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The Relationship between Sodium Excretion and Renin Secretion by the Perfused Kidney[1] (39115)

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(Introduced by W. F. Ganong)

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It is now well established that the control of renin secretion is complex and involves the interaction of a variety of factors including renal perfusion pressure, renal sodium transport, and renal sympathetic neural activity. Renin secretion is also altered by circulating factors including angiotensin II and vasopressin. The exact relationship between renal sodium transport and renin secretion is not well understood, but it has been suggested that the rate of renin secretion varies inversely with the rate of delivery of sodium to the macula densa (1). This hypothesis has been difficult to test directly, since maneuvers designed to alter the delivery of sodium to the macula densa are frequently associated with changes in renal perfusion pressure or renal neural activity, as well. The present study was designed to investigate the effect on renin secretion of altering tubular sodium reabsorption in a denervated kidney preparation that was perfused at constant pressure with blood from a donor animal.

Methods. The experiments utilized 34 male mongrel dogs weighing 19–35 kg. The dogs were anesthetized with pentobarbital sodium (30 mg/kg) and were ventilated via a cuffed endotracheal tube attached to a Harvard respirator. In 20 experiments, urine was collected from one of the dog's *in situ* kidneys through a ureteral catheter, and a kidney from a second dog was used for perfusion. In the remaining 14 experiments, catheters were placed in the right renal vein and right ureter (*in situ* kidney), and the left kidney of the same animal was removed for perfusion. Kidneys to be perfused were freed from surrounding fascia by blunt dissection, and the renal vessels and ureter were isolated and clamped. The kidney was removed and the renal artery attached via a catheter to tubing leading from the dog's femoral artery through an occlusive roller pump. The mean time of ischemia using this technique was 3.1 min (range, 1.5 to 4.8 min). A second catheter was placed in the renal vein, directing blood to one of a pair of calibrated lucite cylinders that automatically filled and discharged alternately. This arrangement permitted the continuous, direct measurement of renal blood flow. From these cylinders, the blood flowed by gravity to a lucite mixing reservoir, and from there it was returned to the dog's femoral vein by means of a second roller pump (Fig. 1). Initially, 500 to 1,000 ml of frozen, thawed canine plasma (19 experiments), or a mixture of plasma and fresh whole blood (15 experiments) was placed in the reservoir and allowed to equilibrate with the dog's own blood prior to perfusion of the kidney. All animals received 2,000 to 4,000 units of sodium heparin intravenously prior to this equilibration procedure. In addition, 12 dogs received intravenous injections of diphenhydramine, 50 mg, prior to the equilibration. All tubing in the circuit was silastic, and lucite surfaces were siliconized. In the kidney perfused in this fashion, GFR was 0.45 ± 0.02 (SE) ml/min/g kidney wt, blood flow was 3.83 ± 0.20 ml/min/g, and fractional reabsorption of sodium was 99.5 ± 0.1%. These values represent the means of initial measurements in all 34 experiments, when perfusion pressure averaged 102 ± 2 mm Hg.

