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Renal Nerve Stimulation Induces α_2-Adrenoceptor-Mediated Antinatriuresis under Inhibition of Prostaglandin Synthesis in Anesthetized Dogs

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HAYASHI, Y., CHIBA, K., MATSUOKA, T., SUZUKI-KUSABA, M., YOSHIDA, M., HISA, H. and SATOH, S. *Renal Nerve Stimulation Induces α_2-Adrenoceptor-Mediated Antinatriuresis under Inhibition of Prostaglandin Synthesis in Anesthetized Dogs*. Tohoku J. Exp. Med., 1999, **188** (4), 335-346 —— The interaction between prostaglandins and α-adrenoceptors in neural control of tubular sodium reabsorption was examined in anesthetized dogs. Renal nerve stimulation (RNS; 0.5-1.0 Hz, 10 V, 1.0-milliseconds duration) reduced fractional excretion of Na^+ (FENa) with minimal changes in hemodynamics and glomerular filtration. Intrarenal arterial infusion of prazosin (0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$), an α_1-adrenoceptor antagonist, inhibited the RNS-induced reduction in FENa. However, the RNS-induced reduction in FENa was resistant to prazosin under pretreatment with indomethacin (5 mg/kg, i.v.), a cyclooxygenase inhibitor. Intrarenal arterial infusion of yohimbine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$), an α_2-adrenoceptor antagonist, failed to inhibit the RNS-induced reduction in FENa in the absence or presence of indomethacin, but combined infusion of prazosin and yohimbine abolished the RNS-induced reduction in FENa in the presence of indomethacin. These results suggest that both α_1- and α_2-adrenoceptors mediate the RNS-induced antinatriuresis, but the α_2-adrenoceptor-mediated portion is impaired by prostaglandins. ———— sympathetic nervous system; kidney; Na^+ reabsorption; prazosin; yohimbine; indomethacin

Elevation of renal sympathetic nerve activity enhances tubular reabsorption of Na^+ and thereby reduces urinary Na^+ and water excretion (DiBona and Kopp 1997). Although receptor binding studies indicate the existence of both α_1- and α_2-adrenoceptors in the rat kidney (Schmitz et al. 1981; Muntz et al. 1986), a selective α_1- antagonist prazosin, but not a selective α_2-antagonist yohimbine or

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rauwolscine, inhibits the enhancement of tubular Na^+ reabsorption induced by renal nerve stimulation (RNS) in dogs (Osborn et al. 1983), rabbits (Hesse and Johns 1984) and rats (Smyth et al. 1985). Thus the neural control of Na^+ excretion is considered to be mediated exclusively by α_1-adrenoceptors. However, Smyth et al. (1986) reported that the RNS-induced antinatriuresis still remained after chronic administration of prazosin, and the residual antinatriuretic response was completely blocked by addition of yohimbine in rats. Furthermore, α_2-adrenoceptors interact with signal transduction evoked by several kinds of hormones in renal tubular cells, and their density is increased in the kidney of spontaneously hypertensive rats (Pettinger et al. 1987). These findings suggest that α_2-adrenoceptors could also contribute to the neural control of tubular Na^+ reabsorption in some cases.

Vasodilator prostaglandins such as PGI_2 and PGE_2 are well known to increase urinary Na^+ excretion (Fulgraff and Meiforth 1971; Haas et al. 1984). It was also demonstrated that RNS enhanced production of prostaglandins in the kidney (Hisa et al. 1985; Hayashi et al. 1987). We therefore postulate that the α_2-adrenoceptor-mediated component of the antinatriuretic response is canceled by the natriuretic property of prostaglandins. In this regard, the present study was performed to investigate whether the inhibition of prostaglandin production reveals the contribution of α_2-adrenoceptors to the neural control of renal Na^+ handling. Effects of prazosin and yohimbine on RNS-induced antinatriuresis were examined in the absence and presence of indomethacin, a cyclooxygenase inhibitor.

