Effect of Acetylcholine on Renin Secretion in Salt-Depleted Dogs[1] (34372)

HITOSHI TAGAWA[2] AND ARTHUR J. VANDER

Department of Physiology, The University of Michigan, Ann Arbor, Michigan 48104

In a previous study (1) it was found that salt depletion increased renin secretion without significant change in renal blood flow. However, recent studies (2, 3) have suggested that changes in sodium balance may induce a redistribution of intrarenal blood flow; specifically, salt depletion may decrease outer cortical flow while increasing juxtamedullary flow. In this manner, total resistance might be little altered whereas the localized vasoconstriction would stimulate renin secretion via either the postulated macula densa or pressure receptors. Acetylcholine has been reported to have effects on blood flow distribution opposite to those of salt depletion [(4) and Dr. N. K. Hollenberg, personal communication]; accordingly the present experiments were designed to determine whether acetylcholine would inhibit renin secretion in salt-depleted dogs.

Methods. Experiments were performed on 12 mongrel dogs of both sexes weighing 13–27 kg, which received mercuhydrin, 80 mg intramuscularly, 2–6 days before the experiments and were maintained on a diet containing 10 meq of Na/day (prescription diet h/d, Hill Packing Co.). All animals were anesthetized with 30 mg/kg sodium pentobarbital intravenously with supplements given as required. Via flank incisions, the left kidney was excised and the right ureter was catheterized with a polyethylene tube. To obtain renal venous blood, a polyethylene catheter (2.4 mm o.d.) was introduced into a femoral vein, passed up the inferior vena cava, and manipulated into the right renal vein; its position was verified at the end of the experiment. An electromagnetic flow probe was placed on the right renal artery, and the renal blood flow (RBF) was continuously recorded by a square-wave electromagnetic flowmeter (Carolina). Care was taken not to cut or injure the visible nerve fibers around the renal artery. A 22-gauge needle attached to a polyethylene catheter was inserted directly into the renal artery proximal to the flow probe and was left in place throughout the experiment, isotonic saline being infused continuously at the rate of 0.2 ml/min with or without acetylcholine. A prime of creatinine was given, followed by continuous intravenous infusion at a rate of 0.2 ml/min for the measurement of glomerular filtration rate (GFR). Clearance periods were 6–15 min long, with arterial and renal venous bloods taken in the midst of each period. Renin activity was assayed in renal venous plasma. Arterial blood was obtained from a carotid artery catheter. Mean arterial blood pressure (MAP) was monitored from a femoral artery catheter using a Statham transducer and Grass polygraph. All red cells taken were suspended in an equal volume of 6% dextran solution in saline and were returned to the dog through an external jugular vein catheter.

Dogs were allowed a period of more than 45 min after completion of all surgical procedures and administration of creatinine prime. After control clearances, acetylcholine (50 or 100 μg/min) was infused directly into the renal artery for 20–60 min and further clearances were performed. After stopping acetylcholine, clearances were performed 10–25 min and more than 45 min later. In some dogs, renin measurements were also made on plasma taken within 7 min after stop of the infusion. This protocol was repeated in some dogs more than 1 hr after the previous infusion.

Plasma renin activity was analyzed by the method described previously (1), and was

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[2] Michigan Kidney Foundation Fellow.

TABLE I. Summary of All Data for Acetylcholine Infusion in Salt-Depleted Dogs.[a]

	Control	Δ (infusion —control)	Δ (recovery—control) <7 min	15–25 min	>45 min
Mean arterial blood pressure (mm Hg)	124.2 ± 4.1	−1.5 ± 2.0	−2.1 ± 1.1	0.9 ± 1.2	0.7 ± 1.3
RPF (ml/kg • min)	6.67 ± 0.39	1.91 ± 0.36[f,b]	0.59 ± 0.23[c]	0.15 ± 0.16	0.34 ± 0.16
GFR (ml/kg • min)	1.67 ± 0.13	−0.08 ± 0.09		0.22 ± 0.10[c]	0.13 ± 0.06[c]
Filtration fraction	0.25 ± 0.02	−0.06 ± 0.01[f]		0.04 ± 0.02[c]	0.01 ± 0.01
Renal vascular resistance (mm Hg • kg • min/ml)	10.02 ± 0.53	−2.13 ± 0.34[f]	−0.99 ± 0.27[e]	−0.28 ± 0.22	−0.32 ± 0.29
Urine flow (μl/kg • min)	16.4 ± 2.0	20.6 ± 3.8[f]	4.5 ± 2.7	1.3 ± 1.1	2.9 ± 0.8[e]
Na excretion (μmoles /kg • min)	0.70 ± 0.11	2.15 ± 0.41[f]		0.21 ± 0.08[d]	0.47 ± 0.13[e]
Renal venous renin (ang. eq, ng/ml of plasma)	18.8 ± 5.1	−4.1 ± 2.7	5.7 ± 3.8	12.9 ± 4.7[d]	8.4 ± 4.6
Change (%)	—	−5.2 ± 7.2	43.5 ±11.8[e]	62.5 ±15.3[f]	23.9 ±12.8

[a] Values are expressed in means ± SEM.

