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Lack of Evidence for Neurogenic Renal Vasodilatation in Anesthetized Dogs[1] (40226)

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Inasmuch as sympathetic cholinergic vasodilator pathways exist in other vascular beds (1, 2), efforts have been made to identify similar pathways in the kidney. Stinson *et al.* (3) suggested that the decrease in renal vascular resistance observed during reductions in renal perfusion pressure below the autoregulatory range in the dog is mediated by cholinergic nerves. However, the results were variable in that only three of six anesthetized dogs with acute renal artery constriction had an atropine response.

Takeuchi *et al.* (4) demonstrated that the renal vasoconstrictor response to greater splanchnic nerve stimulation was abolished by guanethidine and was never replaced by a renal vasodilator response whereas the hindlimb vasculature did reveal a vasodilator response to lumbar sympathetic trunk stimulation after guanethidine. These observations are not in accord with those of Stinson *et al.* (3).

In light of these conflicting reports, we undertook a series of studies to test the hypothesis that sympathetic cholinergic nerves may mediate renal vasodilatation.

Materials and methods. All studies were performed on mongrel dogs 15–25 kg in weight, fed a standard kennel ration. On the day prior to the study all dogs were deprived of food but water was permitted *ad libitum.* On the day of the study the animal was anesthetized with intravenous sodium pentobarbital 30 mg/kg and supplemental doses were added throughout the experiment to maintain anesthesia. The animal was intubated with an endotracheal tube and mechanically ventilated to maintain arterial pH between 7.35 and 7.45. Catheters were inserted into a brachial and a femoral artery for pressure measurements. The left kidney was exposed via a subcostal incision and the renal artery dissected free, taking care to leave the renal nerves intact. An external electromagnetic flow probe was placed on the left renal artery and led to an electromagnetic flow meter (Carolina). This system was calibrated *in vivo* at the end of each experiment. Additional surgical preparation was performed as indicated in the individual protocols. Following surgery, a minimum of 60 min was allowed for equilibration and stabilization.

Protocol A (carotid sinus perfusion). Both carotid sinuses were isolated from the arterial circulation; innervation was preserved. All branches except the external carotid arteries were ligated while each of the common carotid arteries was perfused at constant flow by leading femoral arterial blood through an occlusive roller pump to the individually catheterized common carotid arteries. Outflow from the individual carotid sinuses was via the external carotid arteries through interposed individual Starling resistors. Individual carotid sinus pressures (CSP) were measured by side arm catheters and pressure transducers between the carotid sinus and the Starling resistor. An adjustable clamp was placed around the abdominal aorta above the renal arteries. Brachial artery pressure was taken as mean arterial pressure (MAP) and femoral artery pressure was taken as renal perfusion pressure (RPP). During the Control Period (C), CSP was set 0–10 mmHg below MAP while RPP was lowered approximately 20 mmHg beneath MAP. During the Experimental Period (E), CSP was increased approximately 40 mmHg; RPP was held constant. During the Recovery Period (R), CSP was returned to the Control Period level while RPP was held constant. The Control, Experimental and Recovery Periods were each 15 min long.

Protocol B (cholinergic blockade). A 25 gauge curved needle attached to polyethylene

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tubing was placed in the left renal artery against the direction of flow. RPP and renal blood flow (RBF) were measured during the Control Period (C). Then maximal renal vasodilatation was elicited by lowering RPP below the autoregulatory level for that dog via constriction of an adjustable aortic clamp above the left renal artery. Measurement of RPP and RBF were repeated during the Aortic Constriction Period (AC). Then, with maximal renal vasodilatation present, 2 mg atropine were administered into the renal artery and measurements of RPP and RBF were repeated during the Aortic Constriction and Atropine Period (AC + Atropine). This dose of atropine blocks the renal vasodilator action of acetylcholine infused into the renal artery at 40 μg/min. The Control, Aortic Constriction and Aortic Constriction and Atropine Periods were each 15 min long.

Protocol C (direct renal nerve stimulation). A left renal arterial line was placed as described in Protocol B. During the Control Period (C) RPP and RBF were measured before and during graded direct electrical renal nerve stimulation. The technique for direct electrical renal nerve stimulation is described in detail in our previous publication (5). The stimulus parameters were 10–15 V, 1 ms and 1–3 Hz (low), 4–8 Hz (medium) and 9–15 Hz (high). The duration of renal nerve stimulation at each frequency range was 15 min and the recordings of RPP and RBF during the last 10 min of stimulation were used for data analysis. Then guanethidine was infused into the left renal artery at 0.5 mg/min. Twenty minutes later, RPP and RBF were measured before and during graded direct electrical renal nerve stimulation using the identical stimulus parameters.

Arterial pressures were measured with pressure transducers and recorded with electromagnetic flow meter outputs on a direct writing recorder. Renal vascular resistance (RVR) = RPP/RBF in mmHg/ml/min. The data in the text and figures are expressed as means ± SE. The Student *t* test was used for statistical analysis of paired data within each group (6).

