

Effect of catecholamines and the renal nerves on renin secretion in anesthetized dogs¹

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VANDER, ARTHUR J. *Effect of catecholamines and the renal nerves on renin secretion in anesthetized dogs.* Am. J. Physiol. 209(3): 659-662. 1965.—Intravenous infusion of either epinephrine (5-6 $\mu\text{g}/\text{min}$) or norepinephrine (12-16 $\mu\text{g}/\text{min}$) during maintenance of a constant renal arterial blood pressure by means of suprarenal aortic constriction, or stimulation of the renal nerves produced essentially the same effects on renal function and renal venous plasma renin concentration, the latter being measured indirectly by bioassaying the pressor activity produced by plasma incubation under standardized conditions. Glomerular filtration rate (GFR), renal plasma flow (RPF), and sodium excretion were decreased, and renin concentration was increased. The induction of osmotic diuresis during catecholamine infusion or renal nerve stimulation reversed or prevented the increase in renin secretion but did not alter the changes in GFR or RPF. It is suggested that the increased renin secretion induced by catecholamines and renal nerve stimulation in nondiuretic dogs might be the indirect result of the decrease in filtered sodium produced by these procedures. However, a direct effect of the catecholamines and renal nerves on the renin-secreting cells cannot be ruled out.

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IN A PREVIOUS PAPER, Vander and Miller (7) demonstrated that renin secretion was stimulated by acute reduction of renal arterial pressure or acute elevation of ureteral pressure, and that this enhancement of renin secretion could be prevented by the administration of diuretics. It was hypothesized that the composition of macula densa fluid constitutes the primary input controlling renin secretion. The present experiments were designed to test this hypothesis further by studying the role of the sympathetic nervous system in the control of renin secretion.

METHODS

Animal procedures. Experiments were performed on mongrel dogs anesthetized with 30 mg/kg pentobarbital

administered intravenously with supplements given im and iv as required. Via a right flank incision the right ureter was catheterized with a polyethylene tube which was secured in place with ligatures. A clamp was placed loosely around the aorta proximal to the right renal artery. In five dogs, loop electrodes were placed around the right renal artery in order to stimulate the renal arterial nerve plexus (3). To obtain renal venous blood, a polyethylene catheter (2.4 mm o.d.) was introduced into the left femoral vein, passed up the inferior vena cava, and manipulated into the right renal vein. Arterial blood was obtained from a femoral artery catheter. Blood pressures were monitored from carotid and femoral artery catheters using mercury manometers. Renal excretory and hemodynamic data were obtained for the right kidney only, using standard clearance techniques. Clearance periods were 5-20 min long, with arterial and renal venous blood samples taken in the middle of each period. A prime of creatinine and *p*-aminohippurate was given intravenously, following which these substances were infused in isotonic saline at a constant rate of 0.2 ml/min. Creatinine clearance was used as a measure of glomerular filtration rate (GFR), and total renal plasma flow (RPF) was determined by the Fick principle, using PAH. Experimental observations were not begun until at least 30 min after completion of all operative procedures and the administration of the creatinine and PAH prime. In all dogs control clearances were then performed, after which one of the protocols described below was followed.

Protocol 1. Intravenous infusion of catecholamine and aortic clamping. In seven dogs, following completion of the control clearance periods, either epinephrine (5-6 $\mu\text{g}/\text{min}$) or norepinephrine (12-16 $\mu\text{g}/\text{min}$) was infused continuously intravenously. Simultaneously, as arterial blood pressure increased, the aortic clamp above the right renal artery was tightened an amount sufficient to maintain the mean renal arterial blood pressure at the previous control value (± 3 mm Hg). By this means, the effects of epinephrine and norepinephrine on renin secretion and renal function were studied with mean renal arterial blood pressure as a constant. Further clearances were performed 20-90 min after beginning the catecholamine infusion. After completion of this second group of clearances, one of two procedures was followed: 1) the cate-

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cholamine infusion was stopped, the aortic clamp released, and additional clearances performed 25–40 min later; 2) the catecholamine infusion and aortic clamping were continued, osmotic diuresis was induced, and additional clearances performed 25–40 min later, but never more than 90 min after the catecholamine infusion had been started. The osmotic diuretic used was either mannitol, 300 mM/liter at 5–10 ml/min, or sodium sulfate, 100–150 mM/liter at 10 ml/min.