Arterial pressure was measured in the

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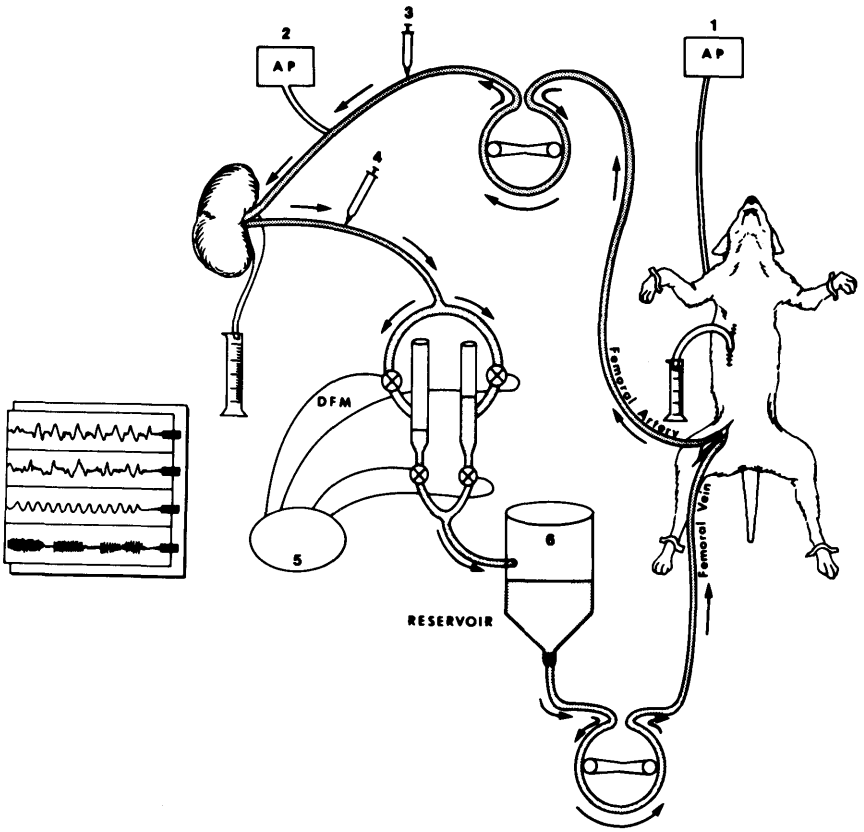

FIG. 1. Schematic representation of the perfusion circuit. A kidney was perfused by a roller pump with blood taken from the dog's femoral artery. Arterial pressure (AP) was measured in the dog's brachial artery (1) and just proximal to the artery of the perfused kidney (2). Arterial and renal venous blood samples were collected at sites (3) and (4). Renal venous effluent was alternately delivered into cylinders of a direct measuring flowmeter (DFM) (5). From the flowmeter, blood was delivered to a mixing reservoir (6) from which it was pumped by another roller pump into the dog's femoral vein. Arterial pressures and cycles of the flowmeter were recorded continuously. The dog's intravascular volume was expanded or contracted by adjusting the second pump either to lower or raise the level of blood in the mixing reservoir. Hemodilution or elevation of plasma protein concentration was accomplished by the addition of Ringer's solution to the reservoir or by administration of concentrated albumin.

dog via a catheter placed in the brachial artery, and in the perfused kidney circuit by a sidearm in the arterial line just proximal to the renal artery. All animals received an infusion of isotonic saline solution at 0.5 ml/min, which contained sufficient inulin and para-aminohippurate (PAH) to achieve plasma concentrations of approximately 25 and 1 mg%, respectively. This infusion also delivered aqueous Pitressin at a rate of 50 mU/hr. All animals received intramuscular injections of desoxycorticosterone acetate (5 mg) and pitressin tannate in oil (2 units) during surgical preparation, and in addition 16 animals were given DOCA injections twice daily for 48–72 hr prior to the experiment.

Arterial pressure of the perfused kidney was held constant by adjusting the roller pump. Contraction or expansion of the dog's blood volume was achieved by altering the rate of return of blood to the dog from the mixing reservoir; in addition, the composition of the blood was altered without changing the animal's extracellular fluid volume by adding Ringer's or albumin solutions to the reservoir. After an equilibration period of 30–60 min, experimental

maneuvers were carried out as described below, the same kidney being studied during several maneuvers.

Effects of volume expansion. After control clearance and renin collections were made, the dog's vascular volume was expanded by increasing the rate at which blood was returned to the dog from the mixing reservoir; the amount of blood administered in this fashion ranged from 20 to 70 ml/kg body wt (mean: 38 ml/kg) and was given over a 15- to 30-min period. After the loading procedure was completed, measurements were repeated.

Effects of graded hemorrhage. The effects of hemorrhage were studied in 20 experiments. The hemorrhage ranged from 12 to 34 ml/kg body wt (mean: 22 ml/kg) and was sufficient to reduce blood pressure markedly in the donor animal. After hemorrhage, additional clearance and renin collections were made.

