Atrial Natriuretic Peptide Is Involved in Renal Actions of Moxonidine

Suhayla Mukaddam-Daher, Jolanta Gutkowska

Abstract—Moxonidine, an antihypertensive imidazoline compound, reduces blood pressure by selective activation of central imidazoline I₁-receptors and inhibition of sympathetic nerve activity and by direct actions on the kidney, with both mechanisms resulting in diuresis and natriuresis. We hypothesized that the hypotensive and renal actions of moxonidine may be mediated by atrial natriuretic peptide (ANP), a cardiac peptide involved in pressure and volume homeostasis through its vasodilatory, diuretic, and natriuretic actions. Renal parameters were measured on an hourly basis over a period of 4 hours in conscious rats that received bolus intravenous injections of moxonidine (1 to 150 μg/300 μL saline). During the first hour, moxonidine dose-dependently stimulated diuresis, natriuresis, kaliuresis, and urinary cGMP, the index of ANP activity. Moxonidine (50 μg) significantly (P<0.001) stimulated urinary volume (0.35±0.04 versus 1.05±0.09 mL/h per 100 g), sodium (14.3±2.5 versus 51.8±6.5 μmol/h per 100 g), potassium (10.5±2.3 versus 32.3±3.2 μmol/h per 100 g), and cGMP (325±52 versus 744±120 pmol/h per 100 g). Pretreatment with a selective imidazoline receptor antagonist, efaroxan, dose-dependently inhibited moxonidine-stimulated renal parameters. Efaroxan (25 μg per rat) significantly inhibited moxonidine-stimulated diuretic and natriuretic effects and urinary cGMP excretion (744±120 versus 381±137 pmol/h per 100 g, P<0.02). The α₂-adrenoceptor antagonist yohimbine (50 μg per rat) partially yet significantly inhibited moxonidine-stimulated diuresis and natriuresis but not cGMP excretion. Plasma ANP was dose-dependently increased by moxonidine and was inhibited by pretreatment with efaroxan (200.8±36.9 versus 100.3±31.7 pg/mL, P<0.03) but not by yohimbine. In conclusion, selective in vivo activation of imidazoline receptors by moxonidine is associated with dose-dependent diuresis, natriuresis, and kaliuresis as well as stimulated plasma ANP and urinary cGMP excretion, thus implicating ANP in the renal actions of moxonidine. (Hypertension. 2000;35:1215-1220.)

Key Words: atrial natriuretic factor ■ receptors, imidazoline ■ receptors, adrenergic, alpha ■ natriuresis ■ cyclic GMP ■ moxonidine

Several important cardiovascular disorders are characterized by increased activity of the sympathetic nervous system (SNS). In essential hypertension, clinical signs of increased SNS tone (eg, higher heart rate) are frequently seen, particularly in younger individuals, as well as several biochemical features such as elevated plasma renin activity or norepinephrine levels. Direct inhibition of peripheral α₁-adrenergic and β-adrenergic receptors and central activation of α₂-adrenergic receptors are routine ways to reduce high blood pressure by lowering peripheral SNS activity. In angina and myocardial infarction, excessive SNS activity is a critical pathophysiological element, and β-adrenergic receptor blockers play important curative and preventive roles. In congestive heart failure, evidences of increased SNS activity have been found several years ago, and the important clinical benefits of angiotensin-converting enzyme inhibitors such as reduced morbidity and mortality rates perhaps could be explained by their quieting effects on the cardiac sympathetic activity. Despite their clinical efficacy and the rationale for their use, drugs activating central α₂-adrenergic receptors (clonidine, α-methyldopa) are not first-choice agents in the therapy of these disorders, particularly because their side effect profile comprises symptoms such as dry mouth, sedation, and mental depression. However, the recent discovery of imidazoline receptors (I-receptors) and a potential role for the activation of I-receptors in mediating the beneficial effects of central α₂-adrenergic agonists has generated a marked interest in this area and has led to the development of new compounds (moxonidine and rilminidine) with improved side effect profiles.1–3

