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Sodium Metabolism and Intrarenal Distribution of Nephron Glomerular Filtration Rates in the Unanesthetized Rat[1] (38075)

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Previous work from this laboratory (1) suggested that the intrarenal distribution of single nephron glomerular filtration rates (SNGFR), measured as the ratio of SNGFR in superficial (S) to that in juxtamedullary (JM) nephrons, is not affected by the level of dietary sodium intake. One complicating factor, however, was the observation that, particularly in rats on high sodium intake, the sodium excretion rate during the experiment was less than half that predicted on the basis of the measured daily sodium intake. Although the sodium excretion rate in these rats was still significantly higher than that of their low-sodium littermates, the data suggested that some factor(s) was operating during the experiment to reduce sodium excretion and possibly blunt physiological changes in intrarenal SNGFR distribution. Since it has been observed that human subjects responded to anesthesia with a reduction in urinary sodium output (2), the present study was undertaken to reexamine the relationship between the intrarenal SNGFR distribution and the level of dietary sodium intake in unanesthetized rats.

Methods. The study was performed on 20 male Sherman rats, in groups of 4 littermates. All animals were fed a sodium deficient diet (General Biochemicals, Chagrin Falls, Ohio) during 3–4 wk. Two littermates were given 0.1%, and the other 1.0% sodium chloride as drinking fluid, for low- and high-sodium intakes, respectively. During this period they were trained to accept the restraining device to be used during the experiment. All quickly learned to lie quietly for the entire 3-to-4-hr training period. During the last week the four littermates were placed in metabolic cages. Fluid (i.e., sodium) intake and urinary output were measured during 3 consecutive days, beginning 2 days after entering the cages.

Successful experiments were completed on 14 of these rats. They were fasted from 4 PM the preceding day, but fluid was allowed *ad lib.* The study began at 10 AM. Under ether anesthesia catheters were placed in both femoral arteries and veins. The bladder was catheterized through a small midline incision. A loose ligature was placed around the left renal pedicle, exposed through a flank incision which was subsequently closed with tape. All other incisions were closed with stitches. Each rat was then placed in a restrainer, where it woke up almost immediately and lay quietly, without evidence of discomfort. To replace surgical fluid losses, 0.9% saline was given in amounts not exceeding 1% of body weight. An intravenous infusion of inulin-methoxy-^{3}H (Intern. Chem. and Nucl. Corp., Irvine, Calif.) in 0.9% saline (10–20 μCi/ml) was started at 0.020 ml/min following a 0.3 ml priming dose. Sixty minutes were allowed for recovery from anesthesia and surgery, and for inulin equilibration. Thereafter, four to six timed urine samples and three evenly spaced arterial blood samples were collected, followed immediately by a rapid intravenous infusion of ^{14}C-ferrocyanide given as previously described (1). Arterial pressure was moni-

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tored by means of a mercury manometer connected to the contralateral femoral artery. The left kidney pedicle was tied exactly at 12 sec. Beginning at the eleventh second, 1 ml of a concentrated sodium pentobarbital solution (60 mg/ml) was rapidly injected into the contralateral femoral vein. The animal died within a few seconds and was pulled out of the restrainer. The flank wound was quickly reopened by pulling the tape and acetone–dry ice was poured over the kidney at about the thirtieth second. Subsequent steps leading to SNGFR measurement and other pertinent techniques have been described elsewhere (1, 3). As usual, 12 nephrons were sampled from several areas at three cortical depths: superficial, intermediate and juxtamedullary, according to previously described criteria (1, 3), except that in this study only JM nephrons in which vasa recta could be clearly identified were sampled. Mean ± standard error (SE) and Student's *t* test used throughout.

Results. Sodium metabolism. Metabolic cage measurements of daily sodium intake and urinary output were made on nine rats on low- and nine on high-sodium intake. Two rats died before this phase of the study was started. Rats on a low sodium diet ingested 0.42 ± 0.02 (SE) mEq Na^+/day, while excreting only 0.26 ± 0.06 into the urine. The mean of the individual differences was 0.17 ± 0.04 ($p < 0.005$). On the other hand, rats on high sodium intake ingested 7.1 ± 0.7 and excreted 7.1 ± 0.7 mEq Na^+/day. Thus, in rats on a low sodium diet extrarenal routes of sodium excretion appear to become prominent, and sodium intake cannot be equated, therefore, with urinary sodium excretion.

