Role of intrarenal $\alpha_2$-adrenoceptors in the renal responses to xylazine in rats

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Menegaz, Rubia G., Daniel R. Kapusta, Antonio M. Cabral. Role of intrarenal $\alpha_2$-adrenoceptors in the renal responses to xylazine in rats. Am J Physiol Regulatory Integrative Comp Physiol 278: R1074–R1081, 2000.—This study examined the contribution of intrarenal $\alpha_2$-adrenoceptor mechanisms to the enhanced urine flow rate (V) and urinary sodium excretion ($U_{NaV}$) responses in ketamine-xylazine-anesthetized rats. Ten minutes after left renal artery (LRA) injection, the $\alpha_2$-adrenoceptor antagonist yohimbine (5 $\mu$g) significantly decreased V from $58 \pm 8$ to $35 \pm 7 \mu l \cdot min^{-1} \cdot g$ kidney $^{-1}$ and $U_{NaV}$ from $2.8 \pm 0.4$ to $2.1 \pm 0.4 \mu eq \cdot min^{-1} \cdot g$ kidney $^{-1}$ without altering right kidney function. The renal effects of the LRA injection of yohimbine were completely abolished in chronic bilaterally renal-denervated (RDNX) rats. In RDNX rats, a higher LRA dose of yohimbine (15 $\mu$g) significantly reduced left and right kidney V, with no effects on $U_{NaV}$. In separate bladder-catheterized rats, yohimbine (0.5 mg/kg), 20 min after intravenous injection, significantly decreased V from $63 \pm 9$ to $13 \pm 2 \mu l \cdot min^{-1} \cdot g$ kidney $^{-1}$ and $U_{NaV}$ from $4.5 \pm 0.5$ to $1.1 \pm 0.1 \mu eq \cdot min^{-1} \cdot g$ kidney $^{-1}$. In RDNX rats, this dose of yohimbine reduced V and $U_{NaV}$, but the magnitude was blunted compared with intact rats. In contrast, 0.1 mg/kg iv yohimbine significantly reduced V and $U_{NaV}$ to similar magnitudes in intact and RDNX groups. Together, these findings indicate that intravenous xylazine acts by renal nerve-dependent and -independent mechanisms to enhance renal excretory function in ketamine-anesthetized rats. Because the effects of the LRA dose of yohimbine were abolished in renal-denervated animals, it appears that xylazine has a direct renal action to augment the renal excretion of water and sodium via a presynaptic $\alpha_2$-adrenoceptor pathway that inhibits the release of neurotransmitters from renal sympathetic nerve terminals.

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produced by yohimbine were then compared between left (experimental) and right (control) kidneys. As demonstrated in previous studies, α2-adrenoceptor antagonists can affect the renal excretion of water and/or sodium via a pathway that involves the renal sympathetic nerves (17, 26–28). Therefore, studies were also performed in chronic bilaterally renal-denervated rats to examine the role of an intact renal innervation in mediating the renal responses to intravenous xylazine infusion.

METHODS

Experiments were performed on male Wistar rats weighing 250–300 g obtained from the Federal University of Espírito Santo. The animals were housed in a temperature- and humidity-controlled room (25°C) with a 12-h light cycle beginning at 0700. Food (standard rat chow) and tap water were provided ad libitum. All experiments were conducted in accordance with our institution’s guide for the care and use of experimental animals and the Brazilian Physiological Society’s principles for research involving animals.

