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Renin Secretion after Papaverine and Furosemide in Conscious Sheep[1] (39644)

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Papaverine has proven to be a useful pharmacological tool to study renin secretion because it blocks the intrarenal vascular receptor that regulates renin release (1). Results of studies in which papaverine has been used in conjunction with the nonfiltering kidney model (2) have favored a predominant role for the vascular receptor in regulating renin secretion (3). These studies have been performed exclusively in anesthetized animals, usually dogs, and data relating to the effects of papaverine on renin secretion and renal function in conscious animals are lacking.

Papaverine induced blockade of the renal vasculature should also provide a useful means for examining the function of the macula densa in regulating renin release. Furosemide-stimulated renin release during isovolemia has been taken as evidence for a functional role of the macula densa (4), but, furosemide is well known to produce hemodynamic changes within the kidney (5). These changes in vascular tone might increase renin secretion by a direct effect on the intrarenal baroreceptor (6). If renin secretion could be stimulated by furosemide in the presence of vascular blockade with papaverine, it would support the concept of a role for the macula densa in regulating renin release.

The present studies were undertaken to define the effects of papaverine in conscious animals and to determine if furosemide would stimulate renin secretion during renal arterial infusion of papaverine and maintenance of constant extracellular volume.

Methods. Six mixed breed sheep (25–40 kg body wt) underwent a right nephrectomy 3–6 weeks before experimentation. One to three days before the animals were to be studied, anesthesia was induced with intravenous pentothal and maintained with halothane-O_2 for implantation of chronically indwelling catheters. The left renal artery was catheterized by the Herd–Barger technique (7) using Silastic tubing (Dow-Corning Co.), and a noncannulating electromagnetic flow probe (Carolina Medical Electronics) was placed around the renal artery at its origin. The flow probe had been calibrated previously *in vitro* using whole blood perfused through segments of sheep carotid arteries. Another Silastic catheter was introduced via a small tributary into the renal vein. The ureter was catheterized with polyvinyl chloride (PVC) tubing close to the renal pelvis. Additional PVC catheters were placed in a carotid artery via a transverse facial artery and into a jugular or saphenous vein. The vascular catheters were maintained patent by daily flushing with 100 U/ml heparin-saline solution.

The experiments were conducted with the animals standing quietly in their cages. Intravenous inulin was continuously administered during the experimental procedure to determine glomerular filtration rate. Zero renal blood flow was determined electronically and both mean flow and arterial pressure were recorded on a Gilson recorder. During the experiment, all urine volumes above control level were replaced continuously with sterile normal saline delivered by a roller pump (Gilson, Minipuls) into a jugular or saphenous vein catheter. All blood samples were immediately replaced with fresh whole blood from another sheep.

Two experimental protocols were followed: To determine the effects of papaverine alone on renin secretion in conscious sheep, saline was first administered into the renal artery at 0.6 ml/min and two 10-min clearance periods were performed. Simultaneous systemic arterial and renal venous blood samples were obtained at the midpoint of each clearance period. Subsequently, the renal arterial infusion was

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changed to papaverine in isotonic saline and delivered at 7 mg/min and two further clearance periods were performed. In some sheep, the papaverine infusion was increased to 10 mg/min for one or two additional clearance periods. The second protocol was similar to that just described except that after two or three clearance periods with renal intra-arterial infusion of papaverine (7 mg/min), 35 mg of furosemide (Lasix) was administered intravenously over 10 min. Three further clearance determinations were made at 20-min intervals with papaverine continuously infused into the renal artery.

Plasma renin activity was determined by radioimmunoassay of *in vitro* generated angiotensin I based on the technique of Haber (8). Reagents were supplied in kit form by New England Nuclear Co. The incubation was carried out for 1 hr at pH 6.0 without addition of substrate. Aliquots of all plasma samples were maintained at 4°C and assayed simultaneously with the incubated samples. The immunoreactive material assayed in the unincubated sample was substracted from the measured angiotensin I in the incubated sample to yield the value for arterial and renal venous plasma renin activities. Figure 1 illustrates that the angiotensin I production was linear for up to 2 hr of incubation without added substrate for a wide range of plasma renin activities. Renin secretion was calculated by multiplying the renal venous minus arterial plasma renin activity by the renal plasma flow.

Plasma and urinary electrolytes were determined by flame photometry. Mean arterial blood pressure and mean renal blood flow were determined by sampling these variables once each minute and averaging over the 10- or 20-min clearance period. Inulin in plasma and urine was determined by the method of Heyrovsky (9). Statistical analysis was carried out on a pair design basis utilizing Student's t test.