Results. Protocol A. The data for studies in 22 dogs are illustrated in Fig. 1. Increasing CSP from 126 ± 4 to 165 ± 4 mmHg ($P < 0.001$) resulted in a decrease in MAP from

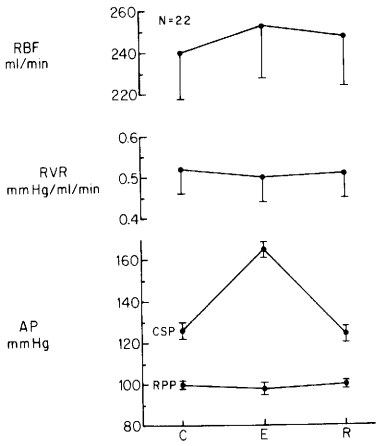

FIG. 1. The effect of increasing carotid sinus pressure (CSP) on renal perfusion pressure (RPP), renal blood flow (RBF) and renal vascular resistance (RVR). C = control, E = experimental, R = recovery, AP = arterial pressure; data are mean ± SE; N = 22 dogs.

135 ± 5 to 116 ± 4 mmHg ($P < 0.01$) while RPP was unchanged, 100 ± 2 vs. 98 ± 3 mmHg. RBF rose slightly from 240 ± 22 to 253 ± 25 ml/min; while this mean change (i.e. mean of the paired differences) was statistically significant ($P < 0.01$), its magnitude (13 ml/min or 5%) was small. RVR fell slightly from 0.52 ± 0.06 to 0.50 ± 0.06 mmHg/ml/min; again, though small (0.02 mmHg/ml/min or 4%), this mean change was statistically significant ($P < 0.01$). Restoring CSP to Control Period levels, 124 ± 4 mmHg, resulted in a return of MAP to Control Period levels, 137 ± 5 mmHg; RPP was held constant at 100 ± 2 mmHg. RBF and RVR returned toward Control Period levels, 248 ± 24 ml/min and 0.51 ± 0.06 mmHg/ml/min, respectively. In five additional dogs, these small increases in RBF and decreases in RVR were unaffected by renal arterial administration of 2 mg atropine.

Protocol B. The data for studies in 12 dogs are illustrated in Fig. 2. In response to a reduction in RPP from 138 ± 4 to 63 ± 5 mmHg ($P < 0.001$), RBF decreased from 222 ± 14 to 160 ± 16 ml/min ($P < 0.001$) and RVR decreased from 0.64 ± 0.03 to 0.42 ± 0.03 mmHg/ml/min ($P < 0.001$). Following renal cholinergic blockade with atropine, RPP was unchanged at 66 ± 5 mmHg. RBF fell slightly to 150 ± 15 ml/min ($P > 0.1$) and RVR rose slightly to 0.47 ± 0.02 mmHg/ml/

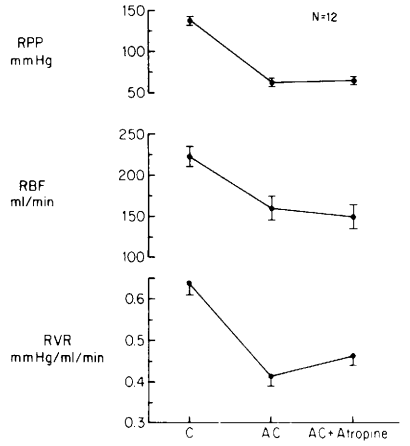

FIG. 2. The effect of renal arterial atropine (2 mg) on the maximum renal vasodilator response to decreased renal perfusion pressure. C = control, AC = aortic constriction, AC + Atropine = aortic constriction plus renal arterial atropine; data are mean ± SE; N = 12 dogs.

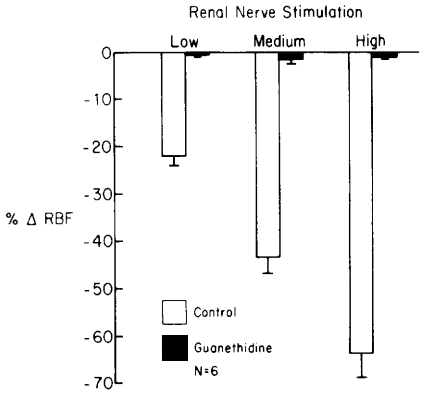

FIG. 3. Effect of graded levels of direct electrical renal nerve stimulation on renal blood flow (RBF) before and during renal adrenergic blockade with guanethidine. Renal nerve stimulation characteristics were 10–15 V, 1 ms and 1–3 Hz (low), 4–8 Hz (medium) and 9–15 Hz (high). %Δ = percent change; data are mean ± SE; N = 6 dogs.