One of the important clinical features of increased SNS activity is sodium and water retention. Many drugs that are beneficial in cardiovascular disorders are also useful because they stimulate sodium and water losses by different mechanisms including altered sodium transport along the tubules. Clonidine tends to stimulate diuresis and natriuresis by
mechanisms that involve actions on the renal tubule to modulate the actions of vasopressin or by mechanisms independent of vasopressin, namely through the release of the atrial natriuretic peptide (ANP), a vasodilator, diuretic, and natriuretic peptide, which stimulates cGMP production in different target cells leading to vasodilation and natriuresis. Previous work from our group and others demonstrated that in sympathetic inhibition, α-adrenergic receptors play a role in the cardiac release of ANP. We have shown that in vivo administration of clonidine or its peripherally acting 2-adrenoceptor agonists, ST-91, induces dose-related increases in plasma ANP levels and results in diuresis and natriuresis and that ANP is inhibited by α2-adrenergic receptor antagonists.

The actions of clonidine were originally exclusively attributed to activation of central α2-adrenoceptors and subsequent decrease of sympathetic nerve activity. However, several studies have shown that its actions are more related to its chemical structure as an imidazoline than to its ability to act as an α2-adrenoceptor agonist. These studies led to the identification of a new class of brain receptors named imidazoline-preferring sites or I-receptors, and specifically by I1-subtype, whose binding activity correlates with the hypertensive effect of clonidine.

Moxonidine, a newly developed antihypertensive imidazoline compound, is chemically and pharmacologically similar to clonidine but shows a 100-fold higher affinity to imidazoline I1 receptors over α2-adrenoceptors. Moxonidine has been shown to decrease blood pressure by selective activation of central imidazoline I1-receptors and subsequent decrease of sympathetic nerve activity and by direct actions on the kidney resulting in diuresis and natriuresis. The aim of the present study was to show that the hypotensive and renal actions of moxonidine may be mediated by another mechanism, namely stimulation of the release of ANP, which plays an integral role in volume and pressure homeostasis in normal and pathophysiological conditions.

Methods

Female Sprague-Dawley rats (weight 200 to 225 g) were purchased from Charles River. Animals were housed in a temperature- and light-controlled room with food and water ad libitum and maintained for ≥3 days before experimentation. Experiments were performed with the approval of the Bioethics Committee of CHUM, according to the Canadian Guidelines.

Renal Parameters

The renal responses to the various treatments and the possible involvement of ANP were investigated by assessment of diuresis, natriuresis, and kaliuresis as well as urinary cGMP (UcGMP), the index of natriuretic peptide activity, on an hourly basis over a period of 4 hours (8 AM to noon) in normotensive conscious rats injected intravenously with imidazoline compounds, moxonidine (I1 receptor agonist), and clonidine (mixed I1/I2 agonist) and compared with a nonimidazoline compound, guanabenz (α2-agonist).

On the day of the study, the rats received bolus injections of increasing doses of moxonidine (1, 10, 50, and 150 μg), clonidine (1, 5, and 10 μg), and guanabenz (1, 10, and 25 μg) in 300 μL of 0.9% saline into the tail vein. The control group received an equal volume of saline. The rats were then placed individually in metabolic cages without food and water. Urine was collected every hour over 4 consecutive hours for volume, sodium, potassium, and cGMP measurements.

After the dose-response studies, the receptor types mediating the effects of treatments were investigated in other groups of rats pretreated with the I1-receptor antagonist efaroxan (5, 10, 25, 50, and 100 μg in 300 μL saline) or the α2-adrenoceptor antagonist yohimbine (10, 25, and 50 μg in 300 μL saline) injected into the tail vein 10 minutes before the administration of the agonists moxonidine (50 μg), clonidine (5 μg), and guanabenz (10 μg).

Telemetric Measurement of Blood Pressure

Systolic, diastolic, and mean arterial pressures and heart rates were measured by Dataquest IV telemetry system (Data Sciences International).