Surgical procedures. The anesthesia and surgery procedures used for studying the renal excretory responses produced by the intravenous infusion of xylazine in ketamine-anesthetized rats have been previously described (8, 9). Briefly, on the day of the experiment, rats were initially anesthetized with thiopental sodium (Thiopental, 50 mg/kg ip supplemented intravenously as needed, Cristalia, São Paulo, Brazil). In contrast to previous studies (8, 9), thiopental was substituted for sodium methohexital (Brevital) due to a difficulty in obtaining the latter anesthesia. Animals were then implemented with catherets (PE-50 fused to PE-10) in the left femoral artery for the recording of arterial pressure and heart rate and the left femoral vein for the administration of drugs and isotonic saline. As a standard procedure in our laboratory, the catherets were tunnelled subcutaneously to the back of the neck. After the catherets were implanted, the rats were administered ketamine (40 mg/kg iv) over a 5-min period. An intravenous infusion (55 µl/min) of isotonic saline containing ketamine (1 mg·kg⁻¹·min⁻¹) and xylazine (50 µg·kg⁻¹·min⁻¹) was then started and continued throughout the experiment. Ketamine- and xylazine-anesthetized rats were then allowed at least 120 min for equilibration of urine flow rate and urinary sodium excretion. For certain studies in which yohimbine was injected as an intravenous bolus, a bladder catheret (flanged PE-240) was implanted for collection of urine. For investigations involving the intrarenal artery injection of vehicle or antagonist, the left kidney was exposed by a left flank incision, and the left and right ureters were isolated and cathereterized (PE-10) near the renal pelvis for collection of urine (23). A 30-gauge needle (90° bend) was advanced into the renal artery though the abdominal aorta for intrarenal administration of yohimbine or saline.

Certain studies were performed on rats in which the influence of the renal nerves on renal excretory function was removed. For this purpose, rats underwent chronic bilateral renal denervation 5 to 7 days before the experiment (12). Briefly, under pentobarbital sodium anesthesia, the left kidney was exposed via a flank incision. The adventitia surrounding the renal artery and vein were stripped, and all visible renal nerves were cut under a microscope (D. F. Vasconcellos 18140, São Paulo, Brazil). The vessels were then treated with an alcohol solution containing phenol (10%). After renal denervation was completed, the flank incision was sutured closed and the procedure was repeated on the opposite side to denervate the right kidney. This renal denervation procedure prevents the renal vasconstrictor response to suprarenal lumbar sympathetic nerve stimulation, prevents the antinatriuretic response to environmental stress, and reduces renal tissue norepinephrine concentration to <5% of control for up to 15 days postdenervation (12). Our laboratories have previously verified that this renal denervation procedure completely removes the influence of the renal nerves on kidney function (24, 22).

Experimental protocols. In previous studies, we have shown that in ketamine-anesthetized rats the intravenous infusion of xylazine significantly increases urine flow rate and urinary sodium excretion (8, 9). The enhanced levels of these renal excretory parameters tended to stabilize ~120 min after the start of drug infusion and remained relatively constant for an additional 90 min (longer time control periods not studied) (8, 9). Therefore, in the experimental protocols described below, rats were allowed to stabilize for at least 2 h after starting the intravenous ketamine and xylazine infusion before starting the experiment. Throughout the experiment, mean arterial pressure and heart rate were continuously recorded using a polygraph (Sensormedics Dynograf Recorder R 711).

Effects of intrarenal yohimbine administration on renal excretory function in intact and bilaterally renal-denervated rats. Experiments were performed to examine the contribution of intrarenal α2-adrenoceptor mechanisms to the enhanced renal excretory responses elicited by the intravenous infusion of xylazine in ketamine-anesthetized rats (n = 8). After the stabilization of urine flow rate and urinary sodium excretion, two consecutive 10-min control urine samples were collected from the catheters implanted in the left and right ureters. After these control periods, yohimbine was injected into left renal artery (5 µg total/5 µl isotonic saline) over 3 min. Immediately after the intrarenal drug injection, five consecutive experimental urine samples (10 min each) were collected from the left (experimental) and right (control) kidneys.

Studies were performed to investigate the role of intact renal nerves in mediating the renal responses produced by left renal artery injection of yohimbine (5 µg total) in ketamine- and xylazine-anesthetized rats. For these studies, the above-mentioned protocol was repeated in the rats (n = 7) having undergone chronic bilateral renal denervation 5 to 7 days before investigation. In addition, studies, changes in the left and right kidney functions produced by left renal artery administration of a higher dose of yohimbine (15 µg total/15 µl isotonic saline) were examined in renal-denervated rats (n = 5).

