiments on normotensive SD rats and shown to dose-dependently inhibit diuresis and natriuresis, as well as cGMP excretion, evoked by acute volume expansion (by rapid injection of 6 ml isotonic saline), the primary stimulus of natriuretic peptide release.

Separate groups of rats were killed by decapitation 15–20 min after injection of moxonidine (100 μg) or an equal volume of saline vehicle. Blood was collected in prechilled tubes containing protease inhibitors in a final concentration: 1 mmol l^{-1} EDTA, 5 μmol l^{-1} Pepstatin A, and 10 μmol l^{-1} phenylmethylsulfonyl fluoride (PMSF), and immunoreactive ANP was measured in extracted plasma by a specific radioimmunoassay as described previously (Gutkowska, 1987). Urinary cGMP was quantified by radioimmunoassay according to a previously described method (Gutkowska *et al*., 1997). Urinary sodium and potassium concentrations were measured with a flame photometer (Perkin-Elmer 51, Norwalk, CT, U.S.A.), and excretions per hour were calculated. Although the rats had almost similar body weight ($\sim$200 g), their renal parameters were normalized to percent body weight to avoid the effect of any body weight variation among the different groups.

Drugs

Moxonidine (kindly provided by Solvay Pharmaceuticals, Hannover, Germany) was dissolved in 0.1 mol l^{-1} acetic acid, and its volume was brought up to the required concentration with normal saline. Clonidine, efaroxan, and yohimbine (Sigma-Aldrich, St Louis, MO, U.S.A.) were dissolved in saline. Anantin (Cedarlane Laboratories Ltd, Hornby, ON, Canada) was dissolved in 50% acetic acid, aliquoted, and stored at -20°C. On the day of the experiment, aliquots were diluted to 5 or 10 μg in 300 μl saline. All solutions were freshly prepared on the day of the experiments.

Data analysis

Statistical analysis of data obtained from normotensive SD and WKY rats and hypertensive SHR with and without

different treatments was performed by ANOVA, followed by Neuman–Keuls multiple comparison test. $P<0.05$ was considered significant. All data are expressed as mean ± s.e.m.

Results

Renal parameters measured over 4 h post-moxonidine and clonidine in normotensive SD and WKY rats and hypertensive SHR revealed that the effects were more pronounced during the first hour of treatment. First hour urine output after injection of saline vehicle was higher in SD ($0.42\pm0.06\,ml^{-1}\,h^{-1}$ $100\,g^{-1}$ body weight, $n=13$, $P<0.001$) than in WKY ($0.12\pm0.04\,ml^{-1}\,h^{-1}$ $100\,g^{-1}$, $n=26$) and SHR ($0.21\pm0.04\,ml^{-1}\,h^{-1}$ $100\,g^{-1}$, $n=16$). Increasing doses of clonidine (Figure 1) and moxonidine (Figure 2) stimulated the excretion of urine, sodium, potassium, and cGMP in a dose-dependent manner, with similar profiles.

The renal responses to 5 μg clonidine and 50 μg moxonidine and inhibition by yohimbine and efaroxan are depicted in Figures 3 and 4. Yohimbine at 25 μg (data not shown) tended to, but did not significantly suppress, the renal parameters evoked by clonidine and moxonidine. At 50 μg, the inhibitory effect of yohimbine was more evident. Efaroxan at 25 μg *significantly* inhibited and at 250 μg (data not shown) it *totally* suppressed both clonidine- and moxonidine-stimulated renal parameters. Thus, α_2-adrenoceptors and I_1-receptors are implicated in these renal responses.

