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Sodium Excretion after Renal Vasodilation by Papaverine in Conscious and Anesthetized Sheep[1] (40182)

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Vasodilating drugs have been used extensively to study renal autoregulation (1–3) renin release (4, 5) and the physical factors underlying Na reabsorption by the kidney (6–9). All of these studies were conducted in anesthetized dogs and all reported increased $U_{Na}V$ after renal vasodilation. Recently, I reported that renal vasodilation by papaverine was not natriuretic in conscious sheep (10). This observation was contrary to the results published by others using papaverine in anesthetized dogs (2, 4, 11).

Because of the disparity of these results, it was important to investigate the effects of renal vasodilation in anesthetized sheep for comparison with the observations made in conscious animals. In addition to our previously published data (10), another group of conscious animals was studied in which papaverine was infused into the renal artery. The results from this latter group were compared to another group of anesthetized sheep which also received papaverine into the renal artery.

These data indicate that renal vasodilation by papaverine does produce different effects on $U_{Na}V$ depending upon whether the animals are anesthetized or not. Papaverine is distinctly natriuretic in anesthetized sheep but does not promote elevated $U_{Na}V$ in conscious sheep. Differences in RBF and GFR between the two groups could contribute to the findings but other factors might also be involved.

Materials and methods. Routine maintenance and surgical preparation of the conscious animals are reported elsewhere (10). Briefly, 10 conscious animals were studied. They were uninephrectomized and catheters were placed in the renal artery and vein and into the ureter. A noncannulating electromagnetic flow probe (Carolina Medical Electronics) was placed around the remaining renal artery. The animals were allowed to recover from surgery and were tested over the subsequent 1-week period.

Chloralose anesthesia (60 mg/kg) was used in another group of 10 sheep and pentobarbital anesthesia (35 mg/kg) in two sheep. The right kidney was removed through a retroperitoneal flank incision and the stump of the right renal artery was cannulated for blood pressure measurement and arterial blood sampling. The left kidney was exposed through a second retroperitoneal incision and the renal artery and vein dissected free of surrounding tissue. A noncannulating flow probe was placed around the renal artery and a 22 g siliconized needle inserted into the artery below the flow probe. An infusion of normal saline at 0.6 ml/min was commenced immediately into the renal artery. The ureter was divided and the end proximal to the kidney was cannulated for continuous urine collections. A saphenous vein was cannulated for infusion of inulin.

In both groups of sheep a priming dose of inulin (2 g) was injected followed by a continuous inulin infusion at 0.5 mg/kg/min. At least 1 hr was allowed for inulin equilibration and stabilization of renal function before experimental samples were collected.

With saline being infused into the renal artery (0.6 ml/min), timed collections of urine were begun. The clearance periods lasted 20 min and blood samples were obtained at the midpoint of each clearance period. The withdrawn blood was immediately replaced with fresh whole sheep blood. During the first 20 min clearance period, saline was infused into the renal artery. Subsequently, papaverine HCl (Lilly) in normal saline was infused at 7 mg/min for a second 20 min clearance period.

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In the anesthetized animals, the renal artery flow probe was calibrated at the end of the experiment by cannulating the renal artery distal to the probe and collecting timed volumes of blood. In the conscious animals, the probes had been calibrated *in vitro* using segments of sheep carotid arteries perfused with whole sheep blood.

Two statistical tests were applied to the data. For comparison of the two groups, the t statistic for group comparisons was used. For comparisons within a group (before and after papaverine), each animal served as its own control and a paired t statistic was used.

Results. In the anesthetized, acutely prepared sheep, RBF and GFR were lower and estimated total renal resistance was higher than in the conscious animals during the period when saline was infused into the renal artery (Table I). The most marked difference was on RBF and renal resistance which were halved and doubled respectively ($P < 0.01$). Although GFR was significantly reduced in the anesthetized group ($P < 0.05$), the magnitude of the difference (30%) was proportionately less than the RBF difference.

