Renal $\alpha_2$-Adrenergic Receptors Multiply and Mediate Sodium Retention After Prazosin Treatment

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SUMMARY Renal nerve stimulation-induced antinatriuresis normally is mediated through post-synaptic $\alpha_1$-adrenergic receptors; however, prazosin-induced $\alpha_1$-adrenergic receptor blockade is associated clinically with sodium retention and not natriuresis. To study whether $\alpha_2$-adrenergic receptors mediate renal nerve stimulation-induced antinatriuresis after chronic prazosin treatment, Sprague-Dawley rats were pretreated for 3 days with prazosin (3 mg/kg/day i.p. plus 0.15 mg/ml drinking water) or vehicle (untreated). In isolated perfused (Krebs-Henseleit; Ficoll, 3.5 g/dl, + albumin, 1.0 g/dl at 36°C) kidneys from untreated rats, subpressor levels of renal nerve stimulation (~1 Hz, 10 V, 1 msec) decreased (p < 0.05) sodium (from 4.50 ± 0.42 to 1.71 ± .23 $\mu$Eq/min) and urinary excretion rate (from 87.2 ± 4.1 to 57.9 ± 3.9 $\mu$l/min). Adding prazosin (30 nM) to the perfusate completely (~90%) reversed this effect (p < 0.05), while $\alpha_2$-adrenergic receptor blockade with yohimbine (300 nM) had no effect. In perfused kidneys from prazosin-treated rats, renal nerve stimulation decreased (p < 0.05) sodium (from 3.24 ± .40 to 1.32 ± .27 $\mu$Eq/min) and urinary excretion rate (from 78.7 ± 5.0 to 54.1 ± 5.3 $\mu$l/min). However, adding prazosin (100 nM) to the perfusate produced only a slight, insignificant reversal of these effects; prazosin plus yohimbine were required to completely reverse the effects. These results suggest that renal nerve stimulation-induced sodium reabsorption was activated by $\alpha_1$-adrenergic receptors in untreated rats and in part by $\alpha_2$-adrenergic receptors in rats pretreated for 3 days with prazosin. Since renal $\alpha_2$-adrenergic receptor density increased (p < 0.05) with prazosin therapy, the most likely explanation is that newly formed $\alpha_2$-adrenergic receptors occupy the postsynaptic sites at which $\alpha_1$-adrenergic receptors ordinarily predominate. (Hypertension 8: 323-331, 1986)

KEY WORDS • renal $\alpha_2$-adrenergic receptors • renal nerve stimulation • sodium retention • chronic prazosin • postjunctional • $\alpha_1$ and $\alpha_2$ adrenergic receptors • neurogenic sodium transport

ACTIVATION of renal $\alpha$-adrenergic receptors induces sodium retention, and renal denervation causes sodium and water excretion. Subpressor levels of renal nerve stimulation (RNS) enhance sodium and water reabsorption through activation of $\alpha$-adrenergic receptors. Recent studies in the dog and rabbit have identified the $\alpha_1$-adrenergic receptor as the receptor subtype mediating the RNS-induced sodium and water retention. Prazosin, a selective $\alpha_1$-adrenergic receptor antagonist, has been used clinically in the treatment of hypertension. Although initial therapy with prazosin causes orthostatic hypotension (termed "the first dose phenomenon"), this hypotension dissipates within 24 hours, which suggests that another receptor mediates sympathetic nervous system regulation of venous tone with continuing $\alpha_1$-adrenergic receptor blockade. Prazosin-induced $\alpha_1$-adrenergic receptor blockade has not been associated clinically with natriuresis, as would be expected if renal $\alpha_1$-adrenergic receptors mediate sodium and water retention. In fact, an increase in sodium retention occurred with prazosin therapy. Together these observations suggest that an alternate
mechanism or receptor subtype mediates effects activated by \(\alpha\)-adrenergic receptors in untreated persons.

