Augmentation of moxonidine-induced increase in ANP release by atrial hypertrophy

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SYMPATHETIC CONTROL of the circulation is altered in the early stage of hypertension, and this may be a contributory factor to the disease. Several classes of drugs acting on the central as well as the peripheral and autonomic nervous systems are very efficient in the treatment of hypertension. One class, a second generation of a group of centrally acting drugs selective for imidazoline receptors, has proven beneficial in this respect (15, 23, 28). Differing from α2-adrenergic receptor agonists of the imidazoline class such as clonidine and dexamethasone, moxonidine has been demonstrated to produce significantly less sedation, dry mouth, and rebound hypertension, which are symptoms caused by activation of the central α2-adrenergic receptors (20, 27).

Imidazoline receptors are divided into I1 and I2 subtypes (24, 29). I1-imidazoline receptors are mainly distributed in the brain stem and are also present in peripheral tissues such as kidney, adrenal chromaffin cells, carotid body, atria, and ventricles (2, 3, 9, 26). Moxonidine is a mixed I1- and α2-adrenergic receptor agonist with 100-fold selectivity for I1 over α2-receptors (12). The selective in vivo activation of imidazoline receptors by moxonidine has been reported to cause diuresis and natriuresis as well as to stimulate plasma atrial natriuretic peptide (ANP) and urinary cGMP excretion (8, 16). Renal actions of moxonidine are blocked by efaroxan (a selective I1-imidazoline antagonist) and partially blocked by yohimbine (an α2-adrenoceptor antagonist). However, an increase in plasma ANP concentration is inhibited by efaroxan but not by yohimbine (16). The renal effects of moxonidine are reported to be enhanced in spontaneously hypertensive rats (17), and I1-imidazoline receptors are also reported to be upregulated during hypertension or heart failure (9). In vitro studies show that clonidine increases ANP release by activating the α2-adrenoceptors and imidazoline receptors in the whole heart (18). However, there is no report touching on the direct effect of moxonidine on ANP secretion. We hypothesized that moxonidine increases ANP release by direct stimulation of atrial I1-imidazoline receptors and via a different mechanism(s) from clonidine. Therefore, the aim of the present study was to use isolated, perfused, beating atria to evaluate the direct effects of moxonidine on hemodynamics and ANP release compared with clonidine and the modification of these effects by atrial hypertrophy.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats were used in this study. Rats weighing 300–320 g that received no anesthesia or surgery were used as a control group. For remnant-kidney hypertensive rats, animals weighing 240–270 g were anesthetized with a 9:1 ketamine-xylazine mixture, and bilateral flank incisions were performed. After removal of the right kidney, two of the three branches of the left renal artery were ligated; this was followed by infarction of approximately two-thirds of the left kidney. Cardiac hypertrophy with hypertension was developed 4 wk after surgery. Sham-operated rats underwent anesthesia, laparotomy, and manipulation of the renal pedicles without removal of renal mass. Rats were killed for perfusion experiments 4–5 wk after surgery. Body weights of rats used for in vitro experiments were similar between the three groups (control, 309.2 ± 4.4; sham operated, 302.3 ± 10.4; remnant hypertensive, 292.3 ± 9.3 g). Experimentation was conducted in accordance with the “Guiding Principles in the Care and Use of Animals” of the American Physiological Society.
Preparation of isolated, perfused, beating atria. Isolated, perfused, beating atria were prepared using a previously described method (7, 11). In brief, after the rat was killed, the left atrium was dissected from the heart and fixed with a Tygon cannula. The cannulated atrium was transferred into an organ chamber, immediately perfused with oxygenated HEPES buffer solution at 36.5°C, and paced at 1.2 Hz (duration, 0.3 ms; voltage, 40 V) as described previously (11). Intra-atrial pressure was recorded on a physiograph via a pressure transducer, and pulse pressure was calculated as the difference between systolic and diastolic pressures. The composition of the HEPES buffer solution was as follows: 118 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l CaCl₂, 1.2 mmol/l MgSO₄, 25 mmol/l NaHCO₃, 10 mmol/l HEPES, 10 mmol/l glucose, and 0.1% bovine serum albumin (BSA). The pericardial buffer solution, which contained [3H]inulin to allow measurement of the translocation of extracellular fluid (ECF), was also oxygenated by placing silicone-tubing coils inside the organ chamber. The atrium was perfused for 100 min to stabilize the secretion of ANP and to maintain a steady-state [3H]inulin level in the extracellular space. The perfusate was collected into preheilled tubes at 2-min intervals at 4°C.

Experimental protocols. Experiments were performed with five groups.