Despite these differences in RBF and GFR, FE_{Na} was similar in both the conscious and anesthetized animals. During the renal arterial saline infusion, FE_{Na} averaged 3.2 ± 1.3% in the conscious group and 2.7 ± 0.6% in the anesthetized animals. $U_{Na}V$ is illustrated in Figure 1 and like FE_{Na} there was no statistically significant difference between the conscious and anesthetized animals. Arterial blood pressure tended to be higher in the anesthetized than in the conscious sheep (100 ± 5 and 88 ± 5 mm Hg respectively, $0.1 > P > 0.05$).

When papaverine was administered into the renal artery at 7 mg/min, RBF increased in both groups. However, even after vasodilation, there remained a statistically significant difference between groups. As was ob-

TABLE I. RENAL FUNCTIONAL VARIABLES IN CONSCIOUS OR CHLORALOSE-ANESTHETIZED SHEEP.

	Saline[a]	Papaverine	Difference	
V[b] (ml/min)				
C[c]	2.6 ± 0.7	1.6 ± 0.4	−0.9 ± 0.6	N.S.
A	1.6 ± 0.2	3.2 ± 0.8	1.7 ± 0.8	$P < 0.05$
	N.S.	$P < 0.05$	$P < 0.01$	
$U_{Na}V$ (μEq/min)				
C	130 ± 46	73 ± 27	−49 ± 38	N.S.
A	106 ± 29	366 ± 113	260 ± 105	$P < 0.02$
	N.S.	$P < 0.05$	$P < 0.02$	
FE_{Na} (%)				
C	3.2 ± 1.3	3.1 ± 1.1	−0.04 ± 0.63	N.S.
A	2.7 ± 0.6	8.5 ± 2.2	5.8 ± 1.9	$P < 0.02$
	N.S.	$P < 0.05$	$P < 0.02$	
GFR (ml/min)				
C	37 ± 4	30 ± 5	−10 ± 2	$P < 0.01$
A	26 ± 3	27 ± 3	0.5 ± 2	N.S.
	$P < 0.05$	N.S.	$P < 0.01$	
RBF (ml/min)				
C	531 ± 53	597 ± 48	70 ± 13	$P < 0.01$
A	286 ± 34	334 ± 33	48 ± 32	N.S.
	$P < 0.01$	$P < 0.01$	N.S.	
BP (mm Hg)				
C	88 ± 5	81 ± 4	−6 ± 2	$P < 0.02$
A	100 ± 5	86 ± 5	−14 ± 5	$P < 0.02$
	N.S.	N.S.	N.S.	
RR (PRU)				
C	0.16 ± 0.01	0.13 ± 0.01	−0.03 ± 0.01	$P < 0.01$
A	0.38 ± 0.03	0.28 ± 0.03	−0.10 ± 0.04	$P < 0.02$
	$P < 0.01$	$P < 0.01$	$P < 0.01$	

[a] Saline was infused into the renal artery for 20 min (0.6 ml/min) then the infusion was changed to papaverine HCl (7 mg/min) in saline for another 20 min.

[b] V = urine flow rate, $U_{Na}V$ = absolute Na excretion, FE_{Na} = fractional Na excretion, GFR = glomerular filtration rate, RBF = renal blood flow, BP = arterial blood pressure, RR = estimated renal resistance.

[c] C = conscious (N = 10), A = anesthetized (N = 10).

served during the saline infusion, the conscious sheep demonstrated a mean RBF which was almost twice the level seen in the anesthetized animals. The decrease in renal resistance was much greater in the anesthetized animals (−0.10 ± 0.04 vs −0.03 ± 0.01 PRU, $P < 0.01$) and the difference between the two groups was similar after papaverine infusion (Table I).

The effects of the papaverine infusion on GFR also were different between the two groups of sheep. In the conscious animals, GFR decreased significantly from an average of 37 ± 4 ml/min to 30 ± 5 ml/min ($P <$ 0.01). These data are consistent with my previously published study (10). On the other hand, GFR was maintained at constant levels after papaverine-induced renal vasodilation in the anesthetized sheep. During the control saline infusion GFR was 26 ± 3 ml/min in this group and was 27 ± 3 ml/min after dilation. Mean arterial BP decreased significantly in both groups of sheep after papaverine ($P < 0.02$). This decrease was much greater, however, in the anesthetized animals where blood pressure fell an average of 14 ± 5 mm Hg while in the conscious group the decrease was 6 ± 2 mm Hg. Despite the greater fall in BP in the anesthetized sheep, there remained no significant differences between the two groups after vasodilation by papaverine.