The present study was undertaken to identify the \(\alpha\)-adrenergic receptor subtype(s) mediating subpressor RNS-induced sodium retention in normal rats and in rats treated with prazosin for 3 days. The nonrecirculating isolated perfused rat kidney model was chosen since it eliminates several extraneous variables (i.e., neural, hormonal) that may alter sodium excretion and allows regulation of perfusate flow and perfusion pressure at a predetermined level.

**Materials and Methods**

**In Vivo Prazosin Pretreatment**

Male Sprague-Dawley rats (Harlan, Houston, TX, USA) weighing 280 to 310 g were divided into two groups of 44 each. The control group received normal Purina rat chow (St. Louis, MO, USA) and tap water for drinking and underwent either 1 or 3 days of vehicle injection (dextrose, 5 g/dl). The prazosin pretreatment group received 1 or 3 days of prazosin pretreatment, consisting of daily intraperitoneal injections of prazosin (3 mg/kg in dextrose, 5 g/dl; Pfizer & Co., New York, NY, USA) and prazosin (0.15 mg/ml) added to the drinking water. Based on a daily water consumption of 10 to 20 ml, each rat would receive an oral dose of 1.5 to 3.0 mg/day. Systolic blood pressure was recorded daily in these rats immediately before and 2 hours after the administration of vehicle or prazosin. Blood pressure was recorded with the tail cuff method in unanesthetized restrained rats with a programmed electrophysymomanometer (Type 7211, Narco Biosystems, Houston, TX, USA). Following these treatments, the rats were either 1) killed by decapitation, and their kidneys removed, quick-frozen (ethanol and dry ice), and stored at -40°C for later radioligand binding studies or 2) anesthetized, and their kidneys surgically isolated and perfused with a nonrecirculating artificial medium. In the prazosin-treated group, only animals treated for 3 days were perfused since 1 day of prazosin therapy did not significantly alter renal \(\alpha\)-adrenergic receptor density.

**Radioligand Binding Studies**

Kidneys from control and prazosin-treated rats were assayed for radioligand binding studies. Renal plasma membranes were prepared, and binding studies were performed as described by Williams et al. with modifications by Schmitz et al. \[15\] \([3H]\)Rauwolscine (0.50–20.0 nM) was used to construct Scatchard plots in duplicate for each assay. The maximal concentration of the radioligand studied was at least five times that of its dissociation constant \(K_d\) to ensure adequate saturation of the receptor sites. \[15\] Renal membranes (100 \(\mu\)l, protein concentration, 2.5–4.0 mg/ml) were incubated with each radioligand for 30 minutes at 25°C in a 50 mM Na\(_2\)HPO\(_4\) and 50 mM KH\(_2\)PO\(_4\) (pH = 7.4) buffer, in a final volume of 150 \(\mu\)l. Incubation was terminated by addition of 5 ml of cold buffer (4°C) and instantaneous filtration through Whatman GF/C glass fiber filters (Clifton, NJ, USA). The filters were washed with three additional 5-ml aliquots of cold buffer (4°C), dried, placed in scintillation vials, and counted in 10 ml of octoxyxynol (Triton X-100)-toluene aqueous scintillation mixture. Counting efficiency was 40.5%. Specific binding in this study refers to the fraction of bound radioligand displaced by 10 \(\mu\)M phenolamine.

The radioligand was diluted to the appropriate concentrations in 10% ethanol, 5 mM HCl, and 0.2% bovine serum albumin. This diluent did not alter the pH of the incubation mixture or the binding of the radioligands to the renal membranes but was effective in decreasing the nonspecific binding of compounds to the plastic tubes used in the assay. \[16\] Maximum specific binding with the lowest ligand concentration was 85 to 90% for rauwolscine. All experiments were performed in duplicate and consisted of a minimum of six radioligand concentrations for the Scatchard plots. Membrane protein was determined according to the procedure of Lowry et al. using bovine serum albumin as the standard.