Group 1 comprised the time-control atria (n = 5), which were perfused with HEPES buffer throughout the experiment.

Group 2 included the moxonidine- or clonidine-perfused atria from control rats. Moxonidine (3, 10, and 30 μmol/l; n = 7) or clonidine (3, 10, and 30 μmol/l; n = 5) was cumulatively introduced into the atrial lumen after a 10-min control collection period, and perfusate was collected for 28 min per dose.

Group 3 was composed of the antagonist-pretreated atria. To modify the effect of moxonidine (10 μmol/l; n = 7) on ANP secretion, the β₁-imidazoline receptor antagonist efaroxan (0.3 μmol/l; n = 7) or the α₂-receptor antagonist rauwolscine (0.3 μmol/l; n = 8) was administered as a pretreatment 30 min after the start of perfusion. Then moxonidine was simultaneously infused after a 10-min control collection period, and the perfusate was collected for 50 min.

Group 4 included the moxonidine- or clonidine-perfused atria from the remnant-kidney hypertensive and sham rats. Moxonidine or clonidine (3, 10, and 30 μmol/l; all n = 7) was cumulatively introduced into the atrial lumen after a 10-min control collection period, and the perfusate was collected for 28 min per dose.

Group 5 comprised the antagonist-pretreated atria from the remnant-kidney hypertensive rats. To modify the effect of moxonidine (10 μmol/l; n = 5) on ANP secretion, the β₁-imidazoline receptor antagonist efaroxan (0.3 μmol/l; n = 5) or the α₂-receptor antagonist rauwolscine (0.3 μmol/l; n = 5) was administered as a pretreatment 30 min after the start of perfusion. Then moxonidine was simultaneously infused after a 10-min control collection period, and the perfusate was collected for 50 min. All agonists and antagonists used in the study were purchased from Sigma Chemical (St. Louis, MO).

Radioimmunoassay of ANP. The concentration of immunoreactive ANP in the perfusate was measured with a specific radioimmunoassay as described previously (7, 11). The molar concentration of the ANP released was calculated as follows because ANP was secreted into the atrial lumen with the translocation of ECF (5)

\[ \text{ANP released (in } \mu\text{M}) = \text{ANP (in pg} \cdot \text{min}^{-1} \cdot \text{g}^{-1})/\text{ECF translocation (in } \mu\text{l} \cdot \text{min}^{-1} \cdot \text{g}^{-1})/3,060 \]

The denominator 3,060 refers to the molecular mass for ANP₁₋₂₈ (in Da), as the ANP secreted was found to be the processed ANP (7).

Measurement of ECF translocation. We previously reported a two-step sequential mechanism of ANP secretion from the atrium: first, atrial release of ANP into the interstitial space occurs by means of atrial stretching; and second, the released ANP is translocated into the atrial lumen concomitant with ECF translocation by atrial contraction (4, 5). The radioactivity of [3H]inulin in the perfusate was measured with a liquid scintillation counter. The amounts of ECF translocated through the atrial wall were calculated as follows

\[ \text{ECF translocation (in } \mu\text{l} \cdot \text{min}^{-1} \cdot \text{g}^{-1}) = \text{total radioactivity in perfusate (in cpm/min)/radioactivity in pericardial reservoir (in cpm/μl)/atrial wet wt (in mg)} \times 1,000 \]

Statistical analysis. The results are given as means ± SE. ANOVA followed by Dunnett’s multiple-comparison test was used (see Fig. 1). Statistical significance of the differences was assessed using one-way ANOVA and a subsequent Bonferroni multiple-comparison test (see Figs. 2, 3, and 5). Student’s unpaired t-test was also used (see Figs. 4 and 6). Statistical significance was taken at a value of P < 0.05.

RESULTS

Effects of moxonidine or clonidine on intra-atrial pressure and ANP release. Figure 1 shows the effects of moxonidine on pulse pressure, ANP secretion, ECF translocation, and interstitial ANP concentration in beating rat atria. After 100 min of stabilization, the perfusate was collected five times every 2 min to serve as a control period. Then, moxonidine (3, 10, and 30 μmol/l) was cumulatively administered. The pulse pressure was abruptly decreased by 3 μmol/l moxonidine and began to recover to control level despite administration of a higher dose (Fig. 1A). ANP secretion increased in a dose-dependent manner (Fig. 1C), but ECF translocation was relatively constant (Fig. 1B). The ANP released from the atrial myocytes into the interstitial space was translocated into the atrial lumen concomitant with the ECF translocation. Therefore, the ANP secretion in terms of ECF translocation (the interstitial ANP concentration) was increased in a dose-dependent manner (Fig. 1D).