The individually measured mean daily sodium excretion rate was then compared in each rat to that obtaining during the experiment in the conscious state. Fig. 1 depicts the observations on the first nine successful studies. Clearly, conscious animals on a high sodium diet exhibited a depressed rate of sodium excretion during the experiment, ranging from 17 to 71% of that predicted on the basis of the mean daily excretion rate measured in the metabolic cage.

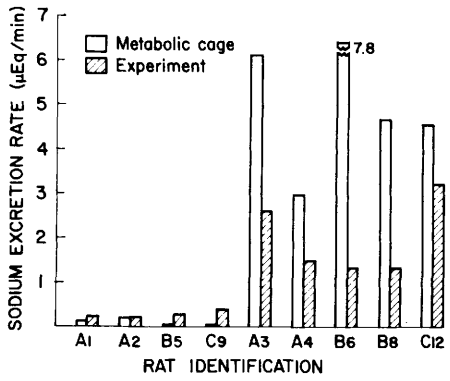

FIG. 1. Comparison of urinary sodium excretion rates in the metabolic cage (24-hr av) and during the experiment in unanesthetized rats, identified as in Table I.

This depression, similar in magnitude to that observed in anesthetized rats, suggested that factors other than pentobarbital anesthesia were responsible for the reduced rate of sodium excretion. Three such factors could be: (1) a residual effect of ether; (2) changes introduced by recent surgery, such as body fluid displacements, discomfort, or residual neurohumoral effects; (3) a circadian rhythm of sodium excretion, whereby more sodium would be excreted at night than during the day. The latter possibility was examined subsequently in littermate groups. Sodium intake and urinary output were measured twice daily, at 10 AM and at 4 PM. This pattern was chosen because it permitted selective examination of the time of day at which the experiment was performed. In addition, during the third day of the balance study (4 PM to 4 PM) food, but not fluid, was removed from the cages to match more closely the conditions obtaining during the experiment. The results of this phase of the study are depicted in Fig. 2. Both sodium intake and urinary sodium excretion exhibited a marked circadian rhythm, with a much higher turnover during the night. This pattern was observed in all rats, regardless of the level of sodium intake. Removal of the food at 4 PM was frequently followed by an increased fluid—and, therefore, sodium—ingestion that night, but not the following morning and afternoon. To preserve the low sodium regimen,

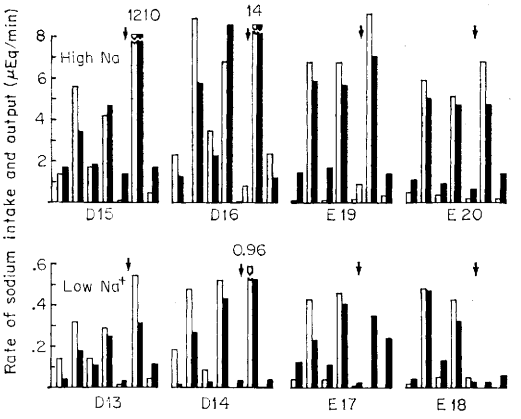

FIG. 2. Metabolic cage study of circadian rhythm of sodium intake (clear columns) and urinary sodium excretion (solid columns). Seven consecutive paired measurements of intake and excretion in each of eight rats are depicted beginning with a 10 AM–4 PM collection. Animals fasted at arrow. Rats E17 and E18 were switched from 0.1% NaCl to distilled water during the fasting period.

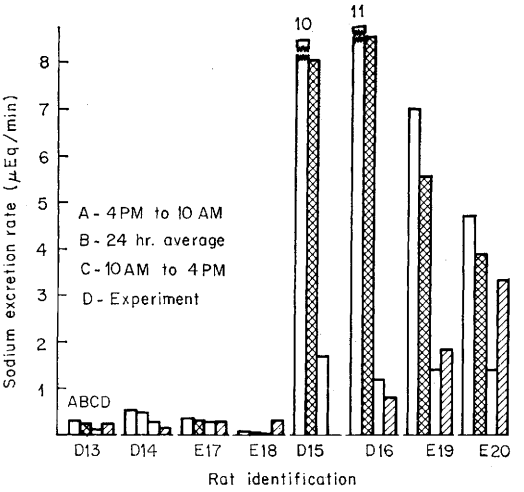

FIG. 3. Comparison of urinary sodium excretion rates in the metabolic cage, showing the circadian rhythm and the 24-hr average, and during the experiment, in fasted, unanesthetized rats.

rats E17 and E18 were given distilled water during the fasting period, and again when fasted for the experiment.