Effects of hemodilution without volume expansion. In 35 experiments, 2 liters of Ringer's solution were added to the reservoir to alter the composition of the blood without changing the total extracellular fluid volume of the dog. Urine collections were resumed 20–40 min after the addition of the Ringer's solution.

Effects of hyperoncotic albumin. In nine experiments, bovine serum albumin in isotonic saline (30g/100 ml) was administered to the dogs in which hemodilution had lowered plasma protein concentration; the amount of albumin given was calculated to restore the plasma protein concentration to control levels. In five of these experiments, the albumin was added directly to the mixing reservoir and allowed to equilibrate with the animal's blood. In the remaining four experiments, it was infused intravenously at 5 to 10 ml/min. Urine collections were continued throughout the infusion and usually for 30 min following its cessation.

The order in which these maneuvers were carried out was varied from one experiment to the next; the measurements made during one maneuver became the control measurements for the next maneuver. Not every maneuver was studied in each experiment, and some maneuvers were studied more than once in a given experiment. For statistical purposes, each observation on the effects of a maneuver was treated separately, so that the large number of observations reflects the fact that in some experiments a given maneuver was repeated in the same animal.

In all experiments, two to five clearance periods of 5- to 15-min duration were collected before and during each maneuver; blood samples were drawn at the midpoints of alternate periods. Plasma and urine samples were analyzed for inulin, PAH, sodium, potassium, and osmolality by methods previously reported (2). Renal plasma flow (RPF) in the perfused kidney was calculated from the hematocrit (Hct) and directly measured blood flow (RBF):

$$RPF = RBF\ (1 - Hct).$$

For the dog's *in situ* kidney, RPF was determined from the clearance and extraction of PAH.

Plasma renin activity (PRA) was measured using a radioimmunoassay for angiotensin I (A–I) (3, 4). PRA was expressed as ng A–I generated per ml of plasma during a 3-hr incubation. Renin secretion rate (RSR) was determined from arterial and renal venous PRA and the RPF ($RSR = (V-A)_{PRA} \cdot RPF$) and expressed as ng A–I per min. Statistical significance was assessed using the paired t test; all results are expressed as the mean ± SEM.

Results. Effects of volume expansion with equilibrated whole blood on renal hemodynamics, $U_{Na}V$, and RSR were studied a total of 50 times in 28 perfusions. Volume expansion was the first experimental maneuver performed in 20 studies, whereas in the remaining 30, it took place after the effects of hemodilution had been studied. Since the results were similar in both cases, the two groups were analyzed together (Table I). Volume expansion increased AP from 85 ± 3 to 125 ± 4 mm Hg. In the *in situ* kidney there were large increases in GFR, RBF, $U_{Na}V$ and U_KV; all these changes were highly significant ($P < 0.001$) (Table I). Arterial PRA fell from 49.4 ± 4.8 to 38.5 ± 3.1 ng/ml/3 hr as a result of the loading

TABLE I. EFFECTS OF INTRAVASCULAR VOLUME EXPANSION, HEMORRHAGE, HEMODILUTION, AND HYPERONCOTIC ALBUMIN ADMINISTRATION ON RENAL HEMODYNAMICS, ELECTROLYTE, EXCRETION, AND RENIN SECRETORY RATE IN PERFUSED AND IN SITU KIDNEYS[a]