Materials and Methods

Animal preparation

All animal protocols were reviewed and approved by the Animal Subjects Committee of Graduate School of Pharmaceutical Sciences, Tohoku University. Mongrel dogs of either sex, weighing 10–16 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). A cuffed endotracheal tube was inserted and the animal was artificially ventilated with room air after administration of decamethonium bromide (0.25 mg/kg, i.v.) to prevent spontaneous active respiratory movement. The right and left cephalic veins were cannulated for the infusions of inulin solution and supplemental dose of the same anesthetic ($5\ mg \cdot kg^{-1} \cdot h^{-1}$). The right brachial artery was cannulated for measurement of systemic blood pressure with a pressure transducer (MPU-0.5, Nihon Kohden, Tokyo) and for collection of arterial blood samples. The left kidney was exposed by a retroperitoneal flank incision and the animal was suspended by clamping one of its spinous processes to facilitate manipulation. The ureter was cannulated to obtain urine samples. For renal nerve stimulation, all visible nerves running along with the renal artery were isolated and cut after ligation, and the distal cut portion was placed on bipolar platinum electrodes. Thus, the nerve activity

passing to the kidney was evoked only by electrical stimulation. An electromagnetic flow probe (2.5–3.5 mm in diameter; Nihon Kohden) was attached at the renal artery to measure renal blood flow (RBF) with a square-wave flowmeter (MF-27, Nihon Kohden). A curved 25-gauge needle connected to polyethylene tubing was inserted into the renal artery for drug infusion. Following the bolus intravenous administration of a priming dose of inulin (50 mg/kg), inulin solution was infused at 1.6 ml/min to maintain plasma inulin concentration at approximately 20 mg/100 ml. At least 1 hour was allowed for stabilization after the completion of surgery.

Experimental protocol

In each experiment, 20-minutes urine collections were performed; arterial blood samples (3 ml each) were obtained in the middle of each urine collection period for determination of plasma inulin and sodium concentrations. After urine and blood samples for basal values were obtained, the first RNS (0.5–1.0 Hz, 10 V, 1.0 milliseconds) was applied and urine and blood were collected by the same manner. Thirty minutes after the cessation of RNS, urine and blood samples for recovery values were obtained. Then the continuous renal arterial infusion of vehicle (0.9% saline, Group 1, $n=6$), prazosin (0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$, Group 2, $n=5$) or yohimbine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$, Group 3, $n=7$) was started, or indomethacin was injected intravenously (5 mg/kg, Group 4, $n=7$). After a 15-minute equilibration period, the same protocol described above was repeated in the presence of the drug; urine and blood samples were collected before, during and after the second RNS. In other experimental groups, dogs were pretreated with indomethacin (5 mg/kg i.v. injected 20 minutes before the first RNS and a supplemental dose of 2 mg/kg i.v. injected 20 minutes before the second RNS), and the effect of intrarenal arterial infusion of prazosin (Group 5, $n=8$), yohimbine (Group 6, $n=6$) or prazosin plus yohimbine (combined infusion, Group 7, $n=6$) was examined in a similar manner as in Groups 1–3.

The doses of prazosin and yohimbine had been confirmed to cause substantial suppression of α_1- and α_2-adrenoceptor-mediated renal vasoconstriction, respectively, in anesthetized dogs (Chiba et al. 1990).

Measurements

Blood samples were transferred into chilled tubes containing diammonium EDTA (6 mg/ml blood) and then centrifuged to obtain plasma samples. Plasma and urinary Na^+ concentrations were determined by flame photometry (flame photometer, 775-A, Hitachi, Tokyo). Plasma and urinary inulin concentrations were determined colorimetrically by the anthrone method (Davidson and Sackner 1963). Glomerular filtration rate (GFR) was calculated as inulin clearance. Fractional excretion of Na^+ (FENa) was calculated by dividing Na^+ clearance by inulin clearance.

Drugs

Indomethacin (Sigma, St. Louis, MO, USA) was dissolved in 0.1 M Na_2CO_3 and the pH was adjusted to 7.4 with 1 N HCl. Prazosin HCl (Sigma) was dissolved in distilled water by heating and diluted with 0.9% saline. Yohimbine HCl (Wako Pure Chemical Industries, Osaka) was dissolved in distilled water and diluted with 0.9% saline.