The characteristics of the \(\alpha\)-adrenergic receptors were determined by the ability of cold antagonists, both specific for \(\alpha\)-adrenergic (prazosin) and \(\alpha\)-adrenergic (rauwolscine) receptors and nonspecific (phenolamine), to compete for \([3H]\)rauwolscine binding to rat renal membranes. Membranes were incubated for 30 minutes at 25°C with \([3H]\)rauwolscine (4–6 nM) in the presence of increasing levels of antagonist. The apparent \(K_d\) values were calculated from the equation \(K_d = EC_{50}/(1 + [R/K_0])\), where EC\(_{50}\) is the concentration of antagonist producing 50% inhibition of \([3H]\)rauwolscine binding, \(R\) represents the concentration of \([3H]\)rauwolscine, and \(K_0\) is the dissociation constant of \([3H]\)rauwolscine determined by equilibrium binding studies.

**Isolated Perfused Kidney**

The right kidney from control rats or rats treated with 3 days of prazosin was perfused with a nonrecirculating perfusate as described by Smyth et al. \[17\] With the rats under pentobarbital anesthesia (50 mg/kg, i.p.), an abdominal cruciate incision was made and the aorta exposed by reflecting the abdominal viscera to the right. A loose tie was placed around the right renal artery. The right ureter was cannulated (polyethylene 50 tubing). The superior mesenteric artery was cannulated in the retrograde direction, and the tip of the glass perfusion cannula advanced to the aorta. Once perfusate flow was begun, the cannula was advanced into the right renal artery and the appropriate ligatures secured. The kidney was removed from the surrounding viscera, cleaned of perirenal tissue, and placed in the perfusion chamber. Perfusate flow was adjusted during the stabilization period to produce a perfusion pressure of 100 mm Hg (ITT Cannon pressure transducer, Model DMP-43 physiograph, Narco Biosystems).

The perfusion medium, a modified Krebs-Henseleit solution, contained the following components (in mM): NaCl, 110; KCl, 4.7; CaCl\(_2\), 2.5; KH\(_2\)PO\(_4\), 1.2;
MgSO₄, 1.2; NaHCO₃, 26; dextrose, 11.0; urea, 6; β-alanine, 10; Na₂HPO₄, 1.2. Bovine serum albumin (fraction V, Pentex Biochemical, Kankakee, IL, USA) 1.0 g/dl, Ficoll 70 (Sigma Chemical Co., St. Louis, MO, USA), 3.5 g/dl, an amino acid mixture that produced amino acid levels similar to that observed in rat plasma, and a β-adrenergic-selective concentration of propranolol (100 nM) were added to the perfusion medium. Creatinine, 50 mg/dl, was added for determination of creatinine clearance as an indication of the glomerular filtration rate, using a previously described method. The solution was oxygenated by passing the perfusate through the hollow core fibers of a C-DAK 135sce artificial kidney (Cordis Dow, Miami Lakes, FL, USA). Equilibration with 95% O₂, 5% CO₂ was achieved by flowing this gas mixture through the dialysis chamber at a rate of 2 L/min per minute. The perfusate was prefiltered and filtered again (5.0 μM) before entering the kidney. The temperature of the perfusate at the level of the kidney was 35 to 37°C with pH of 7.35 to 7.45. A Harvard peristaltic pump (Model 1210, Millis, MA, USA) was used to maintain a pulsatile flow. Perfusate and urinary sodium and potassium concentrations were determined with a Beckman Klina Flame Photometer (Palo Alto, CA, USA).

**Perfused Kidney Protocol**

After a 15-minute stabilization period, four timed, 10-minute urine collections were obtained in preweighed collection tubes at 15, 30, 40, and 50 minutes of the perfusion. In no experiments were interventions begun until after the first urine collection (15–25 minutes) so that this first collection period could verify the renal function of each individual preparation. Following the first collection period and immediately before the second collection period, untreated and prazosin-treated rats received one of the following: 1) no intervention (control), 2) RNS, 3) RNS and yohimbine, and 4) RNS and prazosin, or 5) yohimbine and prazosin.