In a separate group of ketamine- and xylazine-anesthetized rats (n = 7), the same experimental protocol was repeated, with the exception that yohimbine (5 µg) was injected as an intravenous bolus. These studies served as an additional control to verify that the renal responses produced by left renal artery administration of yohimbine resulted from an intrarenal action of the drug and to further demonstrate the stability of the cardiovascular and renal excretory parameters under the time course of the study.

Effects of intravenous yohimbine administration on renal excretory function in intact and bilaterally renal-denervated rats. Experiments were performed to determine the effects of an intravenous bolus injection of yohimbine on the enhanced renal responses produced by an intravenous xylazine infusion in ketamine-anesthetized rats with the intact renal innervation. After the equilibration and stabilization of the renal excretory functions, two consecutive control urine samples were collected (10 min each). Yohimbine (0.5 mg/kg, n = 5; 0.1 mg/kg, n = 6), was then administered as an intravenous bolus. After waiting 10 min for the drug distribution, six
RESULTS

Figure 1 shows the systemic cardiovascular and renal excretory effects produced by the administration of yohimbine (5 µg/5 µl) into the left renal artery of ketamine- and xylazine-anesthetized rats with intact kidneys. Consecutive urine samples (10 min each) were collected before (C1, C2) and after the yohimbine injection (over 3 min) from the left (experimental) and right (control) kidneys. The intrarenal yohimbine injection produced an immediate, but transient, decrease in the left kidney urine flow rate (C2, 53 ± 8; 10 min, 35 ± 7 µl·min⁻¹·g kidney wt⁻¹) and urinary sodium excretion (C2, 3.0 ± 0.4; 10 min, 2.1 ± 0.4 µeq·min⁻¹·g kidney wt⁻¹). In contrast, renal excretory function from the right kidney was not altered by the injection of 5 µg of yohimbine into the left renal artery. In preliminary studies, left renal artery injection of isotonic saline vehicle (5 or 15 µl over 3 min) did not alter any cardiovascular or renal excretory (left or right kidney) parameter (data not shown).

In contrast to the findings shown in Fig. 1, renal excretory function from both kidneys remained unchanged after left renal artery injection of yohimbine (5 µg total) in ketamine- and xylazine-anesthetized rats that underwent chronic bilateral renal denervation (Fig. 2). Mean arterial pressure and heart rate were not altered by left renal artery injection of yohimbine in intact (Fig. 1) or bilaterally renal-denervated rats (Fig. 2). Moreover, in additional studies (data not shown), the intravenous bolus administration of the same dose of yohimbine (5 µg total) did not affect systemic cardiovascular or renal excretory function in ketamine- and xylazine-anesthetized rats that underwent chronic bilateral renal denervation (Fig. 2). These latter findings and the results depicted in Fig. 2 demonstrate the stability of the cardiovascular and renal excretory parameters over the time course of the study. Moreover, these findings indicate that the renal responses produced by left renal artery injection of yohimbine (Fig. 1) occur via an intrarenal action that is dependent on an intact renal innervation.