Figure 2 shows the relative percent changes in pulse pressure, ECF translocation, ANP secretion, and interstitial ANP concentration obtained from the mean of five control values and the last five experimental values for exposure to cumulative doses of moxonidine or clonidine (3, 10, and 30 μmol/l) compared with the time-control group. In the control group,ero pressure was decreased in terms of time. Changes in pulse pressure by moxonidine and clonidine were not significantly different from the corresponding period for the control group except for the lowest dose of moxonidine (Fig. 2A). No appreciable changes in ECF translocation by moxonidine and clonidine were observed (Fig. 2B). The ANP secretion and interstitial ANP concentration were markedly increased in a dose-dependent manner by moxonidine but not by clonidine (Fig. 2, C and D).

Effects of receptor antagonists on moxonidine-induced atrial hemodynamics and ANP release. To determine which receptors are involved in the moxonidine-induced augmentation of ANP secretion, pretreatment with the β₁-imidazoline-receptor antagonist efaroxan (0.3 μmol/l) or the α₂-adrenoceptor antagonist rauwolscine (0.3 μmol/l) preceded simultaneous perfusion with moxonidine (10 μmol/l). Efaroxan or rauwolscine alone did not affect the pulse pressure or ANP secretion (Fig. 3A). Figure 3B shows the relative percent changes in pulse pressure, ECF translocation, ANP secretion, and interstitial ANP concentration by moxonidine in the presence of receptor antagonist. As shown in Fig. 3B, the moxonidine-induced decrease in pulse pressure was inhibited by efaroxan.
Atrial Hypertrophy Augments Effect of Moxonidine

**Fig. 1.** Effects of moxonidine (MOXO) on pulse pressure (A), atrial natriuretic peptide (ANP) secretion (B), extracellular fluid translocation (ECF transloc; C), and ANP concentration (D) in isolated perfused, beating rat atria. After stabilization, the perfusate was collected 5 times every 2-min interval as a control period. Then moxonidine (3, 10, and 30 μmol/l) was cumulatively administered. Pulse pressure was decreased by 3 μmol/l moxonidine and gradually attenuated by higher doses of moxonidine. ANP secretion and concentration were increased in a dose-dependent manner, but ECF translocation was maintained relatively constant. Time-CONT, atria perfused with HEPES buffer; CONT, control period. *P < 0.05; **P < 0.01 vs. fifth period of control value.

Modification of effects of moxonidine or clonidine by atrial hypertrophy. When remnant-kidney hypertensive rats were killed 4 wk after surgery, the left hearts were hypertrophied. The ratio of left ventricle and septum weight to right ventricle (y = 2.52x + 5.06; $r^2 = 0.25$; n = 45; $P < 0.005$). This model (at 4 wk after surgery) caused mild atrial hypertrophy (~15%). The basal pulse pressure, ECF translocation, and ANP secretion values for the hypertrophied atria were 9.50 ± 1.07 mmHg, 59.16 ± 3.61 μl·min⁻¹·g⁻¹, and 15.29 ± 2.26 ng·min⁻¹·g⁻¹, respectively, which were not different from the control group (9.57 ± 1.07 mmHg, 71.63 ± 2.73 μl·min⁻¹·g⁻¹, and 16.93 ± 2.08 ng·min⁻¹·g⁻¹, respectively) and the sham group (8.74 ± 1.02 mmHg, 63.06 ± 6.19 μl·min⁻¹·g⁻¹, and 19.38 ± 4.50 ng·min⁻¹·g⁻¹, respectively). The moxonidine-induced (10 μmol/l) decrease in pulse pressure was dramatically augmented in hypertrophied atria. 

No differences in the relative changes in ECF translocation between hypertrophied and nonhypertrophied control atria were found (Fig. 4B). Increases in ANP secretion and interstitial ANP concentration by moxonidine were markedly augmented in hypertrophied atria (Fig. 4A). The relative percent changes in ANP secretion by moxonidine were positively correlated to the ratio of atrial weight over body weight ($r^2 = 0.67$; n = 7; $P < 0.01$) and left ventricular weight to right ventricle ($y = 2.52x + 5.06; r^2 = 0.25; n = 45; P < 0.005$).