Data analysis

Data are expressed as means ± S.E. Overall statistical differences were evaluated by analysis of variance for multifactor repeated measures. Dunnett's test was used to evaluate differences from the basal value in each experimental period. RNS-induced changes in FENa were calculated as described below (FENa ratio), and effects of drugs on them were evaluated by the paired t-test. A p-value less than 0.05 was considered statistically significant.

$$\text{FENa ratio} = \frac{\text{FENa during RNS}}{\text{Average of FENa before RNS (Basal) and FENa after RNS (Recovery)}}$$

Results

The first RNS reduced urine flow rate and urinary Na^+ excretion (data are not shown) and the calculated FENa with minimal changes in mean arterial blood pressure (MAP), heart rate (HR), RBF, and GFR in each experimental group (Tables 1–3). Figs. 1–3 show ratios of FENa: the value during RNS to average of basal and recovery values. The second RNS during infusion of vehicle (Group 1) also reduced FENa (Table 1), the degree of which was not different from that obtained by the first RNS (Fig. 1).

The second RNS during prazosin infusion (Group 2) failed to reduce FENa (Table 1), consequently prazosin increased FENa ratio about to 1.0 (Fig. 1). During yohimbine infusion (Group 3), however, the second RNS reduced FENa (Table 1) by the same degree as the first RNS (Fig. 1). Basal MAP and FENa decreased during prazosin infusion (Tables 1–3).

Indomethacin (Group 4) tended to reduce basal RBF and FENa but did not affect other parameters (Table 2) or the RNS-induced reduction in FENa (Fig. 2). Under the indomethacin pretreatment, the RNS-induced reduction in FENa was attenuated but still observed during prazosin infusion (Group 5, Table 2); FENa ratio during prazosin infusion was still lower than 1.0 (Fig. 2). The RNS-induced reduction in FENa remained unaffected during yohimbine infusion (Group 6, Table 2, Fig. 2). However, the combined infusion of prazosin and yohimbine under the indomethacin pretreatment (Group 7) abolished the RNS-induced reduction in FENa (Table 3, Fig. 3).

TABLE 1. *Effects of vehicle, prazosin and yohimbine*

	Basal	1st RNS	Recovery	Basal	2nd RNS	Recovery
Group 1 ($n=6$)		Control			Vehicle	
MAP (mmHg)	134±6	137±4	135±5	134±7	137±7	133±7
HR (beats/minutes)	150±18	140±16	130±15	124±14	124±14	117±14
RBF (ml/minutes)	154±17	145±20*	140±31	142±33	131±33*	132±33
GFR (ml/minutes)	18.1±4.4	17.9±4.1	23.9±7.5	15.0±3.5	13.2±4.1	17.0±3.0
FENa (%)	1.88±0.70	0.80±0.32*	1.52±0.60	1.45±0.41	0.74±0.32*	1.18±0.58
Group 2 ($n=5$)		Control			Prazosin	
MAP (mmHg)	135±5	136±5	135±5	115±7**	111±7	109±6
HR (beats/minutes)	151±12	149±11	144±10	153±11	150±11*	141±12
RBF (ml/minutes)	119±17	121±17	126±13	135±13	131±13	117±12
GFR (ml/minutes)	24.1±2.6	19.5±2.4	26.8±3.9	26.1±4.0	26.5±4.7	22.5±3.2
FENa (%)	1.67±0.48	1.19±0.30*	1.76±0.38	0.87±0.20**	0.72±0.15	0.57±0.20
Group 3 ($n=7$)		Control			Yohimbine	
MAP (mmHg)	140±9	141±9	140±9	147±8	148±7	143±7
HR (beats/minutes)	152±5	153±6	148±7	161±8	165±7	159±8
RBF (ml/minutes)	183±27	176±26*	183±29	194±33	190±32	204±32
GFR (ml/minutes)	20.0±2.8	17.3±2.0	20.0±2.6	19.8±2.6	17.2±2.9	21.5±3.4
FENa (%)	0.97±0.21	0.52±0.12*	0.92±0.20	1.08±0.21	0.70±0.17*	1.14±0.24

Values are means±S.E. RNS, renal nerve simulation; MAP, mean arterial pressure; HR, heart rate; RBF, renal blood flow; GFR, glomerular filtration rate; FENa, fractional Na^+ excretion. Vehicle, prazosin (0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or yohimbine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused into the renal artery. * $p<0.05$ vs. each "Basal" value. ** $p<0.01$ vs. values in the "Control" period.