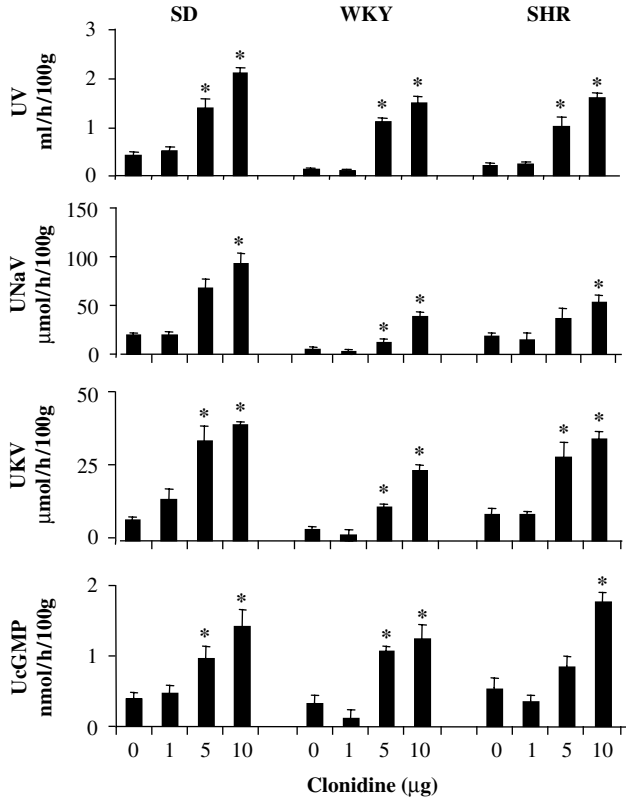

Figure 1 Effect of increasing doses of clonidine on urine output (UV, $ml\,h^{-1}$ $100\,g^{-1}$), sodium (UNaV, $\mu mol\,h^{-1}$ $100\,g^{-1}$), potassium (UKV, $\mu mol\,h^{-1}$ $100\,g^{-1}$), and cGMP (UcGMP, $nmol\,h^{-1}$ $100\,g^{-1}$) excretions during the first hour of drug administration in SD, WKY and SHR ($n=5$–32 rats per group per treatment). $^*P<0.001$ vs corresponding saline control.

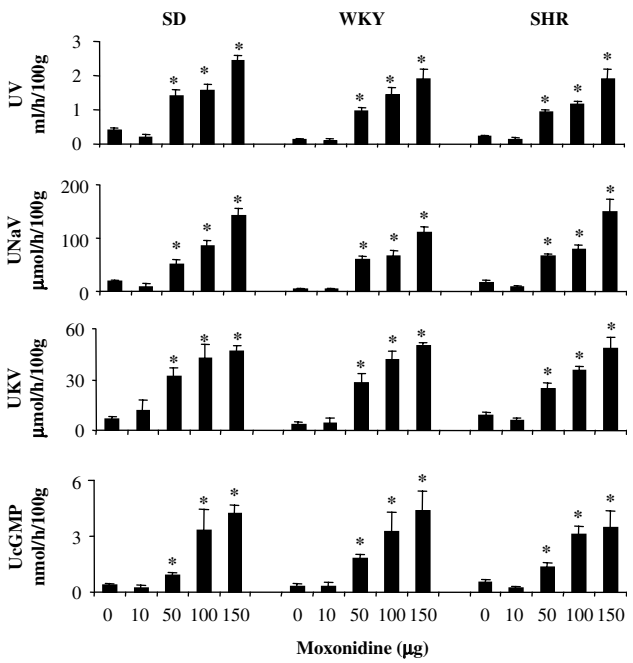

Figure 2 Effect of increasing doses of moxonidine on urine output, sodium, potassium, and cGMP excretions during the first hour of drug administration in SD, WKY, and SHR ($n=5$–30 rats per group per treatment). $^*P<0.001$ vs corresponding saline control.

Compared to saline vehicle, moxonidine at 100 μg significantly ($P<0.001$) increased the renal parameters measured over 1 h post-injection in all groups. Moxonidine-stimulated urine output in SHR ($1.16\pm0.06\,ml^{-1}\,h^{-1}$ $100\,g^{-1}$, $n=16$) was significantly ($P<0.05$) lower than in SD ($1.56\pm0.19\,ml^{-1}\,h^{-1}$ $100\,g^{-1}$, $n=10$) and WKY rats ($1.44\pm0.21\,ml^{-1}\,h^{-1}$ $100\,g^{-1}$, $n=12$) (Figure 5). Sodium and potassium excretions were also significantly ($P<0.05$) lower in SHR compared to SD but not WKY rats.

Figure 5 also shows that pretreatment with the natriuretic peptide receptor (NPR) antagonist, anantin, dose-dependently inhibited the first hour renal parameters stimulated by moxonidine. At 10 μg, anantin inhibited ($P<0.001$) moxonidine-stimulated urine output to $0.38\pm0.06\,ml^{-1}\,h^{-1}$ $100\,g^{-1}$ in SD rats and to 0.12 ± 0.01 and $0.16\pm0.04\,ml^{-1}\,h^{-1}$ $100\,g^{-1}$ in WKY and SHR, respectively. Similarly, anantin totally abolished moxonidine-stimulated sodium, potassium, and cGMP excretions (Figure 5).