A more precise control value could now be used to evaluate the sodium excretion rate during the experiment. Figure 3 depicts the fasting urinary sodium excretion rates measured in eight rats at night and during the day, the corresponding 24-hr average and, in all but one, those measured during the experiment, following ether anesthesia and surgery. Rats E17 and E18 were given inulin in 0.45% saline rather than usual 0.9%, whereas in rats E19 and E20 the inulin concentration in 0.9% saline was reduced by one-half and the infusion rate increased from 0.02 to 0.05 ml/min. These modifications were imposed in an attempt to approach the corresponding sodium intake rates established by the rats' ad lib. fluid intake. It can be observed that the relatively low sodium excretion rates during the experiment in rats on high sodium intake can be entirely accounted for by the physiologically low excretion rates characteristic of that time of day in rats, as a result of the marked circadian rhythm. Moreover, it is also shown that it was possible to induce higher than control excretion rates by increasing the sodium infusion rate within the limits determined by the rats' spontaneous drinking.

Intrarenal SNGFR distribution. The data from individual experiments are summarized in Table I, grouped according to dietary sodium intake. There was no significant difference in body and kidney weights, urine flow and arterial pressure between the two groups. The inulin clearance (C_{In}) was significantly lower in the low-sodium group (2.50 ± 0.20 (SE) vs 3.36 ± 0.30 ml/min, $p < 0.05$); a significant difference persisted when C_{In} was expressed per 100 g BW (0.99 ± 0.04 vs 1.22 ± 0.08, $p < 0.025$) but not when expressed per g kidney wt (1.29 ± 0.09 vs 1.46 ± 0.10, $p < 0.10$).

In contrast with anesthetized rats, the rapid ferrocyanide infusion did not affect blood pressure in the unanesthetized animals. The SNGFR distribution, expressed as the S/JM SNGFR ratio, was 0.905 ± 0.037 and 0.845 ± 0.060 in the low- and high-sodium groups, respectively (Table I). These values do not differ significantly, but, since SNGFR and nephron size are correlated (1, 4), it is necesary to compare the corresponding nephron dimensions. The

TABLE I. Summary of Experimental Data.

Rat	BW g	KW g	V μl/min	C_{In} ml/min	$U_{Na}{}^{+}V$ μEq/min	AP mmHg	SNGFR S/JM
				Low-Na diet			
A1	308	1.00	7.1	3.76	0.24	106	—
A2	336	1.22	3.6	2.32	0.26	106	0.854
B5	252	0.89	7.7	2.38	0.27	112	0.955
C9	212	0.83	15.9	2.14	0.40	104	0.998
C10	217	1.01	56.4	2.46	2.67[1]	105	0.882[1]
D13	230	0.94	6.0	2.24	0.23	99	0.759
D14	223	0.83	7.6	1.96	0.17	120	—
E17	251	1.05	9.0	—	0.27	120	0.879
E18	306	1.10	75.6	2.72	0.30	124	0.987
±	259	0.99	21.0	2.50	0.27	111	0.905
SE	15	0.04	8.7	0.20	0.02	3	0.037
				High-Na diet			
A3	259	1.22	12.4	3.84	2.70	106	0.897
A4	336	1.21	8.4	4.82	1.52	104	0.697
B6	330	1.20	20.3	3.34	1.67	110	—
B8	236	1.13	8.0	3.00	1.36	126	0.843
C12	204	1.02	43.2	2.53	3.24	108	0.883
D16	195	0.80	6.0	1.32[2]	0.81[2]	115	0.808[2]
E19	264	0.94	9.3	2.54	1.84	132	0.675
E20	333	1.31	50.0	3.48	3.35	126	1.076
±	270	1.10	19.7	3.36	2.24	116	0.845
SE	20	0.06	6.1	0.30	0.32	4	0.060
$p <$	NS	NS	NS	0.05	0.001	NS	NS

BW: body weight; KW: kidney weight; V: urine flow; C_{In}: inulin clearance $U_{Na}{}^{+}V$: urinary sodium excretion rate; AP: arterial pressure; SNGFR: single nephron glomerular filtration rate; S: superficial nephron; JM: juxtamedullary nephron.

[1] Excluded because sodium excretion increased markedly during the study.