TABLE 2. *Effects of indomethacin and effects of prazosin and yohimbine in the presence of indomethacin*

	Basal	1st RNS	Recovery	Basal	2nd RNS	Recovery
Group 4 ($n=7$)		Control			Indomethacin	
MAP (mmHg)	126±7	127±7	129±8	134±7	135±7	132±8
HR (beats/minutes)	152±9	153±10	151±8	151±10	151±10	153±12
RBF (ml/minutes)	145±16	148±16	146±15	130±9	129±9	140±11
GFR (ml/minutes)	22.8±1.9	19.8±1.9*	22.1±1.7	22.2±1.7	19.3±2.0*	21.4±2.0
FENa (%)	1.74±0.37	1.18±0.26*	1.44±0.32	1.19±0.31	0.70±0.18*	1.15±0.27
Group 5 ($n=8$)		Indomethacin			Indomethacin+Prazosin	
MAP (mmHg)	144±7	143±7	138±8	120±9††	115±9	110±8
HR (beats/minutes)	141±9	141±10	141±11	150±12†	148±12	143±13
RBF (ml/minutes)	153±21	142±20**	165±23	163±22	158±22	157±21
GFR (ml/minutes)	25.7±3.2	17.8±2.7**	23.8±2.9	17.5±2.1††	15.1±2.2	16.9±2.2
FENa (%)	1.19±0.27	0.79±0.12*	1.15±0.26	0.88±0.17†	0.64±0.13	0.71±0.20
Group 6 ($n=6$)		Indomethacin			Indomethacin+Yohimbine	
MAP (mmHg)	132±5	133±6	129±7	131±7	134±5	130±4
HR (beats/minutes)	142±9	142±9	143±9	152±9†	151±10	148±11
RBF (ml/minutes)	129±13	126±12	138±12	145±14	136±13	147±15
GFR (ml/minutes)	28.1±5.3	20.2±2.8*	25.5±3.2	22.5±2.0	13.2±2.0*	22.6±1.4
FENa (%)	0.60±0.13	0.35±0.09*	0.60±0.17	0.60±0.20	0.41±0.13*	0.55±0.15

Values are means±S.E. In Groups 5 and 6, prazosin (0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or yohimbine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was infused into the renal artery under indomethacin pretreatment (5 mg/kg+2 mg/kg, i.v.). Abbreviations are as in TABLE 1. *$p<0.05$, **$p<0.01$ vs. each "Basal" value. †$p<0.05$, ††$p<0.01$ vs. values in the "Control" or "Indomethacin" (first experimental) period.

TABLE 3. *Effects of combined treatment of prazosin and yohimbine in the presence of indomethacin*

	Basal	1st RNS	Recovery	Basal	2nd RNS	Recovery
Group 7 ($n=6$)	Indomethacin			Indomethacin + Prazosin + Yohimbine		
MAP (mmHg)	152±7	151±7	148±7	120±9††	117±7	114±8
HR (beats/minutes)	143±8	144±8	147±9	160±16	163±17	157±18
RBF (ml/minutes)	143±10	140±10	157±15	164±22	164±23	169±27
GFR (ml/minutes)	27.2±2.8	19.8±1.7*	26.8±2.4	22.4±2.7	21.1±2.5	22.0±1.8
FENa (%)	1.20±0.29	0.80±0.17*	1.33±0.34	0.68±0.20†	0.64±0.18	0.48±0.15

Values are means±S.E. Prazosin (0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and yohimbine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was simultaneously infused into the renal artery under indomethacin pretreatment (5 mg/kg + supplemental dose of 2 mg/kg, i.v.). Abbreviations are as in TABLE 1. *$p<0.05$ vs. each "Basal" value. †$p<0.05$, ††$p<0.01$ vs. the values in the "Indomethacin" (first experimental) period.