Results. Effect of papaverine alone on renin secretion. Twelve experiments were conducted on different days on six conscious sheep. The animals stood or laid quietly in their cages during the entire experiment. The only behavioral effect discernible during papaverine administration was licking of the cage, which generally occurred toward the end of the experimental period. Because urine flow never exceeded control levels during the administration of papaverine only, replacement of urine volume was not undertaken in these experiments. Table I presents data obtained with normal saline or 7 or 10 mg/min of papaverine in a 0.9% NaCl solution infused into the renal artery at 0.6 ml/min. Papaverine at 7 mg/min produced a slight but statistically significant fall ($P < 0.01$) in mean arterial blood pressure in the conscious sheep. Likewise, there was a significant increase ($P < 0.01$) in mean renal plasma flow (RPF) reflecting the decrease in renal resistance secondary to papaverine infusion. Glomerular filtration rate

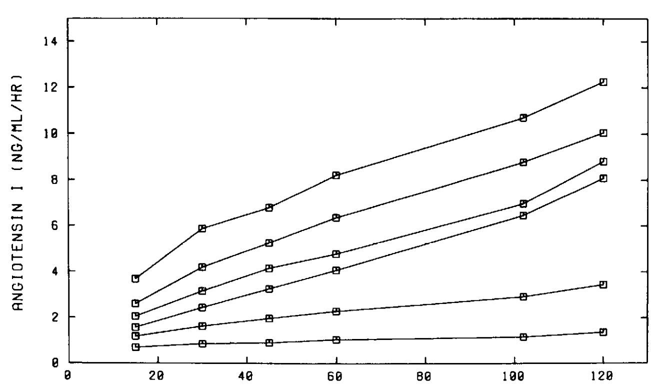

FIG. 1. *In vitro* generation of angiotensin I from sheep plasma for several levels of renin activity. The generation was conducted at 37° for 2 hr to illustrate the linearity of the response without addition of exogenous substrate. The experimental samples for this study were incubated for 60 min and are within the range of renin activities illustrated.

(GFR) fell significantly ($P < 0.01$) resulting in a decrease in the filtered load of Na ($P < 0.01$). Urinary Na excretion and the percentage of filtered Na excreted did not change. Increasing the renal arterial infusion rate of papaverine to 10 mg/min produced no further increase in RPF. In fact, at this level of papaverine administration, there was a slight but significant ($P < 0.05$) decrease in RPF in each animal studied. A further decrease in GFR was observed ($P < 0.05$) but no changes in other measured variables when compared to the 7-mg/min papaverine infusion.

Figure 2 illustrates the changes in renin secretion for each animal in this experiment. No statistically significant changes in renin secretion occurred with either 7 or 10 mg/min of papaverine when compared to the saline control values. In three instances during renal arterial infusion of saline or papaverine, renin was being taken up by the kidney rather than being secreted. These data suggest intermittent secretion of renin in conscious sheep but the observations are too limited to suggest possible significance or controlling factors.

Effects of papaverine and furosemide on renin secretion. The results obtained after intravenous injection of 35 mg of furosemide in conscious sheep which were undergoing a renal arterial infusion of papaverine are illustrated in Fig. 3 and Table II. In these animals urine volume above control levels was continuously replaced by an intra-

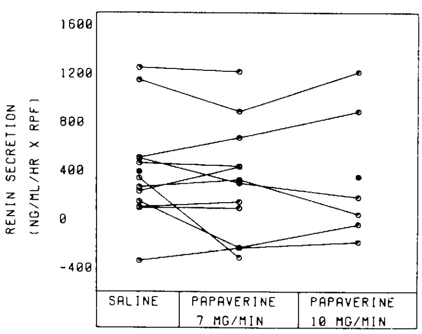

FIG. 2. Individual animal renin secretion rates during renal arterial infusion of normal saline or 7 or 10 mg/min of papaverine. All infusion were at 0.6 ml/min. ●, the mean for each treatment group with the negative values included. No statistical differences exist between groups whether the negative values are considered as such or whether they are considered 0 secretion.