In the conscious group, FE_{Na} was not affected by papaverine. The net change in FE_{Na} being −0.04 ± 0.63% when compared to the saline infusion control period. In the anesthetized group, FE_{Na} rose significantly to 8.5 ± 2.2% after papaverine ($P < 0.02$). This change resulted in there being a significant difference between the groups after papaverine administration ($P < 0.02$).

Figure 1 illustrates the changes which occurred in $U_{Na}V$ after papaverine administration. There was a suggestion that in the conscious animals, papaverine infusion resulted in a decrease in $U_{Na}V$, however, the change (−45 ± 38 μEq/min) was not statistically significant. In the anesthetized group $U_{Na}V$ rose threefold after renal vasodilation by papaverine reaching an average of 366 ± 113 μEq/min. This increase of 260 ± 105 μEq/min was statistically significant ($P < 0.02$). The large rise in $U_{Na}V$ in the anesthetized animals resulted in a significant difference in this variable between the two groups ($P <$ 0.02).

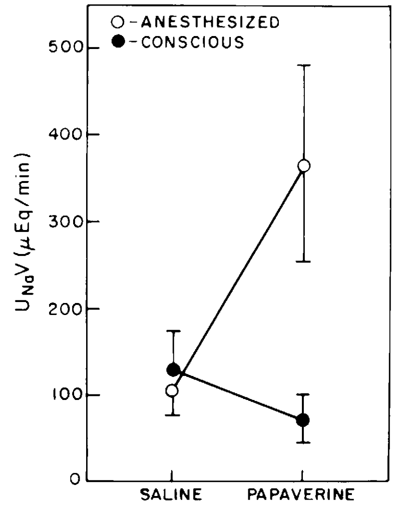

FIG. 1. Absolute Na excretion in conscious (N = 10) and chloralose-anesthetized (N = 10) sheep during renal arterial infusion of saline or papaverine at 7 mg/min.

Much of the previous work done on vasodilators and $U_{Na}V$ has utilized pentobarbital anesthesia. To be certain that we were not observing a peculiarity of chloralose, two sheep were studied during pentobarbital anesthesia. Table II presents the mean data obtained from these two sheep. Qualitatively, the changes were quite similar to those seen in the chloralose-anesthetized group. After papaverine infusion, RBF increased and renal resistance decreased similar to the chloralose-anesthetized group. GFR and arterial BP were essentially unchanged and FE_{Na} and $U_{Na}V$ both increased after papaverine.

Discussion. It is clear that anesthesia can effect changes in physiological variables (12) which may obscure a complete understanding of the mechanism of action of drugs and indeed basic physiological processes. The study was prompted by our recent observation (10) that papaverine administered into the renal artery of conscious sheep did not result in increased $U_{Na}V$ as has been repeatedly observed in anesthetized dogs (4, 11, 13). In the data reported here, only papaverine was used because this vasodilator was used previously in our studies on renin release (10, Blaine and Zimmerman, submitted for pub-

TABLE II. RENAL FUNCTIONAL VARIABLES IN SHEEP ANESTHETIZED WITH NA PENTOBARBITAL.

	Saline[a]	Papaverine
V[b] (ml/min)	0.7	2.9
$U_{Na}V$ (μEq/min)	49	338
FE_{Na} (%)	1.0	7.5
GFR (ml/min)	38	41
RBF (ml/min)	393	456
BP (mm Hg)	127	124
RR (PRU)	0.48	0.31

[a] Saline was infused into the renal artery (0.6 ml/min) for 20 min then the infusion was changed to papaverine HCl (7 mg/min) in saline for another 20 min.