In all groups studied, the kidney was cleaned of perirenal tissue once perfusion was begun. The tissue around the renal artery was left intact since the nerves are located in this region. Two platinum electrodes were placed in contact with the renal artery as close to the end of the cannulated portion as possible. The renal nerves located in this area were then stimulated (Narco Stimulator S1-10) at 10 V and 1.0 msec at a frequency below the threshold level required to induce an increase in perfusion pressure. This level was determined by starting at 0.3 Hz and gradually increasing the frequency until a slight change in perfusion pressure was observed (3–5 mm Hg). The frequency was then adjusted just below this level. Kidneys that failed to demonstrate an effect by 3.0 Hz were excluded. Similarly, the threshold was reestablished at the end of the experiment, and kidneys that failed to reestablish a baseline threshold were excluded. Thus, the continual nerve stimulation could be verified. Although the threshold level of RNS also was determined in the control groups, the stimulator was not turned on again once this was demonstrated.

The following groups were studied in untreated rats and in rats pretreated with prazosin for 3 days. The control groups underwent no experimental intervention after the first urine collection. The effects of subpressor levels of RNS were determined by beginning the RNS immediately after the first urine collection and continuing this for the duration of the experiment. All experiments were conducted in the presence of β-adrenergic receptor blockade (100 nM dl-propranolol; Sigma). To determine the α₂-adrenergic receptor subtype(s) mediating the effect of RNS on sodium and water excretion, specific antagonists of α₂-adrenergic receptor subtype(s) were administered. In the untreated rats, α₂-adrenergic receptor blockade with prazosin (30 nM) was studied in the presence of RNS. Preliminary studies demonstrated that this dose completely reversed the effects of RNS on sodium and water excretion. In rats pretreated with 3 days of prazosin, 30 nM of prazosin failed to alter the effects of RNS. As a result, a higher dose of prazosin (100 nM), which was still α₂-adrenergic receptor-specific, was used. Thus, in the pretreated rats α₂-adrenergic receptor blockade with prazosin (100 nM) was studied in the presence of RNS. α₂-Adrenergic receptor blockade with 300 nM of yohimbine (sigma) was also studied in the presence of RNS in untreated and prazosin-treated rats.

In untreated rats, prazosin (30 nM) completely reversed the effects of RNS. In the prazosin-pretreated group, however, neither prazosin or yohimbine alone significantly altered the effects of RNS. Thus, the combined effect of prazosin (100 nM) and yohimbine (300 nM) on RNS-induced effects was studied in prazosin-treated rats.

**Statistical Analysis**

Statistical analyses were performed with an analysis of variance. Homogeneity of the variances of the groups studied was established with Bartlett’s test. The level of significance between groups was determined with Newman–Keuls multiple comparison.

**Results**

The renal α₂-adrenergic receptor density, as determined by Scatchard analysis of equilibrium binding data, in rats pretreated with 1 or 3 days of prazosin and in the corresponding vehicle controls is shown in Figure 1. After 1 day of treatment there was a trend toward increased α₂-adrenergic receptor density (187 ± 10 vs 153 ± 14 fmol/mg protein; p = 0.072). After 3 days, however, there was a significant increase in renal α₂-adrenergic receptor density in the prazosin-treated rats (209.8 ± 10.8 fmol/mg protein; p < 0.01) compared with that in the control rats receiving vehicle (157.8 ± 11.3 fmol/mg protein). A slight but significant increase in the Kᵦᵣ also occurred after 3 days of prazosin treatment (6.7 ± 0.5 nM; p < 0.05) compared with that seen in the control kidneys (5.0 ± 0.2 nM). Since a consistent and reproducible increase in α₂-adrenergic receptor density could be demonstrated following 3 days of prazosin pretreatment, 3 days of prazosin pretreatment was used for the perfusion studies.
Binding of [3H]rauwolscine was rapid and reversible; equilibrium was achieved in 10 minutes in both prazosin-treated and control kidneys (Figures 2 and 3). The $K_d$ values determined by kinetic analysis (where $K_d = k_2/k_1$) for the prazosin-treated group (4.61 ± 0.5 nM) and the control group (5.96 ± 1.56 nM) were similar to those determined by Scatchard analysis (see Figure 1).