[2] Excluded because C_{In} was too low.

parameter used was the total proximal tubular length (TPL). The S/JM TPL ratios were 0.871 ± 0.032 and 0.890 ± 0.017, respectively ($p > 0.10$). The extraluminal radioactivity in proximal tubules, estimated from the measured radioactivity in distal convolutions as previously described (1), expressed in percent of the total glomerular + proximal tubular radioactivity, was 23.3 ± 1.3 and 23.1 ± 1.4 in S and JM nephrons, respectively, and was unaffected by the level of sodium intake (14 rats). These values were similar to those obtained in 24 anesthetized rats (1).

Paired statistical analysis was carried out between littermates, as shown in Table II.

TABLE II. Paired Analysis in Littermates of the Effect of Sodium Intake (High Minus Low) on Urinary Sodium Excretion and SNGFR Distribution.

Rat pair	SNGFR S/JM	TPL S/JM	$U_{Na}{}^{+}V$ μEq/min
A3-A2	0.043	−0.009	2.44
B8-B5	−0.112	−0.054	1.09
C12-C9	−0.127	0.044	2.84
E19-E17	−0.204	0.132	1.57
E20-E18	0.056	−0.061	3.05
$\bar{X}$	−0.069	0.042	2.20
SE	±0.050	±0.028	±0.38
p	NS	NS	<0.005

TABLE III. Comparisons between Conscious and Anesthetized Rats.

	V μl/min	C_{In} ml/min 100g	SNGFR S/JM	TPL S/JM
Conscious	20.4[1]	1.094	0.862[2]	0.881[2]
$n = 15$	±5.3	±0.051	±0.032	±0.014
Anesthetized	6.1	0.995	0.742	0.869
$n = 15$	±0.4	±0.035	±0.017	±0.010
p	<0.025	NS	<0.005	NS

[1] $n = 17$.
[2] $n = 14$.

Five successfully studied pairs were available. When more than one pairing was possible, those studied closer in time were paired. Rat C10 was excluded because it developed a natriuresis during the study (Table I). Rat D16 was excluded because its C_{In} and $U_{Na+}V$ were abnormally low (Table I). There was no statistically significant difference in S/JM SNGFR (-0.069 ± 0.050) or S/JM TPL (0.042 ± 0.028), but the difference in urinary sodium excretion was significant (2.20 ± 0.38 μEq/min, $p < 0.005$).

Since the S/JM SNGFR was independent of dietary sodium intake, the data were grouped together and compared to the same data obtained in anesthetized rats (Table III). The mean S/JM SNGFR in 14 unanesthetized rats was 0.862 ± 0.032, with a S/JM TPL of 0.881 ± 0.014. In 15 anesthetized rats, the corresponding mean ratios were 0.742 ± 0.017 ($p < 0.005$) and 0.869 ± 0.010 (NS). It would appear, therefore, that pentobarbital anesthesia was associated with a redistribution of SNGFR away from the superficial nephrons. However, it was noted that the unanesthetized rats frequently had a high urine flow during the experiment, independently of the sodium intake (Table I). Urine flow ranged in these rats from 3.6 to 75.6 μl/min, in contrast with a range of only 2.6 to 8.6 μl/min in anesthetized rats (1). A possible effect of urine flow on the intrarenal SNGFR distribution was examined by plotting urine flow against the S/JM SNGFR (Fig. 4). A curvilinear relationship emerged, suggesting that the two variables were interrelated.

Discussion. Rats subjected to chronic sodium loading excrete over 90% of their daily sodium intake into the urine (5–7, and this study). Therefore, measured daily sodium intake is, under this condition, a good index of daily urinary sodium excretion rate. In a previous study (1) it had been observed that rats on a high daily sodium intake of 8–10 mEq (5.5–7.0 μEq/min) excreted only 1.4–1.8 μEq/min during experiments carried out under pentobarbital anesthesia and involving some surgical manipulation. Others made a similar observation from comparisons of measured urinary sodium excretion rates both in metabolic cages and during experiments carried out under inactin anesthesia (5, 7). An antinatriuretic effect of anesthesia and surgery was invoked to explain this phenomenon (7), on the basis of a previous study on human subjects (2). However, the present

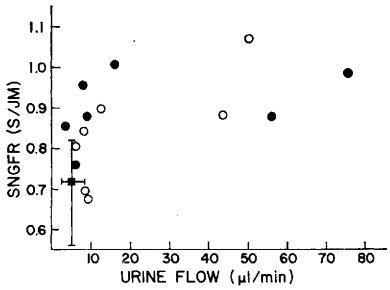

FIG. 4. Plot of urine flow during the experiment against the S/JM SNGFR ratio in unanesthetized rats on low (●) and high (○) dietary sodium intake. The closed square represents the mean values in 15 anesthetized rats (1), their ranges indicated by the vertical and horizontal lines.

