Prazosin-Induced Alterations in Renal α-Adrenergic Receptor Function

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SUMMARY  Chronic (3-day) treatment with prazosin causes an increase in renal α2-adrenergic receptor density and the relocation of renal tubular α2-adrenergic receptors from extrajunctional to postjunctional sites. We investigated whether chronic prazosin treatment (2 mg/kg, i.p.) caused a functional alteration of other renal α2-adrenergic receptors, using the isolated perfused rat kidney. Prazosin significantly reduced blood pressure and increased heart rate during the 3-day treatment. Renal nerve stimulation (2-8 Hz, 10 V, 1 msec) caused a frequency-dependent increase in renovascular resistance, which was potently blocked by prazosin in vitro in both control and prazosin-treated rat kidneys. The vasoconstrictor response to the α2-adrenergic receptor-selective agonist BHT 933 (3-300 μM) was significantly higher in kidneys from prazosin-treated rats. Yohimbine (3-300 nM) potentiated the response to renal nerve stimulation in both treatment groups. The increase in vascular resistance following renal nerve stimulation was lower in kidneys from prazosin-treated rats, but the response to the α1-adrenergic receptor agonist methoxamine (0.3-1 μM) was unchanged. Further studies revealed that renal nerve stimulation-evoked norepinephrine release from prazosin-treated rat kidneys was significantly lower than release from untreated controls. This response could be normalized to control levels by a combination of cocaine (10 μM) and yohimbine (100 nM). Thus, chronic prazosin treatment caused enhanced α2-adrenergic receptor-mediated vasoconstriction and facilitated renal prejunctional inhibitory mechanisms. We conclude that with chronic α1-adrenergic receptor blockade there is increased α2-adrenergic receptor function in at least two and possibly three sites in the kidney; these include postjunctional tubular, prejunctional vascular, and possibly extrajunctional vascular sites. (Hypertension 9 [Suppl III]: III-125-III-129, 1987)

KEY WORDS • α2-adrenergic receptors • renal nerves • α1-adrenergic receptors • prazosin • yohimbine

CHRONIC α1-adrenergic receptor blockade with prazosin in rats increases the density of renal α2-adrenergic receptors.1 Some of these α2-adrenergic receptors, which are normally restricted to extrajunctional sites,2,3 move into and occupy postjunctional sites, the otherwise exclusive domain of α1-adrenergic receptors.2 Thus, in isolated perfused kidneys from untreated rats, α1-adrenergic receptors are the sole adrenergic mediators of neurogenic tubular sodium retention; whereas in kidneys from prazosin-treated rats, α2-adrenergic receptors mediate, at least partially, neurogenic sodium and water retention.1,2

Our previous studies did not examine renal α2-adrenergic receptors at vascular and neuronal sites. Thus, it is possible that the proliferative effects of prazosin on renal α2-adrenergic receptors are not restricted to the tubules. We therefore characterized the junctional locations and functions of renovascular α-adrenergic receptor subtypes in the isolated perfused kidney, and determined whether α2-adrenergic receptor function was altered following 3 days of prazosin treatment. Our results indicate that chronic prazosin treatment caused an increase in the activity of prejunctional inhibitory mechanisms, specifically 1) prejunctional α2-adrenergic receptors and 2) neuronal norepinephrine (NE) uptake.

Methods

Male Sprague-Dawley rats weighing 275 to 330 g (Harlan, Houston, TX, USA) were divided into two groups. The control group (n = 39) was given Purina Rat Chow (Ralston Purina, St. Louis, MO, USA) and tap water ad libitum and received daily injections of dextrose vehicle (5 g/dl, i.p.) for 3 days. Prazosin treatments (2 mg/kg/day) were begun in a separate
group (n = 36) according to the procedure of Smyth et al.1 In some of the rats, systolic blood pressure and heart rate were monitored immediately prior to the prazosin injections and 4 hours later. After 3 days of treatment, the kidneys were isolated and perfused in vitro with a nonrecirculating perfusate, as described previously.1

The effluent was allowed to drip freely from the cut ends of the ureter and renal vein. Perfusate flow was adjusted during the 40 to 50-minute stabilization period to give a perfusion pressure of approximately 100 mm Hg. The oxygenated perfusion medium (35–37°C; pH, 7.35–7.45) was a modified Krebs-Henseleit buffer.1 A β-adrenergic-selective concentration of propranolol (100 nM) was added to the perfusion mixture. A peristaltic pump (Model 1210, Harvard, Millis, MA, USA) was used to maintain a pulsatile flow.