The $K_a$ values determined in the competitive inhibition studies (Figure 4) with unlabeled rauwolscine were similar to those found using kinetic and Scatchard analysis. The $K_a$ values were 4.85 and 3.74 nM, respectively, for the prazosin-treated group and control groups. Specificity of [3H]rauwolscine binding was also similar between two groups as demonstrated by the order of potency of specific antagonists to competitively inhibit radioligand binding. In both groups, the order of potency of inhibition was rauwolscine > > prazosin => phenolamine.

Prestudy blood pressures were similar in control and prazosin-treated rats (Figure 5). Two hours after the first treatment (Day 0), a significant decrease in blood pressure was observed (from 135.2 ± 4.1 to 113.7 ± 2.3 mm Hg) only in the prazosin-treated group. This decrease was not repeated on Day 1 or Day 2 of the prazosin treatment; in fact, blood pressure in this group was significantly greater on Day 1 than that seen following the first treatment (123.3 ± 2.7 versus 113.7 ± 2.3 mm Hg; $p < 0.05$). The blood pressure did, however, remain lower in the prazosin-treated rats than in the control (vehicle-treated) rats.

Because renal hemodynamics and electrolyte excretion were similar in all groups during the first urine collection period (which allowed verification of renal function since no experimental interventions had been administered), these data were pooled in two separate
FIGURE 3. Kinetic analysis of $[^3H]$rauwolscine binding to rat renal membranes obtained from rats pretreated with prazosin for 3 days. Onset and offset curves were obtained as described in Figure 2.

![Onset Curve](image1)

![Offset Curve](image2)

FIGURE 4. Competitive inhibition of $[^3H]$rauwolscine binding (4–6 nM) with adrenergic receptor antagonists in renal membranes obtained from rats treated with vehicle or prazosin for 3 days.

![Inhibition Curves](image3)

FIGURE 5. Effect of daily intraperitoneal injections of vehicle and prazosin on blood pressure in normal rats. Prazosin or vehicle was administered immediately following the blood pressure measurement (Pre). Blood pressure measurement was repeated 2 hours later (Post). Day 0 represents the first dose of vehicle or prazosin administered. Asterisk indicates $p < 0.05$ groups for comparison, as shown in Table 1. Although perfusion pressure was similar in both groups, perfusate flow was slightly greater in kidneys from untreated (20.0 ± 0.3 ml/min; $p < 0.05$) than from prazosin-pretreated rats (18.8 ± 0.4 ml/min). Creatinine clearance was generally, but not significantly, less in the untreated rats than in the prazosin-pretreated rats, while urine volume (43.2 ± 2.4 vs 31.3 ± 2.4 μl/min), urinary sodium excretion (1.09 ± 0.14 vs 0.54 ± 0.07 μEq/min), and urinary potassium excretion (1.84 ± 0.15 vs 0.40 ± 0.05 μEq/min) were significantly less in prazosin-pretreated rats ($p < 0.05$) than in control rats.

The experimental interventions were administered immediately before the second urine collection in kidneys from untreated and prazosin-pretreated rats and had a latent interval of onset, since no significant changes occurred until the third collection period. These effects of RNS and α-adrenergic receptor blockade are presented in detail in Figure 6.
TABLE 1. Effect of 3-Day Prazosin Pretreatment on Renal Hemodynamics and Electrolyte Excretion in the Isolated Perfused Rat Kidney

<table>
<thead>
<tr>
<th>Group</th>
<th>PF (ml/min)</th>
<th>PP (mm Hg)</th>
<th>Cr (ml/min)</th>
<th>V (µl/min)</th>
<th>U_{Na}V (µEq/min)</th>
<th>U_{K}V (µEq/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (n = 29)</td>
<td>20.0±0.3</td>
<td>96.6±1.0</td>
<td>0.69±0.04</td>
<td>43.2±2.4</td>
<td>1.09±0.14</td>
<td>1.84±0.15</td>
</tr>
<tr>
<td>Prazosin pretreatment (n = 33)</td>
<td>18.8±0.4*</td>
<td>95.1±0.7</td>
<td>0.84±0.07†</td>
<td>31.3±2.4†</td>
<td>0.54±0.07§</td>
<td>0.40±0.05‡</td>
</tr>
</tbody>
</table>

