Response of the Isolated Kidney to Saline Infusion[1] (36684)

GERALD F. DIBONA,[2] GEORGE J. KALOYANIDES,[2] AND ROBERT D. BASTRON

Renal-Hypertension-Electrolyte Division, Department of Internal Medicine and Department of Anesthesiology, University of Iowa College of Medicine and Veterans Administration Hospital, Iowa City, Iowa 52240

The natriuresis of volume expansion with isotonic saline is known to be independent of changes in glomerular filtration rate and circulating mineralocorticoid levels (1); micropuncture studies have shown a decrease in proximal tubular sodium reabsorption (2).

We have recently reported studies (3, 4) involving an isolated perfused dog kidney which demonstrate the suitability of this preparation for examining the mechanisms of pressure and volume expansion natriuresis. In the present experiments we applied micropuncture techniques to the study of saline natriuresis to establish the validity of these techniques as applied to the isolated perfused dog kidney.

Methods. Experiments were performed on mongrel dogs weighing 15–30 kg. One dog served as the kidney donor; the second dog was used to perfuse the isolated kidney. The donor and perfusion animals were fed a standard kennel ration; the perfusion animal received 10 mg deoxycorticosterone acetate (DOCA) in oil given intramuscularly on the morning of the study day. The animals were anesthetized with either sodium pentobarbital or sodium pentothal (30 mg/kg) given intravenously with supplemental doses as required. An endotracheal tube was inserted and respirations were regulated with a Harvard respirator adjusted to maintain arterial pH between 7.35 and 7.45.

The preparation of the isolated kidney was similar to that previously described (3, 4).

In all experiments the reservoir was filled with 400 ml of 0.9% NaCl. After perfusion of the isolated kidney was established, the perfusion animal received a priming dose of inulin followed by a constant infusion of inulin in 0.9% NaCl at 1.0 ml/min; aqueous pitressin was added to the infusion to deliver 0.5 mU/kg/min. A minimum of 60 min was allowed for equilibration and stabilization.

Group I, consisting of six experiments, served as a control group for monitoring function of the isolated kidney with respect to time. Renal arterial pressure (P_{RA}) was maintained constant at about 105 mm Hg throughout the experiment.

Group II consisted of nine experiments. After kidney function had stabilized, either a single 30 min or two consecutive 15 min control urine samples with midpoint arterial blood samples were collected after which the perfusion animal was infused with 0.9% NaCl at 2 ml/kg/min for 30 min followed by 0.9% NaCl at 1 ml/kg/min for the remainder of the experiment. The experimental period, either one 30 min or two consecutive 15 min urine samples, was begun 60 min after the initiation of volume expansion. Renal arterial pressure was maintained constant at about 105 mm Hg throughout the experiment. During the control and experimental urine collection periods of each group, single nephron glomerular filtration rate (SNGFR) and fractional reabsorption were measured in proximal tubules of superficial cortical nephrons. After decapsulation of a 2 cm^2 area of cortex, end proximal tubular segments were identified by the injection of 0.2–0.3 ml of a 5% aqueous solution of lissamine green into the arterial cannula of the isolated kidney. Segments so identified were punctured

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with sharpened glass capillary micropipettes of 8–12 μ tip size. A small droplet of Sudan black stained mineral oil was injected to ascertain direction of flow and to verify location in the last surface segment. A column of oil, four to six tubular diameters in length, was injected distal to the micropipette tip. An accurately timed collection (30 to 60 sec) of tubular fluid was made by gentle hand aspiration to keep the distal oil block stationary and the tubular diameter relatively constant. At the end of the collection the micropipette tip was sealed with oil to prevent sample evaporation. To avoid the possible problem of artifactually elevated SNGFR during recollection micropuncture in the dog (5), different end proximal tubular segments were punctured in control and experimental periods. Approximately five to six micropuncture samples were obtained in each period and averaged to give a single value for that period for each dog.

Blood pressure monitoring and recording, urine collection and blood sampling were carried out as previously described (3).

All blood and urine samples were analyzed for sodium and inulin; packed cell volume (PCV) and plasma protein concentration were determined on all blood samples. Sodium was measured with an Instrumentation Laboratories flame photometer. Inulin was measured by the method of Führ, Kaczmarczyk and Krüttgen (6) and plasma protein concentration by refractometry (Goldberg refractometer, American Optical Co., Buffalo, NY). PCV was determined using a microhematocrit centrifuge. Renal blood flow (RBF) was measured directly by timing the flow from the renal vein into a graduated cylinder. Filtration fraction (FF) was determined from the formula FF = C_{in}/RPF where C_{in} is the inulin clearance and RPF is the renal plasma flow calculated according to the formula RPF = RBF $\times$ (1–0.95 PCV). Tubular fluid sample volume was measured with a calibrated micropipette; tubular fluid inulin concentration was measured in duplicate by means of the fluorometric method (7). Single nephron glomerular filtration rate (SNGFR) was calculated according to the formula SNGFR = $V \times (TF/P)_{in}$ where V is the tubular fluid flow rate (nl/min) and $(TF/P)_{in}$ is the tubular fluid to plasma inulin concentration ratio. Proximal fractional reabsorption equals $1 - (P/TF)_{in} \times 100\%$. Proximal absolute reabsorption equals (SNGFR $- V$).

The data in the text and tables are expressed as the mean $\pm$ SE. Student's t test for paired data was used for statistical analysis (8).

