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Effect of DA_1 Receptor Blockade with SCH 23390 on the Renal Response to Electrical Stimulation of the Renal Nerves (42282)

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Abstract. To evaluate the existence of functional renal dopaminergic innervation in the dog, we studied the effects of direct electrical stimulation of the renal nerves (RNS) with and without blockade of the dopamine receptor (DA_1) that mediates the vasodilating and natriuretic response to intrarenal infusion of DA. Before infusion of the DA_1 receptor antagonist, SCH 23390, RNS at 1 Hz did not change renal blood flow (RBF) but caused decreased urinary sodium excretion ($-53 \pm 9\%$, $P < 0.01$) and fractional excretion of sodium ($-47 \pm 10\%$, $P < 0.01$). Stimulation at 4 and 12 Hz elicited marked renal vasoconstriction ($\Delta RBF = -37 \pm 12\%$, $P < 0.05$ and $-57 \pm 12\%$, $P < 0.01$, respectively). When RNS (1 Hz) was performed during DA_1 receptor blockade with SCH 23390, 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv, the responses were not different than those before SCH 23390 infusion (urinary sodium excretion: $-54 \pm 7\%$, $P < 0.01$ and fractional excretion of sodium: $-46 \pm 5\%$, $P < 0.01$). Renal vasoconstriction was also not influenced by SCH 23390 ($\Delta RBF = -35 \pm 11\%$, $P < 0.05$ during 4 Hz RNS and $-58 \pm 12\%$, $P < 0.01$ at 12 Hz RNS). Thus, the present study does not support the concept of functional dopaminergic innervation of the canine kidney.

The kidney is a densely innervated organ with synapses at afferent and efferent arterioles, tubular cells, and juxtaglomerular apparatus (1). The predominant neurotransmitter in these nerves is norepinephrine (2, 3). However, Bell and Lang (4) suggested that dopamine (DA) is a neurotransmitter in renal nerves based on their observation that electrical stimulation of the midbrain and hypothalamus in guanethidine pretreated dogs resulted in renal vasodilation attenuated by the DA receptor antagonist, haloperidol.

Anatomical evidence for a dopaminergic innervation of the canine kidney was provided when Bell *et al.* (5) showed that catecholamine fluorescence in the renal cortex was abolished by 6-hydroxydopamine or reserpine but was restored in some axons by systemic administration of L-dopa. This procedure did not restore fluorescence to atrial noradrenergic axons. Furthermore, Dinerstein *et al.* (6) demonstrated nerve fibers at the glomerular vascular poles in the canine kidney that contained DA but not norepinephrine.

DA causes renal vasodilation and natriuresis mediated by specific DA receptors (7). Both these effects of DA can be completely blocked by the selective DA_1 antagonist, SCH 23390 ((*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-^{1}H-3-benzazepine-7-ol) in anesthetized dogs pretreated with phenoxybenzamine and propranolol (8). It is possible that the natriuresis may be at least in part due to action of DA on tubular DA receptors. Such receptors have been suggested both by functional studies (9) and in ligand binding studies (10, 11).

Recently, Bradley and Hjemdahl (12) demonstrated that electrical stimulation of the renal nerves in the dog results in a frequency dependent release to the renal venous blood of DA as well as norepinephrine. The DA/norepinephrine ratio in renal venous plasma was higher than in venous blood draining canine skeletal muscle (13). However, numerous studies have failed to demonstrate any renal vasodilation or natriuresis in connection with electrical stimulation of the nerves to the canine kidney despite blockade of adrenergic effects (14–19).

To evaluate the possibility of functionally important dopaminergic nerves in the canine kidney, we studied the effects of electrical stimulation of the renal nerves on renal hemodynamics and sodium handling before and during selective blockade by SCH 23390 of

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the DA receptor (DA_1) that mediates the renal vasodilator and natriuretic responses to DA (8).