During the stabilization period, platinum electrodes were placed peripherally for renal nerve stimulation (RNS). A maximal response (40 seconds) was obtained for frequencies of 2, 4, 6, and 8 Hz (10 V and 1.0 msec). In some experiments, the venous effluent was collected on ice prior to the stimulus train and during the 6-Hz stimulation. Aliquots (1.5 ml) of these samples were then frozen (−40°C) for later determination of NE content by a radioenzymatic assay (Cat-A-Kit, Upjohn, Kalamazoo, MI, USA).

Drug infusions were begun immediately following the control frequency response. These infusions were 10 minutes in duration, then continued during the frequency response-curve determination. In one series of experiments, the relative contribution of α1-adrenergic receptors to neurogenic renovasoconstriction was examined by infusing prazosin (1–300 nM) following the control frequency response. In some kidneys, yohimbine (300 nM) was included with prazosin (100 nM) to determine their combined effects. Yohimbine (3 nM–1 μM) was infused alone in other experiments to examine the effects of α1-adrenergic receptor blockade on neurogenic renovasoconstriction. Some experiments were performed with cocaine (10−3 M) to determine the contribution of neuronal uptake on the response to RNS.

Vasoconstrictor responses were obtained in some kidneys with the selective α1-adrenergic receptor agonist methoxamine (300–1000 nM) and the selective α1-adrenergic receptor agonist BHT 933 (30–300 μM). The agonists were infused (2 minutes for each concentration in a stepwise manner) into the perfusion line following a 40 to 50-minute stabilization period. Three such cumulative dose responses were obtained in each kidney.

Homogeneity of variances was established with Bartlett’s test. Multiple comparisons were performed with the Newman-Keuls test. Where applicable, single comparisons were made with the Student’s t test.

The following drugs were gifts: prazosin HCl (Pfizer, New York, NY, USA), methoxamine HCl (Burroughs Wellcome, Research Triangle Park, NC, USA) and BHT 933 (Boehringer Ingelheim, Ridgefield, CT, USA). Yohimbine HCl and cocaine HCl were purchased from Sigma (St. Louis, MO, USA), and sodium pentobarbital (Nembutal) was purchased from Abbott (N. Chicago, IL, USA).

Results
The initial prazosin injections resulted in hypotension and tachycardia, whereas vehicle injections were without effect (Figure 1). Subsequent prazosin injections maintained the significant reduction in resting blood pressure. Heart rate values tended to be more labile, and were increased following daily prazosin injections (see Figure 1).

In control rat kidneys, RNS evoked a frequency-dependent increase in renal vascular resistance (Figures 2–4). Prazosin (1–300 nM) attenuated neurogenic renovasoconstriction in a concentration-dependent manner in kidneys of control and prazosin-treated rats (see Figure 2). Addition of the α1-adrenergic receptor antagonist yohimbine (300 nM) to the perfusate along with the prazosin (100 nM) did not result in any greater inhibition than did prazosin alone (see Figure 2), suggesting that postsynaptic α1-adrenergic receptors are the predominant α1-adrenergic mediator of neurogenic renovasoconstriction.

Yohimbine (3 nM–1 μM) potentiated neurogenic renovasoconstriction in kidneys from both control and prazosin-treated rats (see Figure 3). This potentiation was concentration-dependent up to 300 nM; at higher concentrations (1 μM), the response was diminished. Other experiments demonstrated that 1 μM (but not 300 nM) yohimbine shifted the dose-response curve to methoxamine to the right (not shown), indicating...
that the 1-μM concentration blocks \( \alpha_1 \)-adrenergic receptors.

The observation that the response to RNS was attenuated in kidneys from prazosin-treated rats compared to that in kidneys from controls (see Figures 2–4) led to the studies with \( \alpha \)-adrenergic agonist infusions. The \( \alpha_1 \)-adrenergic receptor agonist methoxamine produced virtually identical concentration-response curves in kidneys from prazosin- and vehicle-treated rats (see Figure 4). The response in each case was steep and could be completely inhibited by prazosin (not shown), and thus there appeared to be no alteration in postjunctional \( \alpha_1 \)-adrenergic receptor functioning.