	GFR (ml/min)		RBF (ml/min)		$U_{Na}V$ (μEq/min)		U_KV (μEq/min)		RSR[b] (ng A-I/min)		AP (mm Hg)		Plasma protein (g%)		Arterial hematocrit	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Intravascular volume expansion																
Perfused kidney Mean	31.0	30.3	248	242	39.1	36.4	33.8	30.1	1381	1236	106	107			30.9	33.5
SEM	±1.2	±1.2	±11	±11	±4.9	±4.9	±2.4	±2.4	±335	±213	±2	±2			±1.0	±1.1
(n = 50) P	NS		NS		NS		<0.005		NS		NS				<0.001	
In situ kidney	27.7	39.5	178	301	25.5	107	21.4	50.2	2975	386	85	125				
	±2.1	±1.8	±25	±25	±6.5	±18	±2.1	±5.1	±586	±366	±3	±4				
(n = 42)	<0.001		<0.001		<0.001		<0.001		<0.005		<0.001					
Hemorrhage																
Perfused kidney	30.1	27.5	225	190	61.4	58.3	35.7	34.4	1358	1064	103	104			28.6	29.4
	±2.0	±2.0	±12	±13	±13.7	±15.2	±4.3	±4.3	±444	±229	±3	±3			±1.8	±1.9
(n = 20)	<0.05		<0.001		NS		NS		NS		NS				<0.02	
In situ kidney	40.4	17.6	304	107	148	12.2	58.5	18.3	1398	1991	128	83				
	±2.6	±2.8	±37	±23	±28	±2.5	±10.4	±3.7	±782	±923	±5	±4				
(n = 18)	<0.001		<0.001		<0.001		<0.001		NS		<0.001					
Hemodilution																
Perfused kidney	29.4	29.7	258	225	15.1	51.3	21.7	37.8	2294	630	103	103	6.07	3.82	37.7	26.7
	±1.3	±1.4	±13	±14	±3.4	±6.5	±1.6	±3.3	±478	±249	±3	±3	±0.18	±0.13	±0.9	±0.8
(n = 35)	NS		<0.001		<0.001		<0.001		<0.001		NS		<0.001		<0.001	
In situ kidney	39.8	23.0	264	136	66.2	32.0	38.4	22.6	890	2414	122	76				
	±2.3	±2.1	±28	±26	±15.7	±8.8	±3.5	±2.4	±489	±545	±4	±4				
(n = 29)	<0.001		<0.001		<0.01		<0.001		<0.05		<0.001					
Hyperoncotic albumin																
Perfused kidney	30.4	28.3	187	247	60.6	35.1	50.4	40.2	1095	1995	103	104	4.02	6.45	22.9	19.7
	±2.9	±2.9	±28	±22	±11.9	±11.2	±7.6	±5.2	±308	±885	±4	±5	±0.50	±0.32	±0.8	±0.5
(n = 9)	NS		<0.01		<0.001		NS		NS		NS		<0.001		<0.02	

[a] Abbreviations: GFR = glomerular filtration rate, RBF = renal blood flow, $U_{Na}V$ = rate of excretion of sodium, U_KV = rate of excretion of potassium, RSR = renin secretory rate, and AP = arterial blood pressure. C = control, E = experimental periods.

[b] RSR was measured in 22 *in situ* kidneys following volume expansion, 11 following hemorrhage, and 18 following hemodilution.

procedure ($P < 0.01$). RSR was measured in 22 of these *in situ* kidneys and fell significantly. In contrast, volume expansion had little effect on the perfused kidney. GFR, RBF, $U_{Na}V$, and RSR were unchanged, but there was a slight decrease in U_KV.

Effects of hemorrhage. Graded hemorrhage of 12 to 34 ml/kg was performed in 20 experiments (Table I). Hemorrhage markedly decreased GFR, RBF, $U_{Na}V$, and U_KV in the dog's *in situ* kidney, as AP fell from 128 ± 5 to 83 ± 4 mm Hg. Venous–arterial PRA difference increased in all of the *in situ* kidneys in which it was measured, the mean increase being from 7.4 ± 4.1 to 27.7 ± 11.5 ng/ml/3 hr ($P < 0.02$). However, because of the marked fall in RPF calculated from the clearance and extraction of PAH, mean RSR increased only from 1398 ± 782 to 1991 ± 923 ng A–I/min (Table I). Arterial PRA increased slightly from 43.3 ± 11.0 to 47.9 ± 10.3 ng A–I/ml/3-hr ($P < 0.10$).

Hemorrhage of the dog had only minor effects on the perfused kidney. GFR fell slightly, and RBF decreased from 225 ± 12 to 190 ± 13 ml/min ($P < 0.001$); since perfusion pressure was maintained constant, this fall in RBF reflected a proportionate rise in renal vascular resistance. Despite these changes in renal hemodynamics, $U_{Na}V$ and U_KV in the perfused kidney were not decreased following hemorrhage (Table I). Also, RSR by the perfused kidney did not change when the dog was hemorrhaged; the mean value was 1358 ± 444 before and 1064 ± 229 ng A–I/min after hemorrhage.