[b] Statistical significance when evaluated by paired analysis: [c] $p < .05$, [d] $p < .02$, [e] $p < .01$, [f] $p < .001$.

expressed as angiotensin equivalents (ng/ml). Hematrocrit (Ht) was measured by microcapillary tube. Renal plasma flow (RPF) was calculated as RBF × (1 − Ht). Renal vascular resistance was calculated as MAP/RBF.

Results. All data for control periods are summarized in Table I. During control periods, *i.e.*, prior to acetylcholine infusion, renin correlated inversely with renal plasma flow (Fig. 1; $r = -0.751$, $p < .001$) and directly with renal vascular resistance ($r = 0.558$, $< .02$), but not with arterial blood pressure, GFR, filtration fraction, urine flow, or sodium excretion.

The effects of acetylcholine are summarized in Table I. Since acetylcholine produced similar results despite differences in dose and duration of the infusion, the data are described together. Invariable findings during the infusion were increases in RPF, urine flow, and sodium excretion, and decreases in filtration fraction and renal resistance; GFR remained unchanged. During the recovery period, RPF and renal resistance returned to control, sodium excretion stayed somewhat above control, and GFR and filtration fraction slightly increased.

As shown in Table I, acetylcholine produced no significant effect on renal venous renin activity when the data for the entire group are evaluated by paired samples analysis. The responses of the individual experiments are illustrated in Fig. 2. It should be

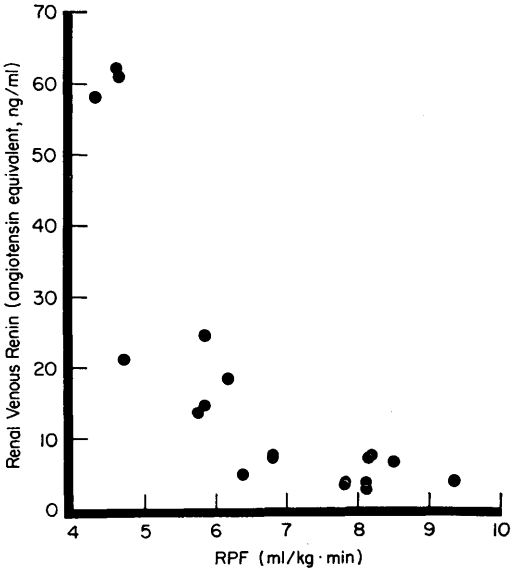

FIG. 1. RPF and renal venous renin activity during control periods in salt-depleted dogs.

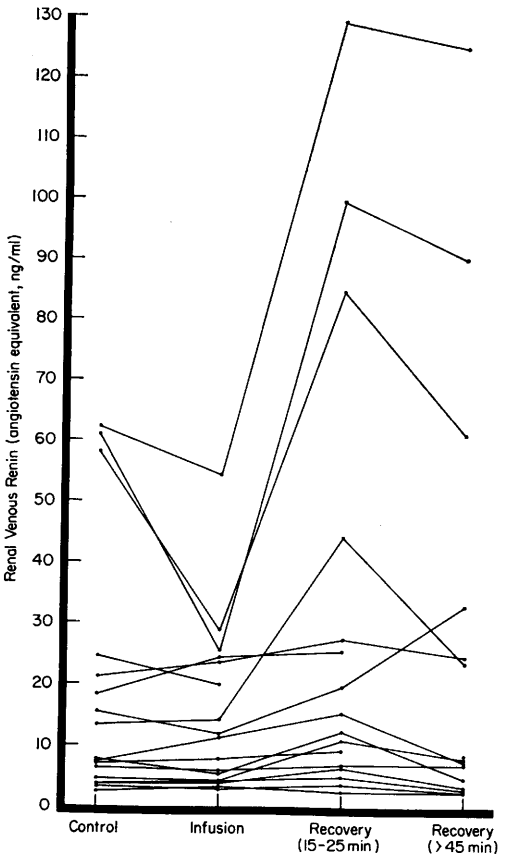

FIG. 2. Effect of acetylcholine on renal venous renin activity in salt-depleted dogs.

emphasized that these data are renal venous renin activities rather than total renin secretory rates. Ideally it would have been desirable to calculate the latter in all experiments, but this would have required the removal of twice as much blood, both renal venous and arterial. Accordingly, a constant or increased renal venous renin, when associated with the increased renal plasma flow induced by acetylcholine, might partially mask an increased secretory rate; conversely, a decreased renal venous renin of small magnitude could well be due to the increased plasma flow. Taking blood flow changes into account, acetylcholine caused definite inhibition of renin release only in those 3 expts. having the highest control renal venous renin activities.