Rats were anesthetized with enflurane gas, and through a midline abdominal incision, the flexible catheter of the transmitter was inserted into the descending aorta just below the renal arteries. The transmitter was inserted into the peritoneal cavity and sutured to the abdominal wall. After surgery, the rats were housed unrestrained in individual cages in a quiet room with a 12:12-hour lighting schedule. The rats were allowed 10 days of recovery. Then, each cage was placed over a receiver panel that monitored output from the transmitter, that is, frequency in Hz. The signals from the receiver were consolidated by multiplex (BCM 100) and were stored and analyzed by a personal computer. The absolute pressure was corrected automatically for changes in atmospheric pressure.

Data were collected every minute, before, and over a period of 4 hours after injection into the tail vein of either saline vehicle (300 μL), 50 μg moxonidine, or 5 μg clonidine.

Drugs

Moxonidine (kindly provided by Solvay Pharmaceuticals GMBH) was dissolved in 0.001 mol/L acetic acid in normal saline. Clonidine hydrochloride, yohimbine hydrochloride, and efaroxan hydrochloride (Sigma Chemical Co) and guanabenz acetate (RBI) were dissolved in normal saline. All solutions were freshly prepared before the injection.

Plasma and Tissue ANP Determination

Rats were killed by decapitation 10 to 15 minutes after moxonidine treatment or saline vehicle. Blood (1 mL) was collected in prechilled tubes containing protease inhibitors in a final concentration: 1 mmol/L EDTA, 5 μmol/L Pepstatin A, and 10 μmol/L phenylmethylsulfonyl fluoride (Sigma Chemical Co). Blood was centrifuged at 4°C and plasma was stored at ~80°C until assayed. The hearts were immediately excised, and left and right atria and ventricles were separated. The tissues were homogenized in 0.1 mol/L acetic acid containing protease inhibitors (as above) at 4°C. After 20 minutes of centrifugation at 30 000g, supernatants were collected, aliquoted, and stored at ~80°C.

Immunoreactive ANP was determined by specific radiomunuassay in serial dilution of tissue homogenates and in plasma after extraction by Sep-Pak C18 cartridges (Millipore) as previously described. Protein content of tissue homogenates was measured spectrophotometrically with BSA used as a standard. UcGMP was measured by radiomunuassay established in our laboratory according to a previously described method. Urinary sodium and potassium concentrations were measured with a flame photometer, and excretions per hour were calculated. Renal parameters were normalized to percent body weight.

Statistical Analysis

Data storage, graphical output, and statistical analysis assessed by 1-way ANOVA were accomplished with the use of RS1 data analysis software (BBN). The pressures and heart rate data were averaged at 15-minute intervals. Statistical analysis was accomplished with 2-way ANOVA (time and treatment) with repeated measures followed by Fisher’s least-squares difference multiple comparison with saline-injected controls, with the use of an SAS statistical analysis package (SAS Institute). Statistical significance was taken as P<0.05. All data are reported as mean±SE.
Results

Renal parameters measured over a period of 4 hours after moxonidine in conscious normotensive rats revealed that the effects were more pronounced during the first hour of treatment. During the first hour, moxonidine stimulated urinary excretion of volume, sodium, potassium, and cGMP in a dose-dependent manner. Clonidine and guanabenz also dose-dependently stimulated the renal responses (Figure 1). Urine volume was 0.35±0.04 mL/h per 100 g body wt in control saline-treated rats and was equally increased by 50 μg moxonidine (1.05±0.09 mL/h per 100 g, n=8, P<0.001), 5 μg clonidine (1.19±0.18 mL/h per 100 g, n=5, P<0.001), and 10 μg guanabenz (1.12±0.10 mL/h per 100 g, n=5, P<0.001). Thus, further studies were performed with the use of these doses.