data reveal that the same phenomenon occurred in experiments performed on unanesthetized rats, during the second hour following termination of ether anesthesia and surgery (Fig. 1). While some lasting effect of these procedures could have been invoked, it is further shown that rats exhibit a marked circadian rhythm of urinary sodium excretion, with lower rates during the morning and afternoon hours (Fig. 2). In fasted rats on high-sodium intake, these lower rates (1.4 ± 0.1, range 1.2–1.7 μEq/min) are practically identical to those observed in experiments on anesthetized rats fed the same high-sodium diet (1) and are further shown to be easily reproduced and even exceeded after recovery from ether anesthesia and surgery, while maintaining sodium intake at rates not higher than those dictated by the rats' previous spontaneous drinking (Fig. 3). Therefore, basal sodium excretion rates during the experiment did not markedly depart from the physiological rates corresponding to that time of day, even in anesthetized animals.

It may be noted that the circadian rhythm of urinary sodium excretion closely paralleled a similar variation in sodium intake (Fig. 2). It is not known whether the excretory rhythm would still be manifest in a situation where sodium intake was not spontaneous, but rather maintained at a constant rate, as in the experimental design of Kuschinsky *et al.* (5) and Daugharty *et al.* (7). The fact that these workers did observe a reduced sodium excretion rate during the experiment suggests, in the light of the present results, that the excretory rhythm was still present.

Since a circadian rhythm was also observed in rats on low sodium intake (Fig. 2), the lower sodium excretion rates observed in high-sodium rats during the daylight hours do not represent a blunting of the differential sodium excretion rate between the two groups. However, some blunting did occur during the experiment, since low-sodium rats frequently had higher, rather than lower, sodium excretion rates relative to those obtaining in the metabolic cage (Figs. 1 and 3). Nevertheless, a significant difference in sodium excretion, eight times higher in high-sodium rats, was preserved (Table I). This difference was similar to that observed in anesthetized animals given identical low- and high-sodium diets (1).

It is of interest to note that, contrary to the findings in anesthetized rats, the intravenous ferrocyanide infusion had no detectable effect on arterial blood pressure in these unanesthetized rats. This result may be attributed to the normal function of the baroreceptors, which is depressed by anesthesia, as suggested by Bing and Poulsen (8), who also observed a better maintenance of arterial pressure in unanesthetized rats given an anti-angiotensin II antibody.

The present study, therefore, having minimized doubts arising from the possibly interfering effect of anesthesia and of arterial pressure fall during the ferrocyanide infusion, reinforces previous evidence (1, 7) that the level of dietary sodium intake does not affect the intrarenal SNGFR distribution. On the other hand, the S/JM SNGFR ratio was significantly higher in conscious than in anesthetized rats (Table III) raising the possibility that anesthesia—or the ferrocyanide-induced arterial pressure drop—evoked nonuniform vasomotor changes throughout the renal cortex. However, while this possibility cannot be excluded, an alternative explanation is provided by the data in Fig. 4. It shows that a high urine flow was accompanied by a high S/JM SNGFR ratio, even in animals on a low-sodium diet. Some parameter associated with diuresis, therefore, might be responsible. Other authors have found that saline diuresis induced by acute, saline loads gave rise to a higher S/JM SNGFR ratio, which was considered to play a role in regulating sodium excretion (9, 10). However, in the present study diuresis without proportional natriuresis had the same effect (Table I). At least in one rat (E18) urinary osmolality, calculated on the basis of measured sodium and potassium concentration, plus estimated urea, was clearly hypotonic, suggesting that partial inhibition of antidiuretic hormone (ADH) does occur in these experiments on unanesthetized rats. Davis and Schnermann (11) found that ADH sup-

pression was associated with a rise in the S/JM SNGFR ratio, while Baines (12) observed that a similar redistribution could be induced by glucosuria. Taken together, these findings make it unlikely that the increase in the S/JM SNGFR ratio detected in this and other laboratories under conditions of acute saline (9, 10, 13) or water (11) diuresis represents a physiological mechanism geared to the more rapid urinary excretion of the excess sodium or water. It appears more likely that this redistribution is a consequence of the increased urine flow as such. If so, a common mechanism, presumably physical in nature, might be implicated. Physical mechanisms capable of bringing about redistribution of SNGFR in association with diuresis have been proposed (11, 12, 14