Figure 3 shows the cardiovascular and renal responses produced by left renal artery injection of a higher dose of yohimbine (15 µg total) in ketamine- and xylazine-anesthetized rats with bilaterally renal-denervated kidneys. Compared with respective control levels for each kidney, left renal artery injection of 15 µg yohimbine did not alter urinary sodium secretion in either kidney (Fig. 3). These findings are similar to those shown in Fig. 2, demonstrating that the antinatriuretic response to 5 µg yohimbine (Fig. 1) is abolished in renal-denervated rats (Fig. 2). In contrast to the effects on urinary sodium excretion, left renal artery injection of 15 µg yohimbine decreased urine flow rate from left and right kidneys of renal-denervated rats (Fig. 3). Note that a significant decrease in urine flow rate was not observed when a lower dose (5 µg) of yohimbine was injected into the left kidney of renal-denervated rats (Fig. 2). Mean arterial pressure and heart rate were not altered by this higher intrarenal dose of yohimbine.
Figure 4 illustrates the cardiovascular and renal responses produced by the intravenous bolus injection of yohimbine (0.5 mg/kg) in intact (n = 5) and chronic bilaterally renal-denervated rats (n = 5). For these studies, consecutive urine samples (10 min) were collected from ketamine- and xylazine-anesthetized rats implanted with a urinary bladder catheter. Compared with respective predrug control levels (C1, C2), 0.1 mg/kg iv yohimbine produced a marked decrease in urine flow rate and urinary sodium excretion in rats with intact and denervated kidneys (Fig. 5). Note, however, that the magnitude decrease in each renal excretory parameter was similar between intact and renal-denervated groups (Fig. 5). This finding differs from those shown in Fig. 4 in that the intravenous injection of 0.5 mg/kg heart rate (tachycardia) but not in the blood pressure response between intact and renal-denervated groups.

Figure 5 shows the systemic cardiovascular and renal excretory effects produced by the intravenous bolus administration of 0.1 mg/kg yohimbine in ketamine- and xylazine-anesthetized rats. It should be emphasized that this is a dose five times lower than that used in studies depicted in Fig. 4. Compared with respective predrug control levels (C1, C2), 0.1 mg/kg iv yohimbine produced a marked decrease in urine flow rate and urinary sodium excretion in rats with intact and denervated kidneys (Fig. 5). Note, however, that the magnitude decrease in each renal excretory parameter was similar between intact and renal-denervated groups (Fig. 5).
yohimbine reduced urine flow rate and urinary sodium excretion to a greater degree in intact than in renal-denervated animals. In fact, the renal responses produced by 0.5 mg/kg yohimbine in renal-denervated rats (Fig. 4) were of similar magnitude to those produced by the injection of 0.1 mg/kg in either intact or denervated rats (Fig. 5). Finally, the intravenous bolus administration of 0.1 mg/kg yohimbine did not produce a statistically significant change in the heart rate or mean arterial pressure response between groups.

DISCUSSION

The findings of the present study demonstrate that $\alpha_2$-adrenoceptor mechanisms within the kidneys are activated and contribute to the diuretic and natriuretic responses produced by the intravenous infusion of xylazine in ketamine-anesthetized rats. As shown in previous studies, a component of the enhanced diuretic response also occurs as a result of xylazine's action to inhibit the secretion-release of arginine vasopressin by stimulating $\alpha_2$-adrenoceptors in the CNS, particularly in the hypothalamic PVN (8). Together, these findings indicate that the enhanced and sustained increase in urine flow rate and urinary sodium excretion produced by the intravenous xylazine infusion are mediated via activation of complex central and peripheral $\alpha_2$-adrenoceptor pathways.

In previous studies, we showed that the intravenous bolus injection of yohimbine (0.5 mg/kg) produces a near complete reversal of the enhanced renal excretory responses produced by intravenous xylazine infusion in ketamine-anesthetized rats (9). In contrast, the intravenous bolus administration of prazosin (0.5 mg/kg), a selective $\alpha_1$-adrenoceptor antagonist, was without effect. Although these observations clearly demon-
strate the involvement of \( \alpha_2 \)-adrenoceptor pathways in mediating the enhanced renal responses, they do not provide information as to the site(s) of action of xylazine.

In the present studies, left renal artery injection of 5 \( \mu \)g total of yohimbine significantly reduced urine flow rate and urinary sodium excretion in the experimental (left) but not in the control (right) kidney of ketamine- and xylazine-anesthetized rats. Compared with pre-drug control levels, the renal excretion of water and sodium from the left kidney was reduced by 34 and 30%, respectively. In contrast, the intravenous bolus injection of yohimbine at the same dose (5 \( \mu \)g total) did not alter renal excretory function in either kidney. Together, these results suggest that intrarenal \( \alpha_2 \)-adrenoceptor mechanisms are activated and contribute to the enhanced diuretic and natriuretic responses produced by xylazine infusion.