Results. In group II, following 0.9% NaCl infusion, there was a fourfold rise in sodium excretion that was not accompanied by significant changes in C_{in}, RPF, FF or P_{RA}. Fractional sodium excretion increased from 0.8 $\pm$ 0.3 to 3.3 $\pm$ 0.9% ($p < .05$). As expected, PCV and plasma protein concentration fell. End proximal fractional reabsorption decreased from 51 $\pm$ 3 to 39 $\pm$ 3% ($p < 0.001$). Since SNGFR did not change absolute reabsorption in the proximal tubule also decreased. The ratio of SNGFR to C_{in}, an indicator of redistribution of glomerular filtrate, showed a slight increase which was not significant.

In group I, the function of the isolated kidney was examined over the same time interval as group II. As shown in Table I, aside from the time related fall in RPF and concomitant rise in FF no other significant changes were observed in any of the other variables measured.

Discussion. These studies show that expansion of the perfusion animal with 0.9% NaCl results in a natriuresis in the isolated kidney which is not associated with an alteration in GFR, RPF, FF or P_{RA}. Proximal tubular fractional and absolute reabsorption decreased significantly and resulted in an increase in distal sodium delivery, findings which are similar to those found previously by others in the intact dog and rat kidney (5, 9). In group II, the change in SNGFR/C_{in} from 3.33 to 3.83 just misses ($p > .05$) statistical significance. Since fresh tubules were punctured in the experimental period (*i.e.*, nonrecollection technique), the possible problem of recollection artifact (5) was avoided. These data do not firmly establish the presence of cortical redistribution of filtrate in volume expansion natriuresis but

TABLE I. Summary of Data from the Isolated Kidney in Groups I and II During Control (C) and Experimental (E) Periods.

Group		$U_{Na}V$ (μEq/min)	C_{in} (ml/min)	RPF (ml/min)	FF	PCV (%)	Plasma protein (g/100 ml)	P_{RA} (mm Hg)	$(TF/P)_{in}$	SNGFR (nl/min)	$(SNGFR/C_{in})$ $\times 10^{-6}$
I ($N = 6$)	C	31.5	35.5	110	0.32	36	4.6	106	1.93	106	3.03
		±12.5	±3.4	±8	±0.01	±3	±0.2	±1	±0.09	±12	±0.30
	E	27.2	34.3	90[a]	0.35[b]	35	4.4	104	1.91	100	3.03
		±12.4	±3.8	±4	±0.02	±4	±0.3	±2	±0.09	±12	±0.39
II ($N = 9$)	C	35.8	32.6	102	0.33	39	4.8	107	2.06	105	3.33
		±12.6	±3.2	±9	±0.03	±1	±0.1	±2	±0.10	±11	±0.31
	E	137.5[b]	30.7	89	0.34	27[c]	3.2[c]	106	1.64[c]	110	3.83
		±30.7	±4.0	±6	±0.03	±2	±0.2	±1	±0.07	±8	±0.35

[a] $p < .02$.
[b] $p < .01$.
[c] $p < .001$.

suggest that, if it occurs, it is of small magnitude.

The experimental design excludes such variables as the renal nerves, renal perfusion pressure and circulating mineralocorticoid or antidiuretic hormone levels from playing a role in the natriuretic response.

Although previous investigators have reported that decreasing hematocrit may depress proximal fractional sodium reabsorption, it is unlikely that the changes in proximal fractional sodium reabsorption observed in the present study can be adequately explained by this mechanism. Knox *et al.* (10) reported that proximal fractional sodium reabsorption decreased from 34 to 28% after a reduction in hematocrit from 49 to 36%. Burke, Robinson and Clapp (11) reported that proximal fractional sodium reabsorption did not change after a reduction in hematocrit from 41 to 31%. In the present experiments, proximal fractional sodium reabsorption decreased from 51 to 39% after a reduction in hematocrit from 39 to 27%; the observed reduction in proximal fractional sodium reabsorption would appear to be greater than that which could be explained solely by a reduction in hematocrit.

The fall in systemic arterial plasma protein concentration could account for the decrease in proximal sodium reabsorption via alterations in peritubular capillary oncotic pressure (12–15). In the saline-loaded rat, systemic arterial plasma protein concentration fell from 6.8 to 5.0 g/100 ml in association with a 30% fall in proximal fractional sodium reabsorption; directly measured efferent arteriolar plasma protein concentration fell from 9.5 to 7.0 g/100 ml (14). In these studies, systemic arterial plasma protein concentration fell from 4.8 to 3.2 g/100 ml in association with a 20% fall in proximal fractional sodium reabsorption. Whole kidney filtration fraction did not change. Since there was no evidence of intrarenal redistribution of filtrate, this suggests that cortical nephron filtration fraction was also unchanged. In these studies, therefore, one can calculate that efferent arteriolar protein concentration fell from 7.2 to 4.9 g/100 ml. This reduction is less than that observed in the rat studies

and could account for the quantitatively smaller decrease in proximal fractional sodium reabsorption. Finally, these results do not exclude the participation of other variables, humoral or otherwise (16), in the natriuretic response to saline loading.

Summary. The isolated perfused dog kidney responds to saline infusion with a decrease in fractional and absolute sodium reabsorption in the proximal tubule. These results appear to be best explained by a reduction in plasma protein concentration.

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