The receptor types involved in the renal actions were determined by injecting the rats with agonist doses that increased urinary output to similar levels and inhibition of the responses by selective antagonists. Figure 2 shows that pretreatment with efaroxan and yohimbine dose-dependently reversed the renal responses evoked by 50 μg moxonidine. Significant inhibitory effect of efaroxan on urine output

![Figure 1. Effect of increasing doses of moxonidine, clonidine, and guanabenz on urine output, sodium, potassium, and cGMP excretions during first hour of drug administration in conscious, normally hydrated rats (n=5 to 8 rats per treatment). Values are expressed as mean±SE. *P<0.001 vs saline control.](http://hyper.ahajournals.org/)

![Figure 2. Urine output, sodium, potassium, and cGMP excretions during first hour of treatment with either 5 to 50 μg per rat efaroxan or 10 to 50 μg yohimbine administered 10 minutes before 50-μg moxonidine injection (n=8 to 26 per group per treatment). Values are expressed as mean±SE. #P<0.001 vs corresponding saline control. *P<0.02, **P<0.001 vs corresponding moxonidine.](http://hyper.ahajournals.org/)
Effect of Yohimbine (50 μg) and Efaroxan (25 μg) Pretreatment on 5 μg Clonidine-Stimulated or 10 μg Guanabenz-Stimulated Renal Parameters Measured During 1 Hour After Injection Compared With Saline-Treated Control

<table>
<thead>
<tr>
<th>Renal Parameters</th>
<th>Saline</th>
<th>Clonidine, 5 μg</th>
<th>Clonidine+ Yohimbine</th>
<th>Clonidine+ Efaroxan</th>
<th>Guanabenz, 10 μg</th>
<th>Guanabenz+ Yohimbine</th>
<th>Guanabenz+ Efaroxan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diuresis, mL/h per 100 g</td>
<td>0.35±0.04</td>
<td>1.79±0.18</td>
<td>1.08±0.22*</td>
<td>0.86±0.2*</td>
<td>1.42±0.12</td>
<td>0.9±0.24†</td>
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<td>Natriuresis, μmol/h per 100 g</td>
<td>14.3±2.5</td>
<td>84.6±11.8</td>
<td>30.3±11.7*</td>
<td>40.6±14.2*</td>
<td>49.2±5.8</td>
<td>22.4±8.6*</td>
<td>21.2±8.3†</td>
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<tr>
<td>Kaliuresis, μmol/h per 100 g</td>
<td>10.5±2.3</td>
<td>52.1±4.5</td>
<td>36.9±6.2*</td>
<td>23.5±8.5†</td>
<td>51.3±4.1</td>
<td>31.2±7.2*</td>
<td>19.2±8.5†</td>
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<tr>
<td>UcGMP excretion, pmol/h per 100 g</td>
<td>325±52</td>
<td>1314±174</td>
<td>804±76†</td>
<td>983±167</td>
<td>946±172</td>
<td>696±104*</td>
<td>661±200</td>
</tr>
</tbody>
</table>

n=8 to 18 rats per treatment. *P<0.03, †P<0.01 vs corresponding agonist.

(P<0.001) and sodium excretion (P<0.03) occurred at a low dose of 10 μg per rat. Efaroxan at 25 μg per rat significantly inhibited moxonidine-stimulated urine volume (1.17±0.08 vs 0.21±0.06 mL/h per 100 g body wt, n=20, P<0.001) and excretion of sodium (51.8±6.5 to 19.3±6.5 μmol/h per 100 g, P<0.001), potassium (32.3±3.2 vs 19.6±7.3 μmol/h per 100 g, P<0.04), and UcGMP (744±120 vs 381±137 pmol/h per 100 g, P<0.02). Efaroxan at 50 μg per rat totally inhibited all renal parameters measured (Figure 2).

Clonidine and guanabenz injections with and without prior inhibition by efaroxan revealed that in contrast to moxonidine, the stimulated renal effects were not altered by 10 μg efaroxan, whereas 25-μg concentrations significantly decreased urinary output and sodium and potassium but not cGMP excretion (Table).