Protocol 2. Renal nerve stimulation. After completion of the control clearances, the right renal nerves were stimulated via loop electrodes around the renal artery. Square-wave impulses were delivered using a Grass SM 6 stimulator. The parameters were: voltage, 20–25 v; duration, 0.5–0.8 msec; frequency, 30–60/sec. Additional clearances were performed 20–30 min after beginning the stimulation, and stimulation was then stopped. In four dogs osmotic diuresis was then induced using either mannitol or sodium sulfate in the doses described above, additional clearances were performed 30 min later, and stimulation was reinstated. Additional clearances were performed 20–30 min after beginning this second course of stimulation. This protocol differed from *protocol 1* in that the osmotic diuretics were not used to reverse an already elevated renin secretion. Rather, an attempt was made to prevent the rise by inducing diuresis before stimulating the nerves. The major reason for this change in procedure was that, in preliminary studies on the technique of stimulating the renal nerves, it was found that steady conditions (as judged by urine flow and renal hemodynamics) could not be maintained for more than approximately 35–40 min of nerve stimulation. This period was too short to obtain accurate clearances during both nondiuresis and diuresis. On the other hand, after 10–20 min of nonstimulation, reinstatement of renal nerve stimulation gave results quite similar to those obtained for the previous stimulation period.

Analytical procedures. Analytical methods for creatinine, PAH, and sodium have been described previously (6). The method used for determination of plasma renin concentration is indirect and has been described in an earlier paper (7). Briefly, 5 ml of renal venous or femoral arterial plasma was acidified to pH 5.1, incubated at 37 C for 31 min, saturated with NaCl and acidified to pH 2 or lower, and extracted with normal butanol. The butanol was evaporated and the residue dissolved in 1 ml distilled water. This solution was then bioassayed for pressor activity in rats given pentobarbital, pentolinium, and Dibenzylamine. The response was matched against standard doses of val⁵-angiotensin II amide (Ciba). The results are expressed in millimicrograms of angiotensin-like activity produced per milliliter of original plasma (angiotensin equivalents per milliliter plasma) under the standardized conditions of the chemical and bioassay procedures described. The characteristics of these methods (reproducibility, sensitivity, concentrations of angiotensinase and angiotensinogen, proof that the reaction product assayed is angiotensin) have been described in detail previously (7). The presence in some of these

experiments of elevated plasma concentrations of catecholamines (resulting from their intravenous infusion) did not interfere with the assay procedure, as evidenced by the following observations: 1) catecholamines added to normal dog plasma *in vitro* in amounts which raised plasma catecholamine concentration to at least twice that found in the experimental dogs during catecholamine infusion could not be detected by the assay procedure, primarily because approximately 90% of the catecholamine activity remained in the aqueous phase during butanol extraction, and also because the assay rats were treated with Dibenzylamine; 2) Dibenzylamine did not alter the pressor activity of the experimental plasmas but abolished comparable pressor responses to standard norepinephrine; 3) as will be described below, the induction of osmotic diuresis abolished or greatly reduced plasma pressor activity despite the continued infusion of catecholamine; 4) the pressor activity of the assay material obtained from renal venous plasma was greater than that of simultaneously obtained femoral artery plasma; 5) the pressor activity of the final assay product was completely abolished by incubation with trypsin.