Materials and Methods. Experiments were conducted on mongrel dogs of either sex weighing 18–27 kg. Food was withheld for 20 hr prior to the experiment. Water was allowed *ad libitum.* Anesthesia was induced with sodium pentobarbital (30 $mg \cdot kg^{-1}$ iv) and maintained by periodic additional administration. The animals were mechanically ventilated. A catheter was placed in a jugular vein and an infusion of 0.9% NaCl at 0.35 $ml \cdot min^{-1} \cdot kg^{-1}$ was begun and continued throughout surgery. A catheter was inserted into the abdominal aorta via the right femoral artery for continuous blood pressure recording (Bell and Howell transducer type 4-327-0010) and an indwelling catheter was placed in the urinary bladder. The left kidney was exposed through a flank incision. The renal artery was carefully dissected and fitted with a calibrated flow probe connected to a Biotronex Laboratory BL-613 electromagnetic flowmeter and, more distally, an arterial occluder for zero flow adjustments. The left ureter was cannulated. The nerves supplying the kidney were carefully dissected and divided close to the origin of the renal artery. The distal nerve bundle was placed on a bipolar electrode connected to a Grass Stimulator (Model SD 9) and isolated from surrounding tissues with Plastibase (Squibb). A marked renal vasoconstrictor response to strong renal nerve stimulation (18–32 V, 1 msec, 10 Hz) before and after each experiment verified that the renal nerves remained functional for the duration of the experiment. At the end of surgery the infusion rate of iv 0.9% NaCl was changed to 0.15 $ml \cdot min^{-1} \cdot kg^{-1}$ and in Group 1 (see below) a priming dose of creatinine (50 $mg \cdot kg^{-1}$) was given followed by a continuous infusion of creatinine, 0.75 $mg \cdot min^{-1} \cdot kg^{-1}$. Approximately 1.5 hr was allowed for equilibration and stabilization. The dogs were divided into two groups:

Group 1: In the first group ($n = 7$) the effect of DA_1 receptor blockade with SCH 23390 on the antinatriuretic response to low level renal nerve stimulation (RNS) was investigated. The control period consisted of two consecutive 10-min urine collections from the left ureter with arterial blood samples drawn in the middle of each urine collection period. Following this period, the renal nerves were stimulated at supramaximal voltage, 1 msec and 1 Hz and beginning 5 min after onset of RNS, two 10-min experimental urine collections and blood samples were taken. Then RNS was discontinued and after 20 min two recovery urine collections and blood samples were taken. An intravenous infusion of SCH 23390 (0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) (SCH 23390 maleate was obtained from Schering Corporation, Bloomfield, N.J.) was started and continued throughout the remainder of the experiment to achieve blockade of renal DA_1 receptors. The DA_1 receptor blockade was verified in each experiment by abolition of the renal vasodilator response to 4 $\mu g \cdot kg^{-1}$ iv DA (DA-HCl was obtained from Calbiochem-Behring, La Jolla, Calif.). All doses were calculated as salts. In a previous study we have shown that this dose of SCH 23390 also blocks the natriuresis produced by DA (8). Ten minutes after the SCH 23390 infusion was begun, the protocol of urine and blood collections before, during, and after RNS was repeated.

Group 2: In the second group of five dogs the renal vascular response to RNS of 4 and 12 Hz before and during DA_1 receptor blockade with SCH 23390, 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv was studied. The nerves were stimulated with an impulse duration of 1 msec and supramaximal voltage and for each RNS period stimulation was carried out until steady state in renal blood flow (RBF) was achieved. DA_1 receptor blockade was verified by abolition of the renal vasodilator response to 4 $\mu g \cdot kg^{-1}$ DA iv. RNS at these frequencies (4 and 12 Hz) decreased urine flow rate to extremely low levels which did not allow for the measurement of glomerular filtration rate (GFR) or sodium excretion.

Analysis. In Group 1, each blood sample was analyzed for hematocrit, Na^+, and creatinine. The volume of urine samples was recorded and the urine analyzed for Na^+ and creatinine. Na^+ was measured on a flame photometer (No. 343, Instrumentation Laboratory, Inc.) and creatinine was measured on a Beckman creatinine analyzer (No. 2). Creatinine clearance was used to estimate the GFR (20). In one experiment where urine flow rate was less than 0.5 $ml \cdot min^{-1}$ during RNS, creatinine clearance was not determined, as in

TABLE I. HEART RATE (HR), MEAN ARTERIAL PRESSURE (MAP), RENAL BLOOD FLOW (RBF), AND GLOMERULAR FILTRATION RATE (GFR) BEFORE AND DURING DA_1 RECEPTOR BLOCKADE WITH SCH 23390, 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv AND EFFECTS OF RENAL NERVE STIMULATION (RNS) AT 1 Hz

	Vehicle			SCH 23390		
	Control	RNS	Recovery	Control	RNS	Recovery
HR, min^{-1}	121 ± 11	120 ± 11	120 ± 11	117 ± 11	113 ± 11	112 ± 11
MAP, mm Hg	130 ± 4	135 ± 4	131 ± 4	126 ± 4	125 ± 4	123 ± 5
RBF, $ml \cdot min^{-1}$	134 ± 31	117 ± 25	111 ± 20	122 ± 34	122 ± 34	125 ± 33
GFR, $ml \cdot min^{-1}$	39 ± 5	37 ± 5	38 ± 5	36 ± 4	33 ± 4	39 ± 6

Note. Data are means ± SE in seven experiments (n = 6 for GFR).

that case it is not accurate as an estimate of GFR (20). The fractional excretion of Na^+ (FE_{Na^+}) was calculated by dividing the clearance of Na^+ by the clearance of creatinine. The effects of RNS were determined as the difference between the mean of the experimental periods and the baseline. The baseline was defined as the mean of the two control periods and the two recovery periods.