The \( \alpha_2 \)-adrenergic receptor agonist BHT 933 was not as potent as methoxamine and produced a relatively flat dose-response curve in the vehicle group (see Figure 4). At its highest concentration, the response to BHT 933 was significantly greater in the prazosin-pretreated group. Thus, kidneys from prazosin-treated rats were characterized by subsensitivity to RNS, an increased response to vascular \( \alpha_2 \)-adrenergic receptor stimulation, and unaltered sensitivity to the \( \alpha_1 \)-adrenergic receptor agonist methoxamine. This profile

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**Figure 2.** Effect of prazosin on the frequency response to renal nerve stimulation in isolated kidneys from rats given vehicle (A) or prazosin for 3 days (B). Baseline ( prestimulation) renovascular resistance = 5.3 ± 0.2 mm Hg/ml/min (n = 13) for the control group and 6.2 ± 0.6 (n = 6) for the prazosin-pretreated group.

**Figure 3.** Effect of yohimbine on the frequency response to renal nerve stimulation in isolated kidneys of rats given vehicle (A) or prazosin for 3 days (B). Baseline renovascular resistance = 5.4 ± 0.4 mm Hg/ml/min (n = 13) for the control group and 5.7 ± 0.3 mm Hg/ml/min (n = 11) for the prazosin-pretreated group.

**Figure 4.** Comparison of the effects of renal nerve stimulation and \( \alpha \)-adrenergic agonist infusions on renovascular resistance in isolated kidneys from prazosin or vehicle-treated rats. Asterisks indicate significant difference from vehicle value (*p < 0.05, **p < 0.01).
prompted us to examine NE release during RNS in the two groups of kidneys. The results revealed that NE release during RNS and the associated rise in perfusion pressure were significantly lower in kidneys from rats receiving 3 days of prazosin (Figure 5).

Yohimbine (100 nM) and cocaine (10 μM) infusions each increased vasoconstrictor responses and NE release following RNS (6 Hz) in kidneys from both control and prazosin-treated rats. The effects of cocaine and yohimbine were not additive in the control group; however, these responses were additive in the prazosin-treated group. Thus, the responses in the two groups increase to the same level following combined cocaine and yohimbine infusion (see Figure 5). These results suggest that prejunctional inhibition of sympathetically mediated renovasoconstriction by chronic prazosin treatment may be caused by a combined enhancement of prejunctional α₁-adrenergic receptor activity and neuronal uptake of NE.

Discussion

Prejunctional α₁-adrenergic receptors inhibit NE release in many tissues. We have now shown that yohimbine potentiates neurogenic renovasoconstriction and NE efflux in normal rat kidneys, consistent with a functionally significant population of renal prejunctional α₁-adrenergic receptors. A previous study demonstrated only a small inhibition of NE release with an α₁-adrenergic receptor agonist in Wistar-Kyoto rats (WKY). It is possible that α₁-adrenergic receptor agonists may elicit only a small response, since prejunctional α₁-adrenergic receptors are being activated simultaneously by endogenous NE. Using yohimbine, we observed a concentration-dependent enhancement of the vascular responses to RNS, reflecting the removal of the tonic activation of prejunctional α₁-adrenergic receptors by neuronally released NE. Muntz et al. recently reported autoradiographic labeling of some rat renal neurons with [3H]rauwolscine. Our results with yohimbine suggest that these renal α₁-adrenergic receptors are prejunctional and inhibit NE release. Our data are in contrast with those reported for the dog, where functioning prejunctional α₁-adrenergic receptors could not be demonstrated in vivo.

Neurogenic renovasoconstriction was subversive in prazosin-treated rats, apparently because of a prejunctional mechanism. To test for this, we blocked two renal prejunctional inhibitory mechanisms, namely prejunctional α₁-adrenergic receptors and neuronal NE uptake, with yohimbine and cocaine, respectively. The combined actions of these drugs normalized the response to RNS in the prazosin-treated group, suggesting that chronic prazosin treatment caused a persistent facilitation of prejunctional inhibitory mechanisms.