Moxonidine-stimulated renal actions were also inhibited by 50 μg yohimbine but not to control levels (Figure 2). Yohimbine partially yet significantly inhibited moxonidine-stimulated diuresis (1.17±0.09 vs 0.73±0.08 mL/h per 100 g, P<0.01) and natriuresis (51.8±6.5 vs 21.5±6.9 μmol/h per 100 g, P<0.02), which implies that the renal actions of moxonidine may also be mediated by α2-adrenoceptors. However, the dose of yohimbine (50 μg) that significantly inhibited the renal responses to clonidine and guanabenz (Table) did not significantly inhibit UcGMP excretion evoked by moxonidine. Taken together, these results may imply that the renal actions of moxonidine are selectively mediated by I1 receptors and that ANP, through its marker UcGMP, is dose-dependently involved in these actions.

Plasma ANP levels measured 15 minutes after intravenous injection with increasing doses of moxonidine (10, 50, 100, 200 μg) are illustrated in Figure 3. Top, effect of increasing doses of moxonidine on plasma ANP, n=4 to 10 rats per group; bottom, effect of 50 μg moxonidine on plasma ANP in rats pretreated with 50 μg yohimbine or 25 μg efaroxan. *P<0.01 vs saline control, **P<0.05 vs moxonidine.

### Figure 3
![Plasma ANP levels](image)

### Figure 4
Blood pressure and heart rate (HR) response in rats treated with 50 μg moxonidine and 5 μg clonidine as compared with saline-treated rats (n=6 rats per treatment). *P<0.002 vs saline control.
and 150 μg) were significantly (P<0.01) stimulated from 67.9±10.1 pg/mL in control saline-treated rats to 143.3±12.0, 231.4±38.3, 314.5±55.7, and 349.7±15.4 pg/mL, respectively (Figure 3). However, administration of 50 μg moxonidine did not significantly alter ANP content in cardiac right and left atria or ventricles (data not shown). Plasma ANP was also increased by 5 μg clonidine (125.7±15.3 pg/mL; P<0.04) and 10 μg guanabenz (138.8±23.6 pg/mL, P<0.02).

Moxonidine-stimulated circulating ANP was inhibited by pretreatment with 25 μg efaroxan (220.8±36.9 vs 100.3±31.7 pg/mL, P<0.03). Pretreatment with 50 μg yohimbine tended to but did not significantly inhibit moxonidine-stimulated ANP (147.5±43.0 pg/mL) (Figure 3). Telemetric measurement of blood pressure (Figure 4) revealed that there was no significant difference between the effects of clonidine (5 μg) and moxonidine (50 μg) as compared with salinized vehicle. Mere handling of the animals and injection of either drug or saline increased blood pressure parameters, but the increase in systolic pressure by moxonidine and clonidine was less than that caused by saline, implying a mild hypotensive effect. Both treatments significantly (P<0.002) reduced heart rate at 15, 30, and 45 minutes after treatment but not at 60 minutes or thereafter. However, no difference was observed between the bradycardic effects of moxonidine and clonidine.

**Discussion**

The results of the present study show that selective in vivo activation of imidazoline receptors by moxonidine, in doses that do not reduce blood pressure, is associated with dose-dependent diuresis, natriuresis, and kaliuresis as well as stimulated plasma ANP and UcGMP excretion, thus implicating ANP in the renal responses to moxonidine. In addition to imidazoline receptors, the mechanisms involved in the renal actions of moxonidine may partially include activation of α2-adrenoceptors and subsequent increase in plasma ANP.

The renal responses to intravenous administration of moxonidine may be both centrally and peripherally mediated. Studies by Penner and Smyth demonstrated that central administration of moxonidine increases sodium excretion without changing blood pressure and that the renal responses to intracerebroventricular moxonidine are attenuated by renal denervation, implying that an intact sympathetic nervous system is important in the renal responses to intracerebroventricular moxonidine, whereby moxonidine may act through the renal nerves to inhibit renal nerve activity, which leads to increased sodium excretion. In support, Kline and Cechetto showed in anesthetized rats that intravenous infusion of rilmenidine, an imidazoline compound that also shows higher selectivity to imidazoline receptors than α2-adrenoceptors as compared with clonidine, decreased mean arterial pressure, heart rate, and renal nerve activity and evoked significant renal responses and that these renal effects were markedly inhibited in the chronically denervated kidneys. On the other hand, moxonidine may act peripherally on imidazoline receptors identified in the kidney proximal tubules and inner medulla, where their action has been associated with inhibition of Na/H exchanger. Intrarenal infusion of moxonidine markedly increases urine flow rate, sodium excretion, and osmolar clearance and modulates noradrenaline release in isolated rat kidneys.