In related studies, Blandford and Smyth (5) demonstrated that the intrarenal infusion of clonidine produced a dose-related increase in urine flow rate. In their studies, however, an increase in urinary sodium excretion and an increase in osmolar clearance were only observed at the highest infusion rate tested (5). Thus these investigators concluded that different mechanisms might be involved in mediating the dissociated effects of clonidine on water and sodium excretion. The concept that two independent mechanisms may mediate the effects of \( \alpha_2 \)-adrenoceptor agonists on the renal excretion of sodium and water has previously been suggested (36, 37). In regards to mechanisms, Blandford and Smyth (5) proposed that clonidine may have acted within the kidneys to stimulate inhibitory presynaptic \( \alpha_2 \)-adrenoceptors and thereby reduce norepinephrine release from renal sympathetic nerve terminals. The inhibition of norepinephrine release would promote water and sodium excretion by reducing the activity of postjunctional \( \alpha_1 \)-adrenoceptors located on the renal tubules and vasculature (11, 16, 34). In agreement with this concept, the results of the present studies indicate that the intrarenal administration of yohimbine blunted the enhanced renal responses to intravenous xylazine by a pathway that involves the renal nerves. This was demonstrated by the observation that the renal artery injection of 5 \( \mu \)g total of yohimbine failed to alter the enhanced diuretic and natriuretic responses produced by intravenous xylazine in bilaterally renal-denervated rats (Fig. 2). From these findings, it may be proposed that in intact rats an intrarenal component of the diuresis and natriuresis produced by intravenous xylazine infusion is mediated by the stimulation of presynaptic \( \alpha_2 \)-adrenoceptors located on renal nerve terminals. Activation of presynaptic \( \alpha_2 \)-adrenoceptors on renal sympathetic nerve terminals would inhibit the neural release of norepinephrine and promote water and sodium excretion, responses that are reversed by the renal artery administration of yohimbine.

In addition to a pathway involving the renal nerves, xylazine may increase urine flow rate in ketamine-anesthetized rats by activating \( \alpha_2 \)-adrenoceptors in the collecting ducts. Along these lines, other \( \alpha_2 \)-adrenoceptor agonists (e.g., clonidine, rilmelnidine, etc.) have been shown to enhance urine output by inhibiting the renal tubular effects of vasopressin on water transport (6, 7, 14, 18, 35). To test this possibility under our experimental conditions, we examined whether a higher intrarenal artery dose of yohimbine could reverse the enhanced levels of urine flow rate that are observed in renal-denervated ketamine- and xylazine-anesthetized rats. Chronic bilaterally renal-denervated rats were used in these investigations to specifically study the role of renal tubular \( \alpha_2 \)-adrenoceptor pathways in mediating the effects of yohimbine (and thus xylazine) on renal excretory function.

In these studies, left renal artery injection of 15 \( \mu \)g of yohimbine significantly reduced urine flow rate from both the left and right denervated kidneys (Fig. 3). Of interest, this higher intrarenal dose of yohimbine did not alter urinary sodium excretion in either the left or right denervated kidney. On the basis of these observations, it is clear that intact renal nerves are of particular importance in mediating the effects of yohimbine and xylazine on the renal handling of sodium. In contrast, because intrarenal yohimbine markedly reduced the enhanced level of urine flow rate in renal-denervated rats, it appears that xylazine may enhance urine flow rate via activating postjunctional \( \alpha_2 \)-adrenoceptors in the distal tubules. Although these observations suggest this possibility, it should be emphasized that the present findings do not specifically prove this point. This stems from the finding that left renal artery injection of 15 \( \mu \)g of yohimbine reduced urine flow rate in both kidneys, thereby indicating that the responses observed in the right kidney occurred subsequent to a leakage into the peripheral circulation. Thus yohimbine may have caused a decrease in urine flow rate in both kidneys via an extrarenal mechanism.