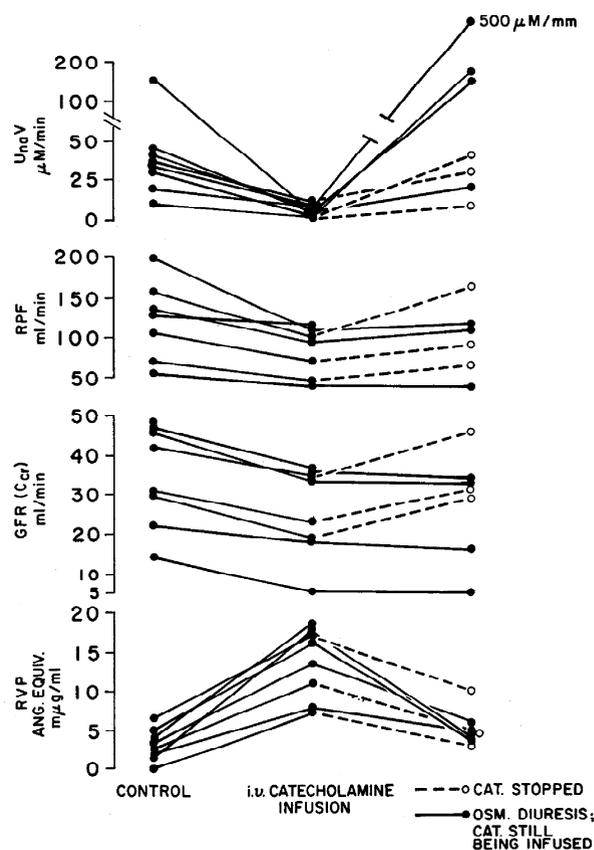


FIG. 1. Effects of intravenous infusion (8 infusions in 7 dogs) of either epinephrine (5–6 $\mu\text{g}/\text{min}$) or norepinephrine (12–16 $\mu\text{g}/\text{min}$) on renal function and pressor activity of incubated renal venous plasma during nondiuresis and osmotic diuresis. Renal arterial blood pressure was maintained constant throughout each experiment by means of an aortic clamp. All data are for the right kidney only. RPF was not determined for one dog.

RESULTS

Protocol 1. Intravenous infusion of catecholamine and aortic clamping. The data for these experiments (8 infusions in 7 dogs) are summarized in Fig. 1 (no significant differences were observed between the effects of epinephrine and norepinephrine). In every case the intravenous infusion of epinephrine or norepinephrine increased RVP pressor activity. Ideally, it would have been desirable to calculate the angiotensin equivalents of renin actually secreted by the kidney per unit time, but this would have required the assaying of both femoral artery and renal venous blood samples for all periods and we did not wish to take such large quantities of blood from the dogs. Vander and Miller have demonstrated (7) that during moderate acute reduction of arterial blood pressure, renal venous pressor activity alone is an adequate indicator of renin secretion. This was also demonstrated to be true under conditions of the present experiments by measuring A-V pressor activity differences and calculating "renin secretory rates" in three of the dogs before and during norepinephrine infusion. Moreover, in none of these experiments was the decrease in renal plasma flow large enough to account for more than a fraction of the observed increase in renal venous pressor activity. As was also true for the increased renin secretion which occurs during acute reduction of renal arterial pressure (7), the renal venous pressor activity increased rapidly after beginning the catecholamine infusion, stabilized in approximately 30 min, remained relatively constant for at least 90 min (no attempt was made to extend the experiments longer), and returned toward control values within 30 min after stopping the infusion. Osmotic diuretics also reduced renal venous pressor activity toward control values within 30 min despite the continued infusion of catecholamine.

In every case, catecholamine infusion decreased GFR, RPF, and sodium excretion. All these changes were rapidly reversed following cessation of the infusion. The administration of osmotic diuretics during catecholamine infusion produced no consistent effect on GFR or RPF, but increased sodium excretion.

Arterial blood pressure (not shown in the figure) proximal to the aortic clamp was increased 10–50 mm Hg, but mean renal arterial blood pressure was maintained constant throughout the experiments by means of the aortic clamp.

Protocol 2. Renal nerve stimulation. The data for these experiments (9 stimulation periods in 5 dogs) are summarized in Fig. 2. It is evident that the renal responses to renal nerve stimulation were similar to those produced by catecholamine infusion. In nondiuretic dogs, renal venous pressor activity increased, whereas GFR, RPF, and sodium excretion decreased. In the four dogs undergoing osmotic diuresis, renal venous pressor activity was increased only slightly by renal nerve stimulation, whereas GFR and RPF were decreased to the same extent as in nondiuretic dogs. Sodium excretion remained well above nondiuretic values. Mean arterial