Statistical analysis. In Group 1 Student's t test for paired samples (21) for used to compare the mean of the experimental periods and the baseline and to compare the effects of RNS before and during DA_1 blockade with SCH 23390. Student's t test for paired samples (21) was used in Group 2 to compare the effects of RNS on RBF in the presence and absence of SCH 23390. The data are expressed as means + SE and $P < 0.05$ was considered significant.

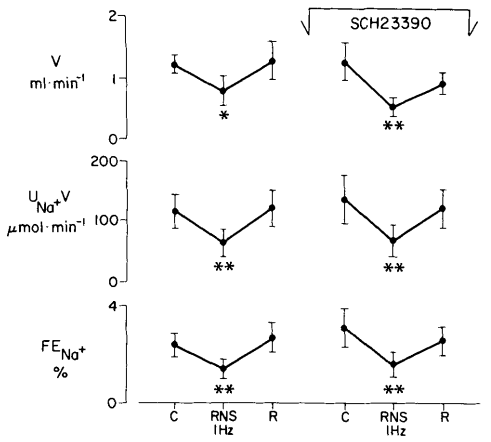

FIG. 1. Changes in urine flow rate (V), urinary sodium excretion ($U_{Na^+}V$), and fractional excretion of sodium (FE_{Na^+}) in response to 1 Hz renal nerve stimulation (RNS) before and during infusion of SCH 23390, 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv. C = control. R = recovery. Data are means ± SE in seven experiments (n = 6 for FE_{Na^+}). *$P < 0.05$; **$P < 0.01$.

Results. *Group 1:* RNS at 1 Hz did not alter heart rate, mean arterial pressure, RBF, or GFR either before or during iv SCH 23390 infusion at 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (Table I). However, as shown in Fig. 1, RNS caused decreases in sodium excretion (−53 ± 9%, $P < 0.01$), urine flow (−43 ± 12%, $P < 0.05$), and FE_{Na^+} (−47 ± 10%, $P < 0.01$). The responses to RNS during SCH 23390 infusion for sodium excretion (−54 ± 7%, $P < 0.01$), urine flow (−52 ± 8%, $P < 0.01$), and FE_{Na^+} (−46 ± 5%, $P < 0.01$) were not significantly different from the control responses (95% confidence limits for the differences in responses was −12 to +15% for sodium excretion, −8 to +26% for the urine flow, and −29 to +17% for FE_{Na^+}).

Group 2: RNS at 4 Hz decreased RBF by 37 ± 12% ($P < 0.05$) before and by 35 ± 11% ($P < 0.05$) during SCH 23390 infusion (Fig. 2). The responses were not statistically different and the 95% confidence interval for the difference in the responses was −22 to +19%. Mean arterial pressure did not change in response to 4 Hz RNS before SCH 23390 infusion, and there was a small increase (from 112 + 6 to 113 + 6 mm Hg, $P < 0.05$) during SCH 23390 infusion. At 12 Hz RBF decreased by 57 ± 12% ($P < 0.01$) and 58 ± 12% ($P < 0.01$), respectively (Fig. 2); whereas, mean arterial pressure was not altered by RNS. Also at this frequency the responses in RBF did not differ significantly (95% confidence interval for the differences in response was −21 to +23%).

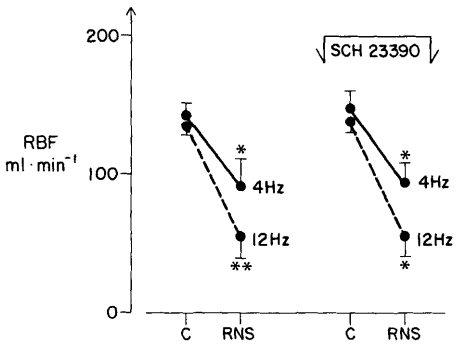

FIG. 2. Effects of renal nerve stimulation (RNS) at 4 Hz (—) and 12 Hz (---) on renal blood flow (RBF) before and during infusion of SCH 23390, 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ iv. C = control. Data are means ± SE in five experiments. $^*P < 0.05$; $^{**}P < 0.01$.