Effects of hemodilution without volume expansion. A total of 35 observations in 31 perfusions was made on the effects of hemodilution with Ringer's solution (Table I). Hemodilution produced a marked fall in the dog's AP, presumably because of decreased intravascular volume resulting from the fall in plasma protein concentration from 6.07 ± 0.18 to 3.82 ± 0.13 g%. Associated with this marked fall in blood pressure were significant decreases in GFR, RBF, $U_{Na}V$, and U_KV in the dog's *in situ* kidney. RSR was measured in 18 of these kidneys, and it increased from 890 ± 489 to 2414 ± 545 ng A–I/min ($P < 0.05$).

Hemodilution had entirely different effects on the perfused kidney. Although GFR did not change, there was a significant fall in RBF. However, $U_{Na}V$ and U_KV increased in every case; these changes in electrolyte excretion were associated with a striking decrease in RSR from 2294 ± 478 to 630 ± 249 ng A–I/min ($P < 0.001$) and a reduction in arterial PRA from 47.5 ± 3.3 to 36.0 ± 3.6 ng A–I/ml/3 hr ($P < 0.005$).

Effects of hyperoncotic albumin administration. Bovine serum albumin was infused intravenously (four experiments) or added to the mixing reservoir (five experiments) after hemodilution had been carried out. Albumin administration increased plasma total protein concentration from 4.02 ± 0.50 to 6.45 ± 0.32 gm/100 ml. Urine flow in *in situ* kidneys, which was already depressed by the hypotension accompanying hemodilution, usually ceased altogether in the experiments in which albumin was added to the mixing reservoir following hemodilution, so that clearance measurements could not be made. Albumin infusion in volume-expanded animals resulted in renal vasodilatation accompanied by transient decreases in urine flow and $U_{Na}V$. Changes in RSR tended to reflect these transitory changes in electrolyte excretion. Because of the variable time course of these events and the lack of urine collection in several experiments, data from these *in situ* kidneys are not included in Table I.

Hyperoncotic albumin administration produced more consistent results in the perfused kidney. Since the results were similar whether albumin was added to the mixing reservoir or infused intravenously, mean data for all nine studies are grouped together (Table I). GFR was unchanged; RBF increased and renal vascular resistance decreased in all but one of the perfused kidneys following albumin administration. $U_{Na}V$ decreased in every experiment, but U_KV was unchanged. RSR increased in six of the nine kidneys, but the overall increase for the group from 1095 ± 308 to 1995 ± 885 ng A–I/min was not statistically significant.

Figure 2 shows the relationship between the changes in cation excretion and RSR in the perfused kidney following hemodilution or hyperoncotic albumin administration.

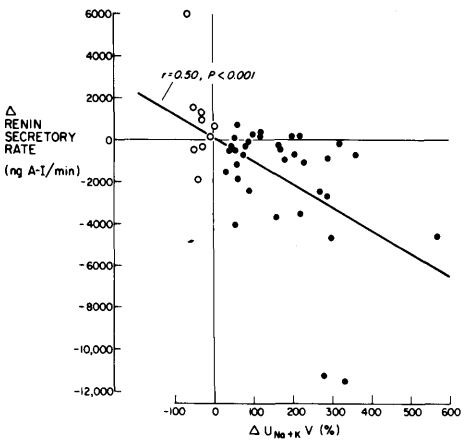

FIG. 2. Overall relationship between changes in cation excretion and renin secretion in perfused kidneys. Each symbol is the mean of multiple consecutive measurements in a single animal. Solid symbols represent measurements following hemodilution (addition of Ringer's solution to the reservoir) and open symbols are measurements after the addition of concentrated albumin in the presence of hemodilution. Overall there was a significant inverse relationship between changes in the secretion of renin and changes in cation excretion.

Overall, there is an inverse relationship between cation excretion and renin release.