On the basis of the present findings from the intrarenal artery yohimbine studies, it would be predicted that the renal nerves (and thus the intrarenal neuronal release of norepinephrine) are also involved in mediating a component of the renal responses produced by the systemic intravenous administration of yohimbine. In fact, this was shown to be the case because the reduction in urine flow rate and urinary sodium excretion produced by the intravenous bolus injection of yohimbine (0.5 mg/kg) in renal-denervated rats was less pronounced than that observed in intact rats (Fig. 4). Of further interest was the observation that when yohimbine was administered as an intravenous bolus at a dose five times lower (0.1 mg/kg), the reduction magnitude in both renal excretory parameters was blunted and remarkably similar between the groups of rats with intact and denervated kidneys (Fig. 5). In these studies, the injection of 0.1 mg/kg of yohimbine to intact and renal-denervated rats (Fig. 5) produced a pattern of renal responses that were comparable to those evoked by a high dose of yohimbine (0.5 mg/kg) in bilaterally renal-denervated rats (Fig. 4).
Perspectives

The ketamine-xylazine protocol provides a novel approach to study the α₂-adrenergic control of renal function (8, 9). In addition to a direct renal action (present findings), it is apparent that other nonrenal mechanisms also contribute in mediating the renal responses to this α₂-adreceptor agonist. For instance, the pattern (magnitude and time course) of the renal responses produced by the intravenous bolus injection of 0.5 and 0.1 mg/kg yohimbine may be due to variance in the ability of these doses to effectively antagonize xylazine at central α₂-adrenoceptors. This is important because stimulation of α₂-adrenoceptors in the CNS is known to produce renal sympathoinhibition and thereby reduce renin release, renal tubular reabsorption of sodium and water, and renal vascular resistance (1, 8, 11). In the CNS microinjection studies, we previously demonstrated that during intravenous infusion, xylazine activates central mechanisms to enhance the renal excretion of sodium and water (8). In intact rats, the high dose (0.5 mg/kg iv) of yohimbine may have crossed the blood-brain barrier in a sufficient concentration to gain access to brain regions (e.g., rostroventrolateral medulla; 23, 25, 31) where it effectively antagonized the central sympatholytic effects of xylazine. On restoration of sympathetic outflow to the kidneys, an increase in renal tubular sodium retention would occur, thereby producing a marked reduction in urine flow rate and urinary sodium excretion. In contrast to this action, it is likely that the low dose of yohimbine (0.1 mg/kg iv) did not gain access into the CNS in a sufficient concentration to effectively prevent the sympatholytic action of xylazine. Thus the renal responses to the low dose of yohimbine may be mediated by other extrarenal mechanisms (e.g., adrenal gland, etc.). As a consequence, the renal sympathoinhibitory effect of xylazine would still predominate and prevent the full reversal of the enhanced renal responses produced by the intravenous yohimbine injection. The profound hypotensive (but only modest tachycardia) response produced by the intravenous bolus injection of 0.1 mg/kg of yohimbine in intact rats (Fig. 5) also provides support to indicate a blockade of peripheral (vascular) but not central α₂-adrenoceptors. Finally, the observation that renal excretory function was augmented in chronic bilaterally renal-denervated rats (partially reversed by yohimbine) clearly reveals an α₂-adrenoceptor action of xylazine that is independent of the renal nerves. Further studies using the ketamine-xylazine model may help to elucidate the sites and mechanisms by which these interesting renal nerve-independent responses are mediated.

In summary, the present investigations indicate that intrarenal α₂-adrenoceptor mechanisms are involved in mediating a component of the enhanced renal excretory responses produced by the intravenous infusion of xylazine in ketamine-anesthetized rats. The intrarenal effects of xylazine on the renal handling of sodium and water are mediated in part by a pathway that involves the renal sympathetic nerves. This could occur via the action of xylazine to activate presynaptic inhibitory α₂-adrenoceptors to inhibit the renal nerve terminal release of norepinephrine.

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