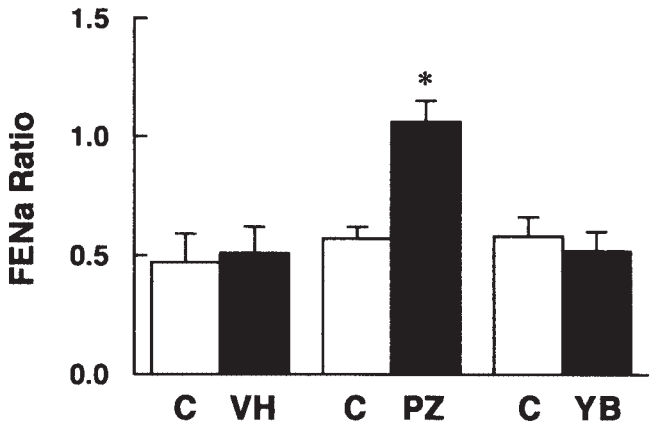

Fig. 1. Effects of vehicle (VH), prazosin (PZ) and yohimbine (YB) on the renal nerve stimulation-induced change in fractional Na^+ excretion (FENa Ratio). Values are means±S.E. C, control. Vehicle (Group 1, $n=6$), prazosin (0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Group 2, $n=5$) or yohimbine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Group 3, $n=7$) was infused into the renal artery. $^*p<0.05$ vs. "C" (before vehicle or drug infusion).

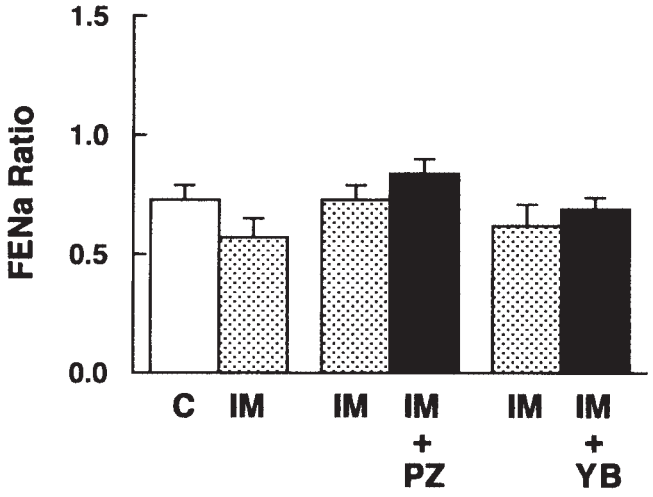

Fig. 2. Effects of indomethacin (IM) and effects of prazosin (PZ) and yohimbine (YB) in the presence of indomethacin on the renal nerve stimulation-induced change in fractional Na^+ excretion (FENa Ratio). Values are means±S.E. Indomethacin (5 mg/kg; Group 4, $n=7$) was administered i.v. Prazosin (0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Group 5, $n=8$) or yohimbine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Group 6, $n=6$) was infused into the renal artery under indomethacin pretreatment (5 mg/kg, i.v. before first RNS+supplemental dose of 2 mg/kg i.v. before second RNS). There were no statistically significant differences between the values in two experimental periods.

Discussion

It is well known that RNS (Kopp et al. 1983; Osborn et al. 1983) or reflex activation of the renal sympathetic nerves (Veelken et al. 1996) reduces urinary Na^+ excretion, which results from norepinephrine release from adrenergic nerve terminals in the kidney (Kopp et al. 1983). Since the RNS-induced antinatriuretic response is associated with a reduction in FENa, the response can be related

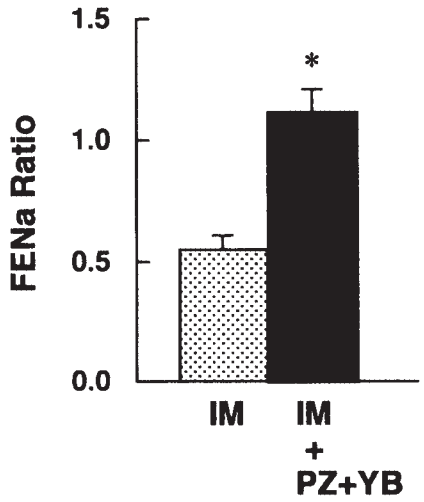

Fig. 3. Effects of combined administration of prazosin (PZ) and yohimbine (YB) on the renal nerve stimulation-induced change in fractional Na^+ excretion (FENa Ratio) in the presence of indomethacin (IM). Values are means ± S.E. Group 7, $n=6$. Prazosin (0.7 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and yohimbine (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) were simultaneously infused into the renal artery under indomethacin pretreatment (5 mg/kg, i.v. before first RNS + supplemental dose of 2 mg/kg, i.v. before second RNS). $^*p<0.05$ vs. "IM" (before infusion of prazosin plus yohimbine).

predominantly to enhanced tubular Na^+ reabsorption when the sympathetic activation causes only a small or no reduction in GFR (Kopp et al. 1983; Osborn et al. 1983). Prazosin almost completely inhibits the RNS-induced antinatriuresis (Osborn et al. 1983; Hesse and Johns 1984; Smyth et al. 1985), suggesting that the neural control of Na+ reabsorption is mediated by α_1-adrenoceptors. This was also confirmed in the present study (Group 2).