**Fig. 2.** Relative percent changes in pulse pressure (A), ECF translocation (B), ANP secretion (C), and ANP concentration (D) by moxonidine (n = 7) or clonidine (3, 10, or 30 μmol/l; n = 5) compared with control group. Values are expressed as percent changes of the last five experimental values exposed to moxonidine or clonidine compared with the mean of five control values. Moxonidine and clonidine caused decreases in pulse pressure that did not differ from the control group except for the lowest dose of moxonidine. There was no significant change in ECF translocation by moxonidine or clonidine. ANP secretion and interstitial ANP concentration were increased in a dose-dependent manner by moxonidine but not by clonidine. *P < 0.05 vs. corresponding group. **P < 0.01 vs. corresponding group.
Fig. 3. Effects of moxonidine on pulse pressure, ANP secretion, ECF translocation, and ANP concentration (A) in the presence of 0.3 μmol/l efaroxan (n = 7) or rauwolscine (n = 8). Both antagonists did not cause any significant changes in basal ECF translocation and ANP secretion. Efaroxan and rauwolscine inhibited the moxonidine-induced decrease in pulse pressure and increase in ANP secretion. Relative percent changes in pulse pressure, ECF translocation, ANP secretion, and ANP concentration by moxonidine in the presence of efaroxan (Efa) or rauwolscine are shown (Rau; B). Antagonistic effects of efaroxan on ANP secretion were greater than those of rauwolscine. CONT, moxonidine-perfused atria without pretreatment of antagonist. *P < 0.05; **P < 0.01 vs. control group; #P < 0.05 vs. corresponding group.

Fig. 4. Comparison of effects of moxonidine on pulse pressure (A), ECF translocation (B), ANP secretion (C), and ANP concentration (D) in hypertrophied atria from remnant-kidney hypertensive rats (HYPER; n = 7) and nonhypertrophied atria from sham rats (SHAM; n = 7). Moxonidine (3 μmol/l)-induced decrease in pulse pressure was dramatically augmented in hypertrophied atria compared with nonhypertrophied atria. However, no significant differences in relative change to ECF translocation between hypertrophied and nonhypertrophied atria were found. Moxonidine-induced increases in ANP secretion and concentration were markedly accentuated in hypertrophied atria. *P < 0.05; **P < 0.01; ***P < 0.001 vs. corresponding sham group.
hypertrophy \((r^2 = 0.72; P < 0.01)\). To determine which receptors are involved in augmentation of the effect of moxonidine on ANP secretion, efaroxan (0.3 \(\mu\)mol/l) or rauwolscine (0.3 \(\mu\)mol/l) was administered as a pretreatment before simultaneous perfusion of moxonidine (10 \(\mu\)mol/l). As shown in Fig. 5, the augmentation of moxonidine-induced ANP secretion and negative inotropism were attenuated by efaroxan but not by rauwolscine. \(*P < 0.05\) vs. control group.

**DISCUSSION**

The present study clearly shows that the preferential activation of imidazoline receptors by moxonidine directly causes dose-dependent increases in atrial ANP release with a different mechanism for clonidine, and this effect is augmented in hypertrophied atria. In addition to imidazoline receptors, the mechanism involved in the moxonidine-induced increase in ANP release may partially include activation of \(\alpha_2\)-adrenoceptors.

Centrally acting antihypertensive drugs such as clonidine (mixed \(I_1\) and \(\alpha_2\)-agonist) were originally thought to reduce blood pressure via the \(\alpha_2\)-adrenoceptors by decreasing sympathetic nerve activity (13). However, Bousquet et al. (1) suggest that the antihypertensive effect is also related to imidazoline receptors. Imidazoline receptors are distinct from adrenergic and histaminergic receptors, as they show a low affinity for catecholamines (1, 19, 25). The \(I_1\)-imidazoline receptors are mainly distributed in the brain stem and peripheral tissues including heart (2, 3, 9, 26). Moxonidine, a second-generation imidazoline derivative, is chemically and pharmacologically similar to clonidine but shows a 100-fold higher affinity for \(I_1\)-imidazoline receptors over \(\alpha_2\)-adrenoceptors (12, 20). In the present study, we evaluated the effects of moxonidine on ANP secretion compared with clonidine. This study clearly shows dose-dependent increases in ANP release by moxonidine but not by clonidine in isolated, perfused, beating atria. There are a few comparable reports that describe the direct effects of moxonidine on atrial ANP release. In vivo study has shown that moxonidine causes diuresis and natriuresis with increasing plasma ANP levels and cGMP excretion in a dose-dependent manner (16). The renal effects and urinary cGMP excretion caused by clonidine were shown to be more potent than those...
caused by moxonidine (16). Mukaddam-Daher and Gutkowska (18) reported (using the Langendorff-perfused heart) that clonidine stimulates ANP release with bradycardia through the α2-adrenoceptors. In that study, using a different atrial model (minced atrium), they also found an increase in basal ANP release by the peripherally acting clonidine analog ST-91. We do not know the reason for the discrepancy in the effects of clonidine on ANP secretion at present. It may be due to use of a different experimental model: Mukaddam-Daher and Gutkowska used an isolated, spontaneously beating whole heart, whereas we used an isolated, electrically stimulated atrium. As seen in Fig. 2A, the effects of clonidine on atrial hemodynamics appear to be relatively weak. Therefore, another possible explanation is that the clonidine-induced increase in ANP secretion may relate to changes in cardiac hemodynamics (mainly ventricles) that would exist because they used the whole heart (18).