Values are means ± SEM  
PF = perfusate flow, PP = perfusion pressure, Cr = creatinine clearance, V = urinary excretion rate; U_{Na}V = urinary sodium excretion, U_{K}V = urinary potassium excretion.  
*p < 0.013, †p < 0.057, ‡p < 0.001, §p < 0.002, compared with values in untreated group.

In kidneys obtained from untreated rats, by the third urine collection period, RNS at subpressor levels had significantly decreased (p < 0.05) urinary volume (from 87.2 ± 4.1 to 57.9 ± 3.9 µl/min) and urinary sodium excretion (from 4.50 ± 0.42 to 1.71 ± 0.23 µEq/min) as compared to that in the control kidneys (see Figure 1). Yohimbine (300 nM), an α₂-adrenergic receptor antagonist, had no effect on RNS-induced increases in sodium and water retention; however, prazosin (30 nM), an α₁-adrenergic receptor antagonist, significantly attenuated these effects. This effect was demonstrated by a significant increase in the urine volume (from 57.9 ± 3.9 to 82.5 ± 3.5 µl/min) and urinary sodium excretion (from 1.71 ± 0.23 to 3.98 ± 0.34 µEq/min) in the group receiving RNS plus prazosin as compared to the increases in the group receiving RNS alone. This prazosin-induced reversal of the RNS effects indicates that these effects were mediated through α₂-adrenergic receptor activation. The group receiving RNS plus yohimbine group had a slight but significant decrease in glomerular filtration rate as compared to that in the control group. Perfusion flow was similar in all groups studied.

In kidneys obtained from rats pretreated with prazosin (3 days), subpressor levels of RNS again significantly decreased (p < 0.05) urinary volume (from 78.7 ± 5.0 to 54.1 ± 5.3 µl/min) and urinary sodium excretion (from 3.24 ± 0.40 to 1.32 ± 0.27 µEq/min) as compared to results in the control group. Perfusate flow was similar in all groups studied.

Figure 6. Alterations in electrolyte excretion and renal hemodynamics following subthreshold renal nerve stimulation (RNS) in the presence and absence of α₁-adrenergic and α₂-adrenergic receptor blockade in 62 rats. Kidneys from untreated (left-hand panel) and prazosin-pretreated rats (right-hand panel) received (from left to right) no intervention (control), RNS, RNS plus 300 nM yohimbine, RNS plus 30 nM prazosin, or RNS plus 300 nM yohimbine and 100 nM prazosin (only in pretreated rats). U_{Na}V = urinary sodium excretion, GFR = glomerular filtration rate, as determined by creatinine clearance. Each group represents mean ± SEM of five to nine experiments. Asterisk indicates p < 0.05.
excretion (1.32 ± 0.27 to 3.63 ± 0.60 μEq/min) in the group receiving RNS plus yohimbine and prazosin as compared to that in the group receiving RNS alone. Neither of these groups, however, had significant changes in perfusate flow or glomerular filtration rate.

Discussion

Previous studies in the rat have indicated the importance of the renal sympathetic nerves in the retention of sodium and water. For example, renal denervation enhances sodium and water excretion, and low levels of RNS significantly enhance sodium and water reabsorption. None of these studies have identified the receptor subtype(s) mediating these sympathetic nervous system effects. Findings in the present study indicate that the α₂-adrenergic receptor mediates the RNS effects on sodium and water reabsorption in normal rats and are consistent with results reported in the dog and rabbit.