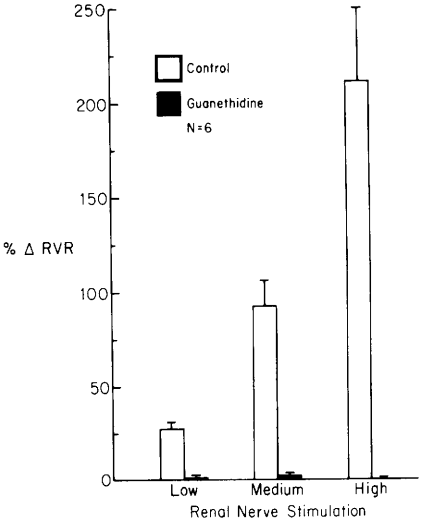

FIG. 4. Effect of graded levels of direct electrical renal nerve stimulation on renal vascular resistance (RVR) before and during renal adrenergic blockade with guanethidine; %Δ = percent change; data are mean ± SE; N = 6 dogs.

min ($P \approx 0.05$). MAP was relatively constant throughout each experiment.

Protocol C. The data for studies in 6 dogs are illustrated in Figs. 3 and 4. Values for RPP, RBF and RVR before renal nerve stimulation were similar for Control and Guanethidine Periods; 144 ± 6 vs. 146 ± 5 mmHg, 242 ± 14 vs. 252 ± 18 ml/min and 0.60 ± 0.04 vs. 0.60 ± 0.06 mmHg/ml, respectively. During the Control Period, graded levels of direct electrical renal nerve stimulation produced dose related decreases in RBF (Fig. 3) and increases in RVR (Fig. 4). After renal adrenergic blockade with guanethidine, the same levels of renal nerve stimulation no longer produced significant changes in RBF or RVR; in no instance was an increase in RBF observed.

Discussion. We were unable to detect a significant sympathetic cholinergic contribution to renal vasodilatation.

From the studies of Kezdi and Geller (7), the increase in CSP of 39 mmHg in this study would be expected to produce a 50% decrease in renal sympathetic nerve impulse frequency. Stimulation of left atrial cardiopulmonary baroreceptors by left atrial balloon distention produced a 27% reflex decrease in renal sympathetic nerve impulse frequency (8) in association with significant increases in RBF and decreases in RVR (9–13). Stimulation of the stellate ganglion results in a 30% reflex decrease in renal sympathetic nerve impulse frequency (14, 15) in association with significant increases in MAP, glomerular filtration rate and urine volume and sodium excretion; however, data on RBF are not available. Increases in hepatic portal venous pressure, which are known to decrease renal sympathetic nerve impulse frequency (16), produce large increases in RBF and decreases in RVR (17). Atropine failed to affect the small increases in RBF and decreases in RVR

observed after increasing CSP in this study, indicating that a role for renal cholinergic pathways could not be detected in this response. These changes are consistent with the anticipated decrease in efferent renal sympathetic vasoconstrictor nerve activity.

Stinson *et al.* (3) found that renal artery constriction which lowered RPP to the lower limit of the autoregulatory range caused a renal vasodilatation manifest by a decrease in RVR; subsequent renal arterial administration of atropine resulted in a variable (ca. 25%) fall in RBF and rise (ca. 40%) in RVR. This response was observed in three of six anesthetized dogs with acute renal artery constriction and three unanesthetized dogs with acute renal artery constriction. Another possible explanation for the variability of the response, in addition to anesthesia, may reside in the inert gas washout method used to measure RBF; the technical limitations of this method have been discussed recently by Stein *et al.* (18). In the current study, using anesthetized dogs with acute reductions in RPP and electromagnetic flowmeter methodology, renal cholinergic blockade did not significantly diminish the maximal renal vasodilator response to reduced RPP. Thus, we could not detect a role for renal cholinergic pathways in maximum renal vasodilatation.

Although previous studies have identified cholinergic innervation of the renal vasculature (19, 20), its functional significance is not readily identifiable. Takeuchi *et al.* (4) utilized an autoperfused dog kidney preparation to demonstrate that the renal vasoconstrictor response to greater splanchnic nerve stimulation was abolished by guanethidine and reserpine and never replaced by a renal vasodilator response; the hindlimb vasculature did reveal a vasodilator response to lumbar sympathetic trunk stimulation after guanethidine. The Protocol C studies utilized an intact normally perfused kidney and direct electrical renal nerve stimulation. The results were confirmatory of those of Takeuchi *et al.*; no renal vasodilator response to renal nerve stimulation during guanethidine was observed. These observations do not support the existence of functional sympathetic cholinergic renal vascular innervation.

Summary. Carotid baroreceptor reflex induced decreases in renal sympathetic nerve activity result in only minimal renal vasodilatation which is unaffected by renal cholinergic blockade with atropine.

Renal cholinergic blockade with atropine does not diminish the renal vasodilation responses to decreases in renal perfusion pressure. Renal adrenergic blockade did not unmask a renal vasodilator response to direct electrical renal nerve stimulation. These findings indicate that the neurogenic contribution to renal vasodilatation is small in comparison to the neurogenic contribution to renal vasoconstriction. Evidence for participation of renal cholinergic neural pathways in renal vasodilatation was not found.

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