When moxonidine was administered cumulatively, the lowest dose of moxonidine decreased the atrial pressure (by 25%), and high doses of moxonidine had no effect. However, when a single dose of moxonidine was administered, a high dose also caused negative inotropism (by 25%). Therefore, it is possible that the 3 μmol/l dose may affect the response to the subsequent higher dose. For example, a low dose of moxonidine may have reset the atrial myocytes to a new, lower basal pressure, after which the pressure increases associated with the higher doses contributed to observed changes in ANP, or the receptors may have already been saturated with the lowest dose; therefore, the cumulative administration of more moxonidine would not make a difference. However, the response of ANP by moxonidine was dose dependent. We do not know the reason; additional studies are needed. In both normal and hypertrophied atria, the lowest dose resulted in a significant drop in pulse pressure that was augmented in hypertrophied atria. These results suggest that moxonidine does affect atrial hemodynamics and may partly alter ANP release.

To determine which receptor subtypes are involved in the stimulation of ANP secretion and negative inotropism by moxonidine, atria were pretreated with specific antagonists for I1-imidazoline receptors or α2-adrenoceptors. Both efaroxan and rauwolscine attenuated the moxonidine-induced increase in ANP secretion, but the potency of efaroxan was greater than that of rauwolscine. Previous studies using receptor antagonists have shown that moxonidine-stimulated diuresis and natriuresis are inhibited by efaroxan and yohimbine (despite a quantitative difference), but increases in plasma ANP levels and cGMP excretion by moxonidine are inhibited only by efaroxan (16). It was shown (10, 18) that the actions of clonidine and ST-91 on ANP secretion were inhibited by both antagonists. However, the actions of ST-91 were mainly mediated by the I1-imidazoline receptor and the actions of clonidine were mainly mediated by α2-adrenoceptors. Therefore, it is difficult for us to specify which receptor subtypes are responsible for the actions of clonidine and moxonidine owing to the different, tissue-dependent specificities of antagonists. We suggest that moxonidine directly increases ANP release via the preferential activation of atrial I1-imidazoline receptors as well as α2-adrenoceptors with different mechanisms from clonidine.

Several studies have shown that I1-imidazoline receptors are altered in certain physiological and pathological states. I1-imidazoline receptors are upregulated in platelets of patients with depression (21) and in postmenopausal women (22). Renal I1-imidazoline receptors are upregulated in genetically hypertensive rats (17), and heart I1-imidazoline receptors are upregulated in the presence of hypertension or heart failure (9). In contrast, the attenuated renal response of moxonidine is reported to possibly contribute to an increase in blood pressure in the one-kidney, one-clip acquired model of hypertension (14). What is the role of I1-imidazoline receptors in the development of hypertension? Mukaddam-Daher and Gutkowska’s study (17) shows that blood pressure is not altered in Wistar-Kyoto rats but is significantly decreased in spontaneously hypertensive rats after treatment with moxonidine. These results indicate that the drugs acting on the I1-imidazoline receptor have beneficial effects in the treatment of hypertension. But Li et al. (14) show that the attenuation of moxonidine induced increases in urine volume, sodium excretion, and osmolar clearance in one-kidney, one-clip hypertensive rats. The roles of imidazoline receptors in the development of hypertension are still controversial. The present study demonstrates augmentation of the moxonidine (but not the clonidine)-induced increase in ANP secretion in hypertrophied atria. We also found that the relative changes in ANP secretion induced by moxonidine are positively correlated with the degree of cardiac hypertrophy. In addition, the accentuation of moxonidine-induced ANP secretion was attenuated by efaroxan but not by rauwolscine. Therefore, we speculate that the augmentation of the effects of moxonidine on pulse pressure and ANP release in hypertrophied atria may be related to the upregulation of atrial I1-imidazoline receptors, and these results provide an advantage for us in using moxonidine for the treatment of hypertension.

In summary, moxonidine directly increases ANP release with negative inotropism mainly through atrial I1-imidazoline receptors and α2-adrenoceptors, and these effects are augmented in hypertrophied atria. These results suggest that atrial I1-imidazoline receptors play an important role in the regulation of blood pressure.

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