Treatment with prazosin for 3 days significantly increased the renal density of α₂-adrenergic receptors in the rat. Kidneys obtained from these rats still demonstrated an enhanced retention of sodium and water following RNS. Unlike results in the untreated rats, however, the addition of prazosin to the perfusate in treated rats failed to alter significantly these RNS effects. Yohimbine-induced α₂-adrenergic receptor blockade produced a similar, slight but nonsignificant reversal of the RNS effects. In fact, in these kidneys from prazosin pretreated rats, yohimbine as well as prazosin was required to reverse the RNS effects back to control levels. These results suggest a role of α₂-adrenergic as well as α₁-adrenergic receptors in RNS-induced sodium and water reabsorption following 3 days of prazosin pretreatment.

Stimulation of renal α₂-adrenergic receptors by agonist infusion will attenuate the renal effects of vasoressin. In the isolated perfused rat kidney, α₂-adrenergic receptor activation by epinephrine infusion (in the presence of α₁-blockade and β-blockade) completely reversed the renal effects of vasoressin. This effect probably was due to inhibition of cyclic adenosine 3',5'-monophosphate (cAMP) formation. We have previously shown that RNS, at the same subpressor levels as those used in the present study, fails to activate α₂-adrenergic receptors, as demonstrated by the failure to antagonize vasoressin's effects. In the present study, subpressor levels of RNS enhanced sodium and water retention in normal rats by α₁-adrenergic receptor activation. These results suggest an arrangement of tubular α₁-adrenergic and α₂-adrenergic receptors as depicted schematically in Figure 7A. The failure of α₁-adrenergic receptors (associated with the antagonism of vasoressin) to be activated by RNS is consistent with an extrajunctional location of these receptors. Similarly, the activation of α₂-adrenergic receptors associated with sodium retention by low levels of nerve stimulation would be consistent with the postulate that these receptors are located postjunctionally.

A similar arrangement has been demonstrated for vascular α-adrenergic receptors. α₁-Adrenergic receptors have been activated by nerve stimulation and α₂-adrenergic receptors mediating vasoconstriction by circulating catecholamines. In normal pithed rats, the pressor response to infused catecholamines was insensitive to α₁-antagonists but selectively blocked by α₂-antagonists. The pressor response following nerve stimulation was selectively blocked by α₂-antagonists. Thus, the arrangement of vascular α₁-adrenergic receptors in normal rats would be consistent with that shown in Figure 7A. However, vasoconstriction would be substituted for the end-organ response, and the α₁-adrenergic receptors would be associated with calcium channels.

This exclusivity in location of vascular α₂-adrenergic receptors to an extrajunctional location, however, appears to be altered in genetic forms of rat hypertension. In the normotensive Wistar-Kyoto rat, the pressor response to field stimulation in the isolated perfused tail artery was unaltered by α₂-adrenergic receptor antagonists. The same response in tail arteries from spontaneously hypertensive rats, however, was significantly attenuated by α₂-adrenergic as well as α₁-adrenergic receptor antagonists. These results

![Figure 7](http://hyper.ahajournals.org/)

**Figure 7.** Hypothetical tubular neuroeffector (NE) junction in normal rats (A) and following 3 days of prazosin treatment (B) G protein = inhibitory protein of adenylate cyclase
are consistent with the schema in Figure 7B, with vasoconstriction substituted for the end-organ response. In turn, α₂-adrenergic receptors, if also elevated in number extrajunctionally, may explain the increased sensitivity observed in this tissue to catecholamines and the subsequent increase in peripheral resistance.