The present study shows that in addition to the reported mechanisms by which moxonidine increases sodium and water excretion, moxonidine may also increase plasma ANP, which in turn would act on the kidney to cause diuresis and natriuresis through the release of cGMP. Acute intravenous administration of moxonidine to normotensive rats dose-dependently and significantly increased plasma ANP and enhanced diuresis, natriuresis, and kaliuresis as well as UcGMP, the index of ANP activity, thus implicating ANP in the renal actions of moxonidine. These effects were inhibited by efaroxan at doses 10 to 50 times lower than those required to inhibit the renal actions of ST-91, a peripherally acting clonidine analogue, confirming the selective involvement of 11 receptors in the renal responses.

The contribution of α2-adrenoceptors to the actions of moxonidine was investigated with the use of yohimbine, a selective α2-antagonist, and comparing its inhibitory effects on the renal parameters evoked by moxonidine with those of clonidine (mixed 11/α2-agonist) and guanabenz (α2-agonist). Although the 3 agonists used evoked similar renal responses, yohimbine significantly inhibited all the renal parameters stimulated by clonidine and guanabenz and moxonidine-stimulated diuresis and natriuresis but not kaliuresis and UcGMP excretion. In addition, efaroxan inhibited the diuretic and natriuretic effects of guanabenz and clonidine almost to control levels but failed to inhibit UcGMP. These findings imply that different mechanisms may be involved in the renal effects of the 3 compounds and suggest a dissociation between the actions of imidazoline receptors and those of α2-adrenoceptors. In the kidney, activation of α2-adrenoceptors stimulates free water clearance, whereas activation of imidazoline receptors stimulates osmolar clearance. Diuresis after in vivo activation of α2-adrenoceptors results from inhibition of cAMP and subsequent antagonism of vasopressin in the renal cortical and medullary collecting tubules, whereas the natriuretic response to moxonidine occurs independent of the renal actions of vasopressin, through stimulation of prostaglandins and, as the present study shows, ANP.

ANP may be implicated in the actions of moxonidine. Both ANP and moxonidine enhance glomerular filtration rate and/or reduce tubular reabsorption of sodium and water as well as suppress renal nerve activity, effects markedly attenuated by sympathetic blockade by prazosin in humans and animals. Furthermore, the lower heart rate observed with moxonidine treatment may also be explained as a consequence to the marked elevation of ANP in plasma, as ANP has been reported to have negative chronotropic actions through increased cGMP, stimulation of Ca- and voltage-activated potassium channel (BK) activity through activation of cGMP-dependent protein kinase (PKG), or inhibition of cardiac sympathetic nerve activity by inhibiting ganglionic transmission. The sites of imidazoline receptors involved in ANP release have not been determined in this study. However, intravenous moxonidine may activate imidazoline receptors in the ventral medulla to cause sympathoinhibition in
various organs including the heart. Hansson et al. reported that chemical and surgical cardiac sympathectomy leads to an increased level of ANP in the Purkinje fibers of bundle branches. In addition, imidazoline receptors may be present in the heart and may directly or indirectly affect ANP release from cardiac myocytes and/or ANP granules identified in the conduction system. Further studies are required to identify the presence of imidazoline receptors in the heart.

In summary, this study presents new evidence that moxonidine, by selective activation of imidazoline receptors, increases sodium and water excretion and that these renal actions are associated with elevated plasma ANP and its marker, UcGMP. These results may suggest the presence of imidazoline receptors in the heart, the primary site of ANP release.

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**References**

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