After acetylcholine was stopped, most dogs, regardless of their renin responses during the infusion, showed increases in renal venous renin for 15–25 min, followed by decreases toward control after 45 min. For the entire group, this increase in renin activity was significant ($p<.02$).

Discussion. The effects of acetylcholine on renal hemodynamics and sodium excretion observed in these experiments are similar to those reported by numerous investigators (5, 6). The possible mechanisms by which acetylcholine and other vasodilators induce natriuresis in the absence of an increased GFR have been discussed recently; the concepts involving changes in intrarenal blood flow distribution (4) or intrarenal pressures (7) appear favored by most evidence at present.

In the present investigation, the inverse correlation of control renin with renal blood flow and its direct correlation with renal resistance were highly significant. Since such correlations were not observed previously for dogs on a normal salt diet (8), this suggests that the increased renin secretion associated with salt depletion might be causally related to renal vasoconstriction. However, the failure of acetylcholine-induced vasodilation to inhibit renin secretion does not support this hypothesis. Only in 3 of 17 experiments was definite inhibition of renin secretion observed; these dogs had the highest control renal resistances in our series, a finding which inicates that, during salt depletion, increased renal resistance might stimulate renin secretion, but only when the resistance is greatly elevated.

Although the failure of acetylcholine to inhibit renin secretion consistently does not support the hypothesis that vasoconstriction is the major stimulus to renin secretion during salt depletion, it does not rule it out, because of the possibility that acetylcholine might induce simultaneous opposing effects, one—vasodilation—inhibiting, and the other stimulating renin secretion. Depending upon conditions, the net effect on renin might be no change [the usual response in our experiments and in dogs on a normal-sodium diet (9)], inhibition (observed in 3 infusions), or stimulation; Ayers *et al.* (10) reported marked stimulation of renin secretion by acetylcholine in dogs with chronic renal hyper-

tension. Opposing effects would also explain why there was usually a transient increase in renin secretion after stopping the infusion;[3] this might well represent a "rebound" effect, *i.e.*, a transient outpouring of renin after removal of inhibition, although the inhibition, itself, might have been masked by an opposing stimulatory effect. We have observed a similar "rebound" phenomenon when angiotensin infusions, which inhibit renin secretion, are discontinued (unpublished observations).

There are several possible mechanisms by which a stimulatory effect of acetylcholine might be mediated: (a) Were acetylcholine to simultaneously reduce juxtamedullary flow while increasing outer cortical flow (4), there might occur an increased renin secretion from this inner cortical area. (b) Acetylcholine might act directly upon the granular cells (or macula densa) to stimulate renin release. (c) Acetylcholine might increase renin release by stimulating intrarenal sympathetic ganglia or releasing catecholamines from sympathetic nerve endings, since it has been demonstrated that sympathetic nerves and circulating catecholamines can stimulate renin release (11-13). Our data do not provide any direct information on these possibilities, and a more extensive study of other vasodilators, flow distribution, and the renal nerves will be required to evaluate this possibility of a stimulatory acetylcholine effect.

Summary. In anesthetized salt-depleted dogs, renal venous renin activity correlated directly with renal vascular resistance and inversely with RPF, but not with arterial blood pressure, GFR, filtration fraction, urine flow, or sodium excretion. Acetylcholine, 50–100 μg/min, infused directly into a renal artery caused significant vasodilation and natriuresis, but no significant change in renal venous renin for the group as a whole. However, definite inhibition of renin release was observed in the 3 dogs with the highest control renal resistance. For the entire group, cessation of acetylcholine infusion was followed by a transient significant increase of renin above the initial control value. The data do not support the hypothesis that vasoconstriction is the major stimulus to renin release during salt depletion, but do not rule it out because of the possibility that acetylcholine might induce simultaneous opposing effects, one inhibiting and the other stimulating renin release.

[3] It is extremely unlikely that this increased renin secretion is due to additional salt depletion secondary to the acetylcholine-induced natriuresis, since total sodium loss during infusion never exceeded 1.3 mmoles.

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