Plasma ANP levels measured 15–20 min after moxonidine or saline-vehicle injections are shown in Figure 6. Plasma ANP tended to be higher in SHR ($853\pm96\,pg\,ml^{-1}$, $n=14$) than in WKY ($309\pm59\,pg\,ml^{-1}$, $n=5$) and SD ($417\pm58\,pg\,ml^{-1}$, $n=18$) rats. Moxonidine stimulated plasma ANP in SHR ($1878\pm229\,pg\,ml^{-1}$, $n=14$, $P<0.001$) and WKY ($1433\pm187\,pg\,ml^{-1}$, $n=5$, $P<0.01$) to higher levels than corresponding SD ($1021\pm112\,pg\,ml^{-1}$, $n=19$).

Discussion

The results of the present study indicate, in conscious freely-voiding rats, that: (1) acute intravenous administration of moxonidine and clonidine evokes diuresis, natriuresis, kaliuresis, and urinary cGMP excretion that are inhibited by

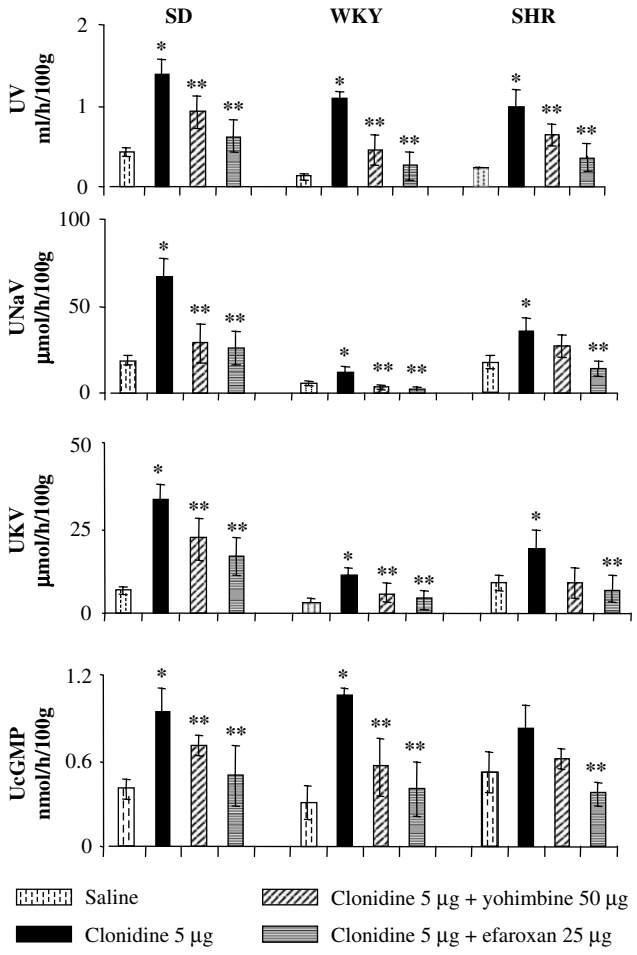

Figure 3 Urine output, sodium, potassium, and cGMP excretions during the first hour of treatment with clonidine with and without pretreatment with yohimbine and efaroxan in SD, WKY, and SHR ($n=8$–26 rats per group per treatment). *$P<0.001$ vs corresponding saline control. **$P<0.01$ vs corresponding clonidine.