In the indomethacin-pretreated group (Group 5), however, prazosin failed to abolish the RNS-induced reduction in FENa. The first RNS (before prazosin infusion) slightly reduced RBF (by about 10%) in the indomethacin-pretreated group (Group 5) but not in the non-pretreated group (Group 2). The vasoconstriction response might enhance tubular reabsorption by reducing peritubular capillary hydrostatic pressure and thereby blunt the inhibitory effect of prazosin. However, this cannot explain the failure of prazosin to abolish the RNS-induced response, because the reduction in RBF was not observed during the second RNS along with prazosin infusion either in the indomethacin-pretreated or non-pretreated group. We had previously demonstrated that i.v. injection of indomethacin at the similar dose used in this study effectively reduced prostaglandin release from the dog kidney (Hisa et al. 1984). Thus the present study indicates that inhibition of prostaglandin production unmasks a prazosin-resistant portion of the neural control of tubular reabsorption.

Combination of prazosin and yohimbine completely inhibited the RNS-induced reduction in FENa in the indomethacin-pretreated group (Group 6). This result suggests that the prazosin-resistant portion of the neurally induced

antinatriuretic response under inhibition of prostaglandin production is mediated by α_2-adrenoceptors; that is, the α_2-adrenoceptor-mediated portion of tubular reabsorption is impaired by prostaglandins. Stimulation of α_2-adrenoceptors is considered to reduce the intracellular cAMP level by inhibiting adenylate cyclase (Limberd 1988). A microdissection study in the rat kidney showed that stimulation of α_2-adrenoceptors suppressed the prostaglandin-induced elevation of cellular cyclic AMP level (Umemura et al. 1986). Stimulation of α_2-adrenoceptors in the rat kidney is also reported to suppress increases in urinary cAMP and Na^+ excretion induced by furosemide (Smyth et al. 1985), the natriuretic action of which is suggested to be mediated by prostaglandins (Miyanoshita et al. 1989). In the dog kidney, recent reports from our laboratory has suggested that renal prostaglandin production is one of the major determinants for the formation of cAMP (Tanahashi et al. 1999a) and the activation of adenylate cyclase induces natriuresis with increasing renal cAMP release (Tanahashi et al. 1999b). It is therefore likely that the adenylate cyclase-cAMP pathway plays a pivotal role in the interaction between α_2-adrenoceptor-mediated mechanism and the renal prostaglandin system at the renal tubular sites.

In this study indomethacin alone did not affect the RNS-induced reduction in FENa (Group 4), whereas modulation by prostaglandins of the neural control of renal functions was demonstrated in rats (Barber et al. 1986). Furthermore, yohimbine alone failed to suppress the RNS-induced response (Group 3). In this regard, the prostaglandin system or the α_2-adrenoceptor-mediated mechanism, although they could compete with each other, may not be able to play an essential role in the neural control of tubular Na^+ reabsorption in the dog kidney. However, a question remains as to why yohimbine did not suppress the RNS-induced reduction in FENa in the presence of indomethacin (Group 6). This discrepancy is difficult to explain. We can only speculate that stimulation of α_1-adrenoceptors predominates over inhibition of the α_2-adrenoceptor-mediated tubular reabsorption.

In summary, the present study demonstrated that prazosin attenuated but failed to abolish the RNS-induced reduction in FENa in the indomethacin-pretreated dogs, and that the combination of prazosin and yohimbine abolished the RNS-induced response whereas yohimbine did not affect the RNS-induced response. These results suggest that both α_1- and α_2-adrenoceptors mediate the neural control of renal tubular reabsorption, but the α_2-adrenoceptor-mediated component is impaired by prostaglandins.

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