Continuous α₁ blockade also results in a shift in the distribution of renal tubular α₁-adrenergic receptors from extrajunctional to postjunctional sites, where they assume the function (sodium and water retention) of α₁-adrenergic receptors. In the present study, we examined the functional distribution of renovascular α₁-adrenergic receptors in prazosin-treated rats. Our results indicate that α₁-adrenergic receptors mediate neurogenic renovasoconstriction in the rat, confirming and extending previous studies that show that renovascular α₁-adrenergic receptors are the predominant postjunctional α₁-adrenergic receptor subtype in the renovascular bed of the rat. The pharmacological observations of the present and previous studies are also supported by recent autoradiographic evidence in the rat, which shows only a sparse vascular distribution of renal α₁-adrenergic receptors concentrated at the arteriolar level. The present study suggests that these α₁-adrenergic receptors are probably extrajunctional, since a combination of yohimbine and prazosin was no more effective than prazosin alone in blocking RNS. We could find no evidence of migration of vascular α₁-adrenergic receptors from extrajunctional to postjunctional sites in prazosin-treated rats, since prazosin potently blocked the vasoconstrictor effects of RNS in kidneys from prazosin-treated rats, and added yohimbine had no effect. We did, however, uncover evidence of a small but statistically significant increase in the functional response to agonist stimulation of vascular α₁-adrenergic receptors. The physiological relevance of this shift in vivo is presently unknown.

Previous results from our laboratory suggest a resemblance between spontaneously hypertensive rats.

Figure 5. Enhancement of renovasoconstriction and norepinephrine (NE) release by cocaine (COC; 10⁻⁵ M) and yohimbine (YOH; 10⁻⁷ M) in kidneys from vehicle- and prazosin-treated rats. Graphs indicate the effect of renal nerve stimulation (RNS; 6 HZ, 10V, 1 msec) on perfusion pressure (top) and venous effluent NE concentration (bottom). The numbers in the bars indicate the number of kidneys in each group. Perfusion flow rate = 15.6 ± 0.8 ml/min in the control (C) group and 16.5 ± 0.7 in the prazosin-treated group. Letters (a) indicate p < 0.05 compared with corresponding control; asterisks indicate p < 0.05 between groups as indicated.
(SHR) and prazosin-treated rats. Additional similarities between these two models include elevated renal α2-adrenergic receptor density,1,14 enhancement of the neuronal NE uptake system in the kidney of SHR13 and prazosin-treated rats (present data), and enhancement of renal prejunctional α2-adrenergic receptor function (Ekas et al.3 and present data).

Why are renal prejunctional inhibitory mechanisms facilitated in SHR and prazosin-treated rats? It has been shown that SHR have an increased sympathetic drive compared to WKY,16 characterized by a higher rate of renal sympathetic nerve firing,17 hypernoradrenergic innervation of the vasculature,18 and increased NE release per unit of renal nerve stimulation.3 Since receptor sensitivity is often inversely proportional to its level of stimulation,19 one would expect high junctional NE concentrations to lead to prejunctional α2-adrenergic receptor desensitization. However, in the case of prejunctional α2-adrenergic receptors, such a desensitization due to increased sympathetic activity would enhance further NE release. Teleologically, supersensitivity of prejunctional α2-adrenergic receptors as a reaction to chronically increased NE release is more likely because it would inhibit further NE release. The available experimental evidence supports this latter premise in that renal prejunctional α2-adrenergic receptors and cocaine-sensitive NE uptake processes are supersensitive in SHR compared to WKY.3,15

We have demonstrated in the present study that these prejunctional inhibitory mechanisms are also facilitated in prazosin-treated rats. These animals probably also have increased sympathetic nerve activity because of hypotension-induced activation of the baroreceptor reflex. This is evidenced by the development of tachycardia following prazosin injections (see Figure 1). Reports of several clinical studies have also noted that prazosin therapy results in increased sympathetic activity as demonstrated by increased plasma catecholamine concentrations.20,21

Thus, facilitation of prejunctional inhibitory mechanisms appears to be a compensatory response to increased renal sympathetic drive. SHR probably overcome these mechanisms because of hyperinnervation18 and increased storage and release of NE per unit of nerve stimulation,5 whereas prazosin-treated rats do not overcome prejunctional inhibition of NE release, at least within 3 days of treatment. Whether increased sympathetic drive causes the increase in renal tubular α2-adrenergic receptors in SHR and prazosin-treated rats is unknown.

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