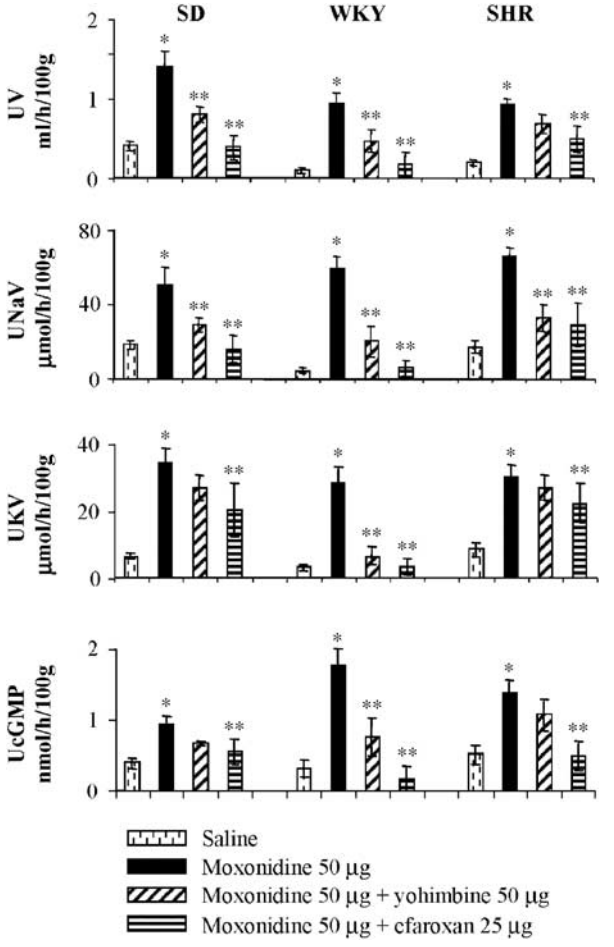

Figure 4 Urine output, sodium, potassium, and cGMP excretions during the first hour of treatment with moxonidine with and without pretreatment with yohimbine and efaroxan in SD, WKY, and SHR ($n=8$–26 rats per group per treatment). *$P<0.001$ vs corresponding saline control. **$P<0.01$ vs corresponding moxonidine.

efaroxan and yohimbine, implicating both I_1-receptors and α_2-adrenoceptors in the renal effects of these drugs, and making distinction between the contributions of either receptor not easy. (2) Regardless of the receptor type, the urinary effects of moxonidine are not consistently different in SHR from WKY and SD normotensive controls, and are, therefore, not influenced by hypertension *per se*. (3) Renal responses to moxonidine are associated with elevated plasma ANP, and are (4) dose-dependently inhibited by the natriuretic peptide antagonist. These studies show, for the first time, that natriuretic peptides mediate the renal effects of acute moxonidine treatment.

Several groups have reported that acute intravenous injections of moxonidine in normotensive and hypertensive rats evoke diuresis and natriuresis (Allan *et al*., 1993; Hohage *et al*., 1997a, b; Mukaddam-Daher & Gutkowska, 1999; 2000). These actions may be mediated centrally and peripherally (Smyth & Penner, 1998). Intravenous moxonidine crosses the blood–brain barrier to act preferentially on imidazoline receptors in the brainstem rostroventrolateral medulla (RVLM) (Haxiu *et al*., 1994), although an effect on α_2-adrenoceptors cannot be ruled out. Activation of both receptor types inhibits central sympathetic output to the peripheral vasculature, the heart, and kidneys. Inhibition of renal sympathetic nerve activity leads to diuresis and natriuresis, by modulating renin release, sodium reabsorption, or renal hemodynamics (Dibona, 2002). Activation of α_2-adrenoceptors in the RVLM can promote urinary sodium excretion by a renal nerve-dependent mechanism and increase the urine flow rate by a pathway that involves vasopressin secretion from the paraventricular nucleus (PVN) of the hypothalamus (Menegaz *et al*., 2001). Activation of α_2-adrenoceptors in micturition centres of the lumbosacral and supraspinal regions leads to bladder hyperactivity (Kontani *et al*., 2000). Imidazolines may also directly act on imidazoline receptors and/or α_2-adrenoceptors present in the kidney cortex and outer medulla (Bidet *et al*., 1990; Limon *et al*., 1992; Li & Smyth 1993a; Greven & von Bronewski-Schwarzer, 2001). Selective activation of renal I_1-receptors by intrarenal infusion of moxonidine markedly increases the urine flow rate and sodium excretion (Allan *et al*., 1993) by a direct tubular effect (Greven & von Bronewski-Schwarzer, 2001). Although moxonidine binds to renal I_1-receptors with higher affinity than to α_2-adrenoceptors, activation of α_2-adrenoceptors may inhibit vasopressin-dependent

(Edwards *et al*., 1992; Nielsen *et al*., 2002) and vasopressin-independent (Junaid *et al*., 1999) aquaporin-mediated water reabsorption, or stimulate local nitric oxide release in the renal medulla (Zou & Cowley, 2000).