The arrangement of renal tubular α-adrenergic receptors may be altered in a similar fashion in certain situations. Following 3 days of prazosin pretreatment, subpressor levels of RNS increased sodium and water retention by activation of both α₁-adrenergic and α₂-adrenergic receptors. Activation by these low levels of nerve stimulation are consistent with a postsynaptic location of α₁-adrenergic as well as α₂-adrenergic receptors, which are associated with the retention of sodium. This arrangement is shown in Figure 7B. These postsynaptic α₁-adrenergic receptors, however, probably are not associated with the adenylate cyclase system. Previous studies in the isolated perfused rat kidney have demonstrated that infusion of epinephrine alone (in the presence of α₁-blockade and β-blockade) in the absence of adenylate cyclase activation has no effect on sodium and water excretion. The renal effects of vasopressin (decreased sodium and water excretion) are mediated through adenylate cyclase–cAMP activation. In this situation α₂-adrenergic receptor stimulation reverses the effects of vasopressin (increases sodium and water excretion) through inhibition of cAMP formation. Thus, activation of α₁-adrenergic receptors associated with the adenylate cyclase system produced an effect only in the presence of an activated adenylate cyclase. In the present study, α₁-adrenergic receptor activation by subpressor levels of RNS increased sodium and water reabsorption in the presence of an unactivated adenylate cyclase system, which suggests that these receptors were not acting through inhibition of this enzyme.

Prazosin, an α₁-antagonist, was added to the perfusate to determine the contribution of α₁-adrenergic receptors to the RNS-induced sodium and water retention. This blocker has been demonstrated to be highly selective for postsynaptic α₁-adrenergic receptors. Thus, this blocker quantifies the contribution of α₁-adrenergic receptors to the RNS-induced effects. However, yohimbine, an α₂-adrenergic receptor antagonist, may have underestimated the contribution of α₂-adrenergic receptors to RNS-induced sodium retention following 3 days of prazosin pretreatment. Although yohimbine demonstrates a high selectivity for α₂-adrenergic receptors, both presynaptic and postsynaptic α₂-adrenergic receptors would have been antagonized. Presynaptic α₂-adrenergic receptors mediate a feedback inhibition of catecholamine release. Results of the present study show that yohimbine partially reversed the RNS-induced sodium retention at postsynaptic α₂-adrenergic-receptors; however, simultaneous blockade of presynaptic α₂-adrenergic-receptors may have masked this effect. The blockade of these receptors prevents feedback inhibition of catecholamine release. The resultant further increase in catecholamine release would act on the unblocked α₁-adrenergic receptors to enhance sodium retention further, an effect opposite to that produced by yohimbine on postsynaptic α₁-adrenergic receptors. This mechanism may explain why both α₁-adrenergic and α₂-adrenergic receptor blockade were required to reverse the effects of RNS in kidneys from prazosin-pretreated rats. Unfortunately, there was no way to prevent this occurrence since presynaptic and postsynaptic α₁-adrenergic receptors could not be preferentially blocked.

Initial therapy with prazosin is associated with orthostatic hypotension, also known as the first dose phenomenon. This effect has been related to a decrease in venous return due to dilatation of resistance and capacitance vessels. This hypotensive effect is greatly diminished after further treatment. Sympathetic nerve activity maintains arterial and venous tone through activation of α₁-adrenergic and α₂-adrenergic receptors. Thus, α₂-adrenergic receptor blockade initially would remove this component of sympathetically mediated tone. An increase in renal α₁-adrenergic receptors with repeated prazosin therapy was demonstrated in the present study; however, a similar increase in venous α₁-adrenergic receptors would explain the absence of this orthostasis with chronic prazosin therapy. With chronic therapy an increase in venous α₁-adrenergic receptors may maintain venous tone despite continued α₁-adrenergic receptor blockade. This contention is speculative and requires further study.

In summary, in normotensive rats subpressor levels of RNS increased sodium and water retention by activation of α₁-adrenergic receptors. Prazosin treatment for 3 days increased renal α₁-adrenergic receptor density. In these same rats, RNS-induced sodium and water retention was mediated through activation of α₁-adrenergic as well as α₂-adrenergic receptors. This α₂-adrenergic receptor–mediated sodium retention may explain the sodium retention observed with chronic prazosin treatment despite continued α₁-adrenergic receptor blockade. We postulate that during chronic prazosin therapy, newly synthesized α₂-adrenergic receptors assume postsynaptic locations and functions of otherwise exclusive locations and roles of α₁-adrenergic receptors.

Acknowledgments

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