[b] V = urine flow rate, $U_{Na}V$ = absolute Na excretion, FE_{Na} = fractional Na excretion, GFR = glomerular filtration rate, RBF = renal blood flow, BP = arterial blood pressure and RR = estimated renal resistance. N = 2.

lication). The control periods from the latter study provided the data for the effects of papaverine on $U_{Na}V$ in conscious sheep. These data are included in this study to confirm our previous findings (10). To define the effects of renal vasodilation in anesthetized sheep, another group was studied at the same time as the conscious animals. These animals were of similar size, age and sex. The principal difference between the two groups was that the conscious sheep were chronically prepared with indwelling cathethers and a renal artery flow probe, and the anesthetized sheep were prepared acutely on the day of the experiment. Although the experiment is not a pair-design in the sense that the same animals were studied while conscious and anesthetized, statistical comparisons between the groups are provided for clarity. The important group as far as this study is concerned is the anesthetized sheep. Within this group a pair-design analysis is possible and these data provide information as to the effects of anesthesia on $U_{Na}V$ after papaverine administration in sheep and places in perspective the data obtained from conscious animals.

When sheep were anesthetized with either chloralose or pentobarbital and papaverine was infused into the renal artery a marked natriuresis was observed. In conscious sheep, renal vasodilation did not promote increased $U_{Na}V$. Thus, the anesthetized sheep responds to renal vasodilation in a manner similar to the anesthetized dog.

It is apparent from the data of Table I that certain other differences existed between conscious and anesthetized sheep. GFR and RBF were significantly less in the anesthetized animals compared to conscious sheep, and BP tended to be elevated in the anesthetized group. This can be attributed to several factors: recent surgical intervention and the direct effects of anesthesia (12, 14) in the anesthetized group and compensatory renal hypertrophy in the conscious group. These data would suggest augmented sympathetic tone to the kidney during anesthesia and this could contribute to the differing effects of papaverine in conscious and anesthetized sheep. The greater RBF in the conscious animals is especially noteworthy, because the same level of papaverine infusion was used in both groups. Thus, the effective concentration of papaverine was higher in the anesthetized than in the conscious animals. Despite this disparity, renal vasodilation occurred in both groups but $U_{Na}V$ increased in only the anesthetized animals. We showed previously (10) that increasing the papaverine infusion to 10 mg/min in conscious sheep produced no further increase in RBF nor did it promote increased $U_{Na}V$. In that study, the effective concentration of papaverine in the RBF was 23 μg/ml and in the present group of anesthetized sheep the effective concentration was 21 μg/ml. Thus, a similar effective concentration of papaverine to that used in this study did not promote increased $U_{Na}V$ in our earlier study.

Changes in renal perfusion pressure might also affect $U_{Na}V$. Arterial blood pressure fell in both groups after papaverine but this effect was most marked in the anesthetized sheep and they clearly demonstrated an increase in $U_{Na}V$.

The significant decrease in GFR after papaverine infusion observed in the conscious animals would contribute to their failure to increase $U_{Na}V$. It would seem unlikely that this is the entire explanation, however, because FE_{Na} failed to change after vasodilation. Also, in four of the anesthetized sheep GFR fell but the animals still exhibited a marked natriuresis.

Marchand *et al.* (15) recently reported that secretin-induced renal vasodilation was not associated with augmented $U_{Na}V$ and those data taken together with the present findings would suggest that vasodilation *per se* is not a sufficient condition for increasing $U_{Na}V$.

Summary. Ten conscious and ten chloralose-anesthetized sheep were studied while either 0.9% NaCl solution or 7 mg/min papaverine HCl solution was infused into the renal artery of a sole remaining kidney. During saline infusion, conscious sheep had significantly higher renal blood (RBF) and glomerular filtration rate (GFR) than anesthetized animals ($P < 0.01$ and < 0.05 respectively). Renal resistance (RR) was significantly greater in the anesthetized animals ($P < 0.01$) but arterial blood pressure (BP), absolute ($U_{Na}V$) and fractional Na excretion (FE_{Na}) were not different between the two groups. Infusion of 7 mg/min papaverine for 20 min resulted in an increase in RBF in both groups but this was statistically significant ($P < 0.01$) only in the conscious sheep. BP declined significantly in both groups ($P < 0.02$) but GFR fell only in the conscious animals ($P < 0.01$). Papaverine had very little effect on FE_{Na} and $U_{Na}V$ of conscious sheep but produced dramatic rises in both variables when the animals were anesthetized ($P < 0.02$).

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