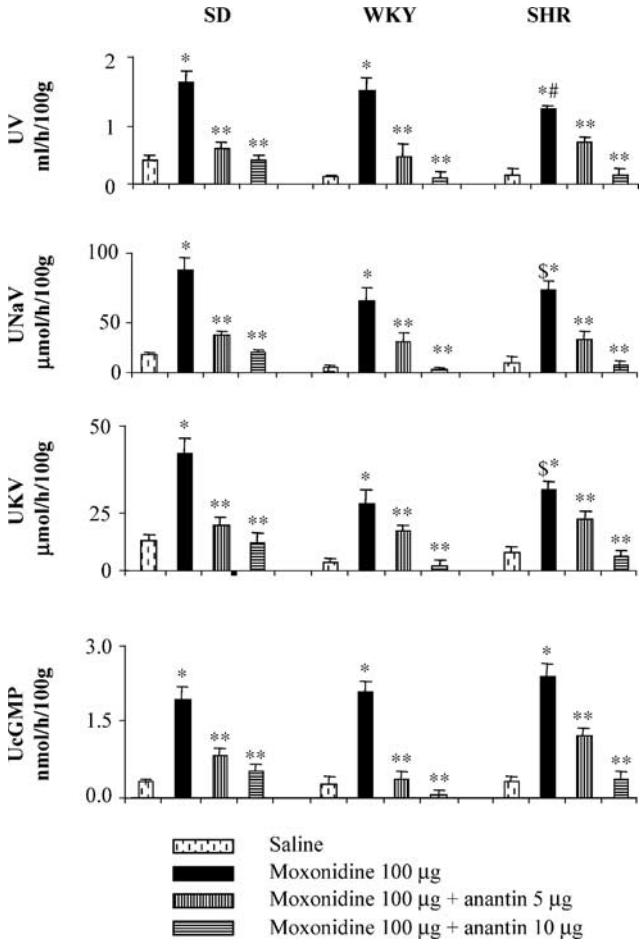

Figure 5 Urine output, sodium, potassium, and cGMP excretions during the first hour of treatment with anantin, administered 10 min before 100 μg moxonidine injection in SD, WKY, and SHR ($n=5$–26 rats per group per treatment). $^{*}P<0.001$ vs corresponding saline control. $^{**}P<0.001$ vs corresponding moxonidine; $^{\#}P<0.05$ vs corresponding SD and WKY; $^{\$}P<0.05$ vs corresponding SD.

Alternatively, Smyth *et al*. (2003) recently reported that low-dose moxonidine infused directly into the left renal artery resulted in similar levels of urine and sodium excretion from the left and the right kidneys, and accordingly suggested that an extra-renal diuretic and natriuretic factor mediated its renal effects. The present study shows that acute intravenous moxonidine in conscious rats increases plasma natriuretic peptides and urinary cGMP excretion. These renal effects are inhibited by anantin, a natriuretic peptide antagonist, providing clear evidence that the extra-renal factors, proposed by Smyth *et al*. (2003), are natriuretic peptides. The present findings substantiate the hypothesis that, regardless of the receptor type involved, intravenous moxonidine injections result in elevated levels of circulating natriuretic peptides, which would act on their receptors to stimulate diuresis and natriuresis.

Natriuretic peptides participate in cardiovascular regulation through direct vasodilating and renal effects, as well as by influencing the sympathetic nerve activity and heart rate (Jamison *et al*., 1992; Imaizumi & Takeshita, 1993; Melo *et al*., 2000; de Bold *et al*., 2001). Intravenous administration of ANP in rats results in suppression of efferent activity in adrenal, renal, and splenic sympathetic nerve fibers, and the effect is absent in decerebrated rats, indicating that circulating ANP modulates autonomic outflows through hypothalamic neurons that lack a blood–brain barrier (Niijima, 1989). Also, circulating natriuretic peptides act on the kidney to cause diuresis and natriuresis by stimulating the glomerular filtration rate and renal blood flow, exerting direct actions on renal proximal tubules and inner medullary collecting duct cells to inhibit sodium and water reabsorption, and by inhibiting renin and vasopressin release and aldosterone synthesis and secretion (Jamison *et al*., 1992; Imaizumi & Takeshita, 1993; Melo *et al*., 2000; de Bold *et al*., 2001).

In the present study, antagonism of natriuretic peptides resulted in complete inhibition of moxonidine-stimulated urinary parameters. Although three NPR subtypes (NPR-A, NPR-B, and NPR-C) are present in the kidney (Jamison *et al*., 1992), NPR-A (also known as GC-A) was shown to be the receptor subtype that mediates the acute diuretic and natriuretic effects of the cardiac natriuretic peptides ANP and brain natriuretic peptide (BNP). In NPR-A knockout mice, rapid volume expansion, a primary stimulus of natriuretic peptides (ANP and BNP) release, fails to stimulate