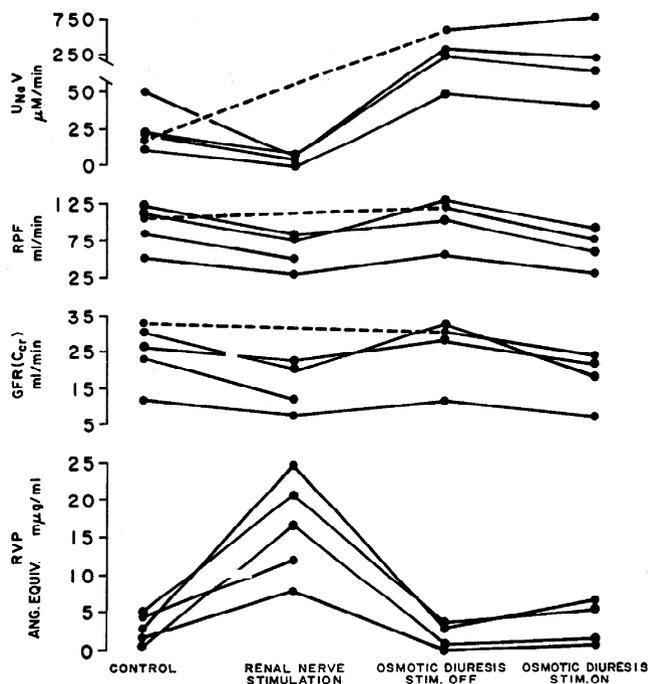


FIG. 2. Effects of renal arterial nerve plexus stimulation (9 stimulation courses in 5 dogs) on renal function and pressor activity of incubated renal venous plasma during nondiuresis and osmotic diuresis. All data are for the right kidney only. Dotted lines indicate no nondiuretic renal function data for one dog during renal nerve stimulation.

blood pressure (not shown in the figure) increased 4–10 mm Hg during nerve stimulation in nondiuretic dogs but showed no consistent change in diuresing dogs.

DISCUSSION

The present studies have demonstrated two experimental procedures which enhanced renin secretion in nondiuresing anesthetized dogs:

1) Intravenous infusion of catecholamine during maintenance of constant mean renal arterial blood pressure; these experiments are in accord with those of Scornik and Paladini (4), who demonstrated significantly increased blood angiotensin concentrations following intravenous infusion of norepinephrine alone and even larger increases during norepinephrine infusion coupled with renal arterial blood pressure reduction. While my experiments were in progress, Wathen et al. (8) reported that infusion of either epinephrine or norepinephrine into a renal artery increased renin secretion.

2) Stimulation of the renal nerves; these experiments are consistent with those of Taquini, Blaquier, and Taquini (5), who have demonstrated that denervation of rat kidneys caused a decrease in their renin content.

The administration of osmotic diuretics reversed or prevented the elevation of renin secretion resulting from these procedures, but did not alter GFR or RPF.

The arterioles of the juxtaglomerular apparatus are well supplied with nonmyelinated nerve fibers presumed

to be sympathetic (1, 2), and it is possible that both catecholamine infusion and renal nerve stimulation have in common a direct action on the juxtaglomerular apparatus. However, the ability of osmotic diuretics to inhibit the increased renin secretion resulting from these procedures is suggestive evidence that the stimulus for renin secretion is not a direct result of sympathetic stimulation of the juxtaglomerular apparatus, but rather an indirect result of alterations in renal function. It is also unlikely that the input is either RPF or afferent arteriolar pressure per se, since the diuretics produced no significant changes in either RPF or GFR in these experiments. The data are consistent with the theory proposed previously (7), that renin secretion is controlled by an intrarenal factor responsive to the resultant of sodium filtration and proximal reabsorption, and that this factor is probably the sodium concentration, osmolality, or flow rate of macula densa fluid. Thus, under the conditions of these experiments, in the nondiuresing dogs, catecholamines

and renal nerve stimulation decreased GFR and, thereby, decreased intratubular sodium.² During osmotic diuresis, on the other hand, the decrease in filtered sodium was more than balanced by the diuretic-induced proximal tubular inhibition of sodium reabsorption (sodium sulfate had the additional effect of increasing plasma sodium concentration).

It should be stressed that, although these experiments are consistent with the macula densa theory, they do not rule out the possibility that catecholamines and the renal nerves directly stimulate the juxtaglomerular cells. Renin secretion may be controlled by a variety of direct inputs (catecholamines, renal nerve stimulation, decreased perfusion pressure, macula densa fluid). Regardless of the mechanism, it is evident that the sympathetic nervous system may play an important role in the control of renin secretion.

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² The decrease in sodium excretion which occurs during catecholamine infusion or renal nerve stimulation is due primarily to a decreased GFR; the problem of a direct tubular effect of catecholamines and the renal nerves remains unsettled.