Discussion. In the present study low frequency RNS before DA_1 receptor blockade decreased urine flow, sodium excretion, and FE_{Na^+} without altering mean arterial pressure, RBF, or GFR, as reported by others (22, 23). RNS at 1 Hz increases renal venous outflow of DA as well as norepinephrine (12) and the antinatriuresis can be blocked by the α_1-adrenoceptor antagonist, prazosin (18). Thus, it appears that the antinatriuresis induced by low level RNS is mediated by action of norepinephrine at postsynaptic α_1-adrenoceptors located on the renal tubules (18). In the present study RNS was performed before and during infusion of SCH 23390 at a dose that completely blocks the vasodilating and natriuretic effect of intrarenally infused DA (8). Thus, a functional role of neuronally released DA, mediated via DA receptors, would be expected to result in enhanced vasoconstriction and antinatriuresis in response to RNS during DA_1 receptor blockade with SCH 23390. However, we found that neither the antinatriuretic response to low frequency RNS nor the vasoconstriction at higher stimulation frequencies was altered by SCH 23390. Thus, the present study does not support the concept of dopaminergic innervation of the canine kidney that affects whole kidney hemodynamics or sodium handling. It is still possible, however, that a local effect of DA may have occurred which was too small to influence total RBF and natriuresis.

In the present study RNS was performed when postsynaptic DA_1 receptors were blocked. Another possibility of DA function in renal nerves is on presynaptic DA (DA_2) receptors inhibiting norepinephrine release from renal nerves (24–26). However, norepinephrine release during RNS was not altered by administration of the DA_1/DA_2 antagonist, racemic sulpiride, in the rat (25). Also, the vasoconstrictor response to RNS was not affected by the selective DA_2 antagonist, pimozide, in the rat (24) or by either the selective DA_2 antagonist, (*S*)-sulpiride, or the relatively nonselective DA receptor antagonist, (*R*)-sulpiride, in the dog (26). Thus, there is no evidence for functional activity of renal DA_2 receptors.

In contrast, three groups have reported renal vasodilation that could be attributed to dopaminergic nerves in the dog and rat. First, Bell and Lang (4) studied anesthetized guanethidine pretreated dogs. Electrical stimulation of the ventral hypothalamus caused short-lived increases in RBF that were unaffected by atropine or mepyramine, but were attenuated by the DA receptor antagonist, haloperidol. The authors did not determine whether denervation of the kidney abolished the response nor did they prove that the antagonism of the renal vasodilation did not result from a nonspecific action of haloperidol. Chapman *et al.* (27) studied renal cortical blood flow in rats by a hydrogen washout technique and observed slight vasodilation during electrical stimulation of renal nerves after administration of prazosin. The vasodilation was reversed by sulpiride but was unaffected by propranolol or atropine. However, as noted by Holdaas and DiBona (19), the hydrogen washout technique for measurement of RBF in the rat was not validated and they used a kidney preparation which did not exhibit normal RBF autoregulatory behavior. Further, biochemical and anatomical studies do not support renal dopaminergic innervation in the rat (28, 29). Recently, Hom and Jandhyala (30) reported that cerebroventricular infusion of ouabain increases RBF and reduces renal vascular resistance in chloralose anesthetized, vagotomized dogs. The renal vasodilator effect was blocked by intravenous administration of racemic sulpiride and was also abolished by acute renal denervation, suggesting activation of renal dopaminergic fibers. However, intravenous infusions of the α-adrenoceptor antagonist, phentolamine, as

well as cerebroventricular infusion of sulpiride (that did not block peripheral DA receptors) abolished the renal vasodilating response. The renal vascular resistance after acute renal denervation or iv phentolamine but before ouabain infusion was lower than in the control experiments and approximately as low as after maximal vasodilation with ouabain in the control experiments. Thus, it is conceivable that the effect of ouabain was a decrease of renal sympathetic tone mediated via central DA receptors.

In a number of studies renal vasodilating fibers were sought after blockade of adrenergic nerves during RNS. DiSalvo and Fell (14) noted that after phentolamine and reserpine RNS induced renal vasoconstriction was blocked but no vasodilation was observed. Takeuchi *et al.* (15) used reserpine and guanethidine, but the renal vasoconstriction was not replaced by a vasodilatory response. Gomer and Zimmerman (16) used phenoxybenzamine, bretylium, guanethidine, and reserpine, and Zambraski *et al.* (17) used guanethidine, and neither group observed renal vasodilation during RNS. Finally, Holdaas and DiBona failed to demonstrate a renal vasodilator response after prazosin pretreatment (19).

The antinatriuretic effect of low level RNS can be completely blocked by guanethidine (31), phenoxybenzamine (23), phentolamine (18), or prazosin (18). In none of these studies was the antinatriuresis in response to RNS replaced by natriuresis after adrenergic blockade. These studies again do not provide evidence for renal dopaminergic nerves.

Thus, while there is experimental support for the existence of renal dopaminergic nerve fibers, the failure of RNS in the dog to cause renal vasodilation or natriuresis after blockade of renal adrenergic effects combined with the failure of blockade of either renal presynaptic DA (DA_2) receptors or postsynaptic DA (DA_1) receptors to alter the response to RNS does not support a functional role for such fibers.

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