Discussion. The purpose of these experiments was to test the hypothesis that changes in the delivery of sodium to the macula densa result in alterations in renin secretion. To vary the delivery of sodium to the macula densa, compositional changes were made in the blood perfusing an isolated kidney preparation by diluting the blood with isotonic Ringer's solution. Clearance and micropuncture studies indicate that this maneuver decreases sodium reabsorption in the proximal tubule (5–7); hemodilution thus provides a means of increasing the delivery of sodium to the macula densa if GFR remains constant, as in the present studies. Furthermore, this increased delivery out of the proximal tubule should be reflected in enhanced sodium excretion if distal reabsorption is not increased to an equivalent degree.

The present results clearly demonstrated that the increase in sodium excretion in the perfused kidney caused by hemodilution was associated with a striking fall in renin secretion. Since the perfused kidney model permitted rigid control of renal perfusion pressure, and was obviously free of influences of the renal nerves, these results can best be interpreted within the context of the macula densa hypothesis (1). According to this formulation, the decreased proximal tubular sodium reabsorption resulting from hemodilution caused an increase in the sodium load to the macula densa, which resulted in the observed decrease in renin secretion.

To test this hypothesis further, a small number of experiments was performed in which plasma protein concentration was returned to control levels by the addition of a concentrated albumin solution. This maneuver produced a significant decrease in sodium excretion in the perfused kidney; renin secretion increased in two-thirds of the kidneys, but the mean change was not statistically significant. Nevertheless, there was a readily demonstrable relationship between electrolyte excretion and renin secretion by the perfused kidney following hemodilution or albumin administration (Fig. 2). Taken together, therefore, the data provide strong support for the macula densa hypothesis.

In general, volume expansion and hemorrhage produced the expected changes in the donor animal, but did not affect the perfused kidney. Thus, volume expansion increased arterial pressure, GFR, and sodium excretion and suppressed renin secretion. Hemorrhage, on the other hand, resulted in hypotension, decreased GFR and sodium excretion, but did not significantly increase renin secretion. The failure to observe a significant increase in renin secretion following hemorrhage may be attributable to the low rates of urine flow in these experiments, leading to an underestimation of renal plasma flow and, hence, of the calculated renin secretion rate. In this context, it should be noted that the venous–arterial difference in plasma renin activity across the *in situ* kidneys always increased following hemorrhage.

No attempt was made in the present study to determine the mechanism by which volume expansion suppressed renin secretion in the *in situ* kidneys. It is possible that the effect was mediated via the macula densa mechanism as outlined above, since there

were reciprocal changes in sodium excretion. However, it is probable that the renal baroreceptor mechanism and the renal sympathetic nerves also played a role. In any case, it is clear that circulating factors were not responsible since, as mentioned above, renin secretion by the perfused kidneys was not affected by volume expansion. In addition, no evidence was found for the existence of a circulating factor, as reported by others (8), which increases sodium excretion in response to volume expansion, since this maneuver, which caused a marked natriuresis in the *in situ* kidney, did not alter sodium excretion in the perfused kidney.

Summary. The effect of altered tubular sodium reabsorption on renin secretion (RSR) was examined under conditions in which other factors influencing renin release could be controlled or excluded. To do this, isolated canine kidneys were perfused at constant pressure with blood circulating from donor animals. Volume expansion or hemorrhage of the donor dogs produced large changes in the animal's blood pressure, renal function, sodium excretion ($U_{Na}V$), and RSR, but were without effect on renal hemodynamics, $U_{Na}V$, or RSR in the perfused kidney. Hemodilution without volume expansion, resulted in hypotension, decreased $U_{Na}V$ and increased RSR in the donor dogs, and increased $U_{Na}V$ and suppressed RSR in the perfused kidney. These effects of hemodilution in the perfused kidney were partially reversed when plasma protein concentration was restored to control levels with hyperoncotic albumin, and, overall, there was a significant inverse relationship between electrolyte excretion and RSR. These results provide new evidence for the hypothesis that the rate at which sodium is delivered to the macula densa is an important determinant of the rate of renin secretion.

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