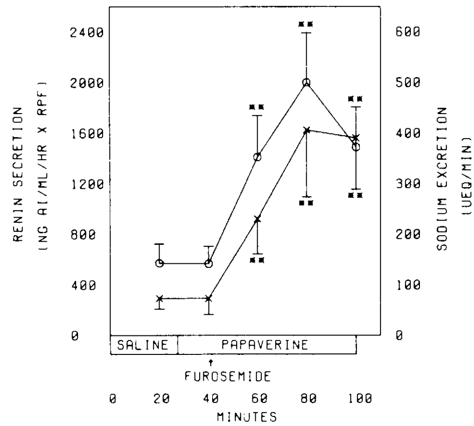

FIG. 3. Renin secretion (O——O) and Na excretion (×——×) during renal arterial infusion of saline or papaverine (7 mg/min) and after an intravenous injection of 35 mg of furosemide with papaverine continuously infused into the renal artery. All renal arterial infusions were at 0.6 ml/min. Statistical analysis is based on the difference between the papaverine alone period and the periods after furosemide injection. Mean ± SEM, ** = $P < 0.01$, $N = 5$.

venous saline infusion. Arterial blood pressure and RPF were not significantly different from the papaverine only control period after furosemide administration. GFR was significantly different only during the final 20-min clearance period. Renal sodium excretion increased rapidly and was elevated significantly at each clearance period after furosemide administration. Likewise, large statistically significant increases in renin secretion paralleled the increase in Na excretion.

Discussion. The present experiments were conducted in conscious animals to eliminate the effect of anesthesia on renin secretion (10, 11). Additionally, it was noted that papaverine infused into the renal artery did not increase Na excretion as has been previously reported in experiments on anesthetized dogs (12, 13). The explanation for this discrepancy between the effects of papaverine on renal Na handling in conscious sheep and anesthetized dogs is not clear. We have recently studied renal arterial infusion of papaverine in anesthetized sheep and observed increased Na excretion similar to that reported for anesthetized dogs (unpublished). It is assumed that the decreased filtered load of Na which results from the decrease in GFR would result in a

TABLE I. RENAL FUNCTION AND ARTERIAL BLOOD PRESSURE DURING INFUSION OF NORMAL SALINE OR PAPAVERINE INTO THE RENAL ARTERY.[a]

		Papaverine	
	Saline	7 mg/min	10 mg/min
BP (mmHg)	93 ± 5[b]	90 ± 5 (−3 ± 0.7*)[c]	93 ± 6 (0.3 ± 1.0)
RPF (ml/min)	438 ± 30	462 ± 22 (40 ± 10**)	442 ± 24 (−32 ± 15*)
GFR (ml/min)	34 ± 3	28 ± 3 (−7 ± 1**)	21 ± 4 (−4 ± 2*)
Filtered Na (μEq/min)	5072 ± 407	4098 ± 405 (−975 ± 254**)	3227 ± 349 (−482 ± 312)
U_{Na} V (μEq/min)	80 ± 24	75 ± 21 (−4 ± 15)	79 ± 26 (−32 ± 25)
Filtered Na excreted (%)	1.6 ± 0.4	1.8 ± 0.5 (0.4 ± 0.3)	2.4 ± 0.6 (0.4 ± 0.5)
N	12	12	6

[a] All renal arterial infusions at 0.6 ml/min. Statistical analysis compares the difference between papaverine (7 mg/min) and saline alone and the difference between 10- and 7-mg/min papaverine infusions.
[b] Mean ± SEM.
[c] Mean difference ± SEM difference.

TABLE II. RENAL FUNCTION AND ARTERIAL BLOOD PRESSURE DURING RENAL ARTERIAL INFUSION OF SALINE OR PAPAVERINE AND AFTER INTRAVENOUS FUROSEMIDE.[a]

	Saline	Papaverine	Papaverine and furosemide		
	20 min	40 min	60 min	80 min	100 min
BP (min Hg)	85 ± 8[b]	82 ± 7	92 ± 12 (10 ± 5)[c]	90 ± 10 (4 ± 2)	92 ± 11 (6 ± 3)
RPF (ml/min)	393 ± 52	433 ± 377	395 ± 48 (−38 ± 28)	407 ± 35 (−26 ± 33)	411 ± 44 (−22 ± 31)
GFR (ml/min)	31 ± 4	23 ± 3	24 ± 2 (1 ± 4)	19 ± 3 (−4 ± 4)	18 ± 3 (−5 ± 2*)
Filtered Na (μEq/min)	4603 ± 514	3421 ± 391	3654 ± 4077 (233 ± 622)	2920 ± 508 (−493 ± 681)	2742 ± 423 (−679 ± 270*)
U_{Na} V (μEq/min)	73 ± 21	74 ± 33	230 ± 69 (157 ± 40**)	406 ± 132 (333 ± 103**)	390 ± 101 (317 ± 71**)
Filtered Na excreted (%)	1.9 ± 0.7	2.3 ± 1.0	7.8 ± 3.5 (5.4 ± 1.8**)	14.3 ± 3.7 (12.0 ± 3.0**)	14.9 ± 3.1 12.5 ± 2.3**

[a] Saline was administered into the renal artery at 0.6 ml/min for the first 20 min; then the infusion was changed to papaverine (7 mg/min) in normal saline for the duration of the epxeriment. Furosemide (35 mg) was injected slowly as a single bolus at 60 min. Statistical analysis compares each of the three periods after furosemide injection to the period during which only papaverine was infused. * = $P < 0.05$, ** = $P < 0.01$, $N = 5$.
[b] Mean ± SEM.
[c] Mean difference ± SEM difference.

decrease in Na excretion if fractional reabsorption remained constant or nearly so. In fact, in 8 of the 12 experiments conducted with papaverine alone infused into the renal artery, Na excretion did decrease. It appears that any inhibitory effect papaverine might have on Na reabsorption by the nephron is augmented considerably by anesthesia.