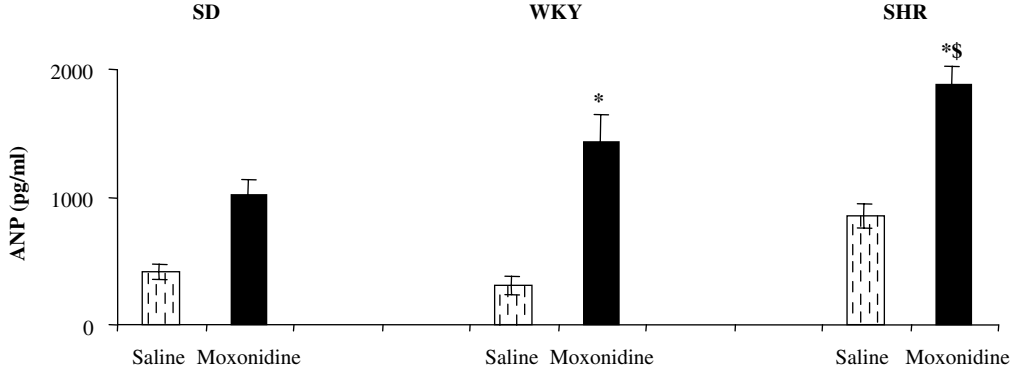

Figure 6 Effect of moxonidine on plasma ANP levels 15–20 min after moxonidine injection in SD, WKY, and SHR ($n=5$–18 rats per group per treatment). $^{*}P<0.01$ vs corresponding saline control; $^{\$}P<0.001$ vs corresponding SD.

water and sodium excretion (Kishimoto *et al*., 1996). Therefore, we may propose that natriuretic peptides mediate the effects of moxonidine, most likely through their NPR-A. In fact, it would be interesting, at this point, to study the effects of moxonidine in NPR-A null mice.

Investigating whether the renal effects of moxonidine are altered in hypertension, the present experiments demonstrate that the responses in SHR were not consistently different from those in two normotensive control strains. Intriguingly, differences were more influenced by strain than pressure. Moxonidine-stimulated diuresis was lower in SHR compared to normotensive SD and WKY rats. On the other hand, natriuresis and kaliuresis were lower in SHR than in SD but not in WKY rats. The lack of stimulated renal effects by moxonidine in SHR is contrary to the expectation that inhibition of hypertension-associated renal sympathetic overactivity, which tends to promote sodium and water retention to a greater extent than in normotensive rats (Roman & Cowley, 1985), may result in enhanced renal responses to moxonidine. Furthermore, plasma natriuretic peptide levels, which were already higher in SHR than in WKY and SD rats, were further elevated by moxonidine, but the increase in circulating levels was not reflected by the urinary parameters. This is not surprising, however, because diuresis and natriuresis are the net product of multiple hemodynamic, neural, hormonal, and local factors that may be altered in hypertension (Li & Smyth, 1993b; Dibona, 2002), including renal I_1-receptors, α_2-adrenoceptors, and NPRs. Previous studies have shown that the density of renal α_2-adrenoceptors is elevated in SHR (Stanko & Smyth, 1991), but not in 1K1C hypertensive rats (Li & Smyth, 1993a). Idazoxan-labelled imidazoline receptor binding is lower in 1K1C hypertensive rat kidneys compared to sham controls (Li & Smyth, 1993a). On the other hand, renal NPRs in SHR are upregulated in the inner medulla (Guillaume *et al*., 1997), and are either unchanged (Tremblay *et al*., 1993) or reduced in glomeruli (Guillaume *et al*., 1997). Therefore, renal receptor regulation may counter-balance hormonal levels.

The reduced renal responses to moxonidine in SHR may also be explained by a greater drop in blood pressure in SHR following treatment. We have shown in previous studies that injections of 50 μg moxonidine in normotensive SD rats do not significantly reduce blood pressures (diastolic, systolic and mean), measured by radiotelemetry (Mukaddam-Daher & Gutkowska, 2000). Also, 50 μg moxonidine, which only slightly decreased systolic blood pressure measured by the tail-cuff method 30 min after injection in WKY rats ($\sim$10 mmHg), resulted in a significant ($\sim$40 mmHg) decrease in SHR (Mukaddam-Daher & Gutkowska, 1999). However, although the drop in blood pressure may explain, in part, the diuretic effect of moxonidine, it may not explain the natriuretic effect, which was not different between SHR and WKY.

Most importantly, whereas characterization of the receptor type mediating the renal effects of moxonidine in these conditions is not conclusive, this study proves, for the first time, that natriuretic peptides are directly involved in the renal actions of acute intravenous moxonidine in conscious rats, and that its actions are not altered by hypertension.

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