As postulated by Vander and Carlson (4), a decrease in the filtered load of Na should be reflected in a decreased load of Na at the macula densa and result in an increased renin secretion. In the present experiments papaverine alone resulted in a 19% decrease in filtered Na but no increase in renin secretion was observed. This observation could be interpreted to suggest that small changes in filtered load of Na do not affect the macula densa receptor. However, lacking specific information on proximal tubular reabsorption of Na under the influence of papaverine (*vide supra*), it is difficult to draw a firm conclusion regarding the Na load presented to the macula densa. It is possible that proximal reabsorption decreased sufficiently to allow no change in macula densa Na load despite the decreased filtered load of Na.

The reasoning behind combining papaverine and furosemide to study the role of the macula densa is that by blocking vasomotion of the renal vasculature and maintaining renal perfusion pressure constant, any influence on renin secretion by the vascular receptor mechanism would be minimized. If alterations in NaCl transport by the macula densa segment can be effected under these conditions, it would be reasonable to assume that any renin secretion which occurred would be due to alterations in ion transport and not secondary to pressure changes.

The elevated renin secretion observed after furosemide administration was not associated with changes in arterial blood pressure or renal blood flow. Urinary Na excretion rose markedly and closely paralled the increase in renin secretion. These data suggest that the stimulated renin secretion under these conditions results from alterations in tubular ion transport and not from changes in vascular tone or distending pressure at the baroreceptor.

Corsini *et al.* (14) recently reported that furosemide stimulated renin secretion in filtering and nonfiltering kidneys of anesthetized dogs. Papaverine blocked this response in the nonfiltering kidney but renal vasodilation by acetylcholine did not block the response in filtering kidneys. Papaverine was not studied in this context in dogs with filtering kidneys. Although the data of Corsini *et al.* would suggest that part of the furosemide-stimulated renin release was secondary to intrarenal baroreceptor activation, their data using acetylcholine induced renal vasodilation and the results from this study would support the concept of a role for the macula densa in regulating renin secretion.

Because these animals did not undergo renal denervation or adrenalectomy, it is possible that the renal nerves or circulating catecholamines could have influenced renin secretion during furosemide stimulation. This possibility would appear unlikely, however, because volume depletion was prevented by replacing urinary and blood losses. Also, Johnson *et al.* (15) have demonstrated that papaverine blocks the stimulatory effect of epinephrine on renin secretion.

These studies do not indicate the nature of the signal perceived by the renal tubular mechanism after furosemide administration; however, since furosemide inhibits transcellular NaCl transport it is likely that this is the variable being sensed. The recent observation by Burg and co-workers (16) and Kokko and co-workers (17) that Cl is the actively transported ion in the ascending limb of the loop of Henle and that furosemide inhibits active Cl transport by this segment, suggest that the macula densa mechanism might be Cl-sensitive rather than Na-sensitive. This is supported by our earlier suggestion (6) that the macula densa probably transports NaCl in a manner similar to the ascending limb of the loop of Henle, since renin secretion is not inhibited by aldosterone (18).

Summary. Conscious sheep with chronically implanted vascular catheters and a renal artery flow probe were used to study the effects of papaverine alone or papaverine plus furosemide on renin secretion. Papaverine infused into the renal artery at 7 mg/min produced a significant renal vasodilation which was not further enhanced by increasing the papaverine infusion to 10 mg/min. GFR decreased significantly as did the filtered load of Na. The increased Na excretion reported in anesthetized dogs was not observed in conscious sheep. Renin secretion was not altered despite these changes in renal function. When 35 mg of furosemide was administered intravenously in addition to the renal artery papaverine infusion, no further change in RPF or GFR was observed. Na excretion was significantly elevated at each clearance period after furosemide administration and these increases were closely paralleled in time by large increases in renin secretion. The increased renin secretion observed from the papaverine blocked kidney after furosemide administration appears to be mediated by changes

in ion transport by the renal tubules and not by activation of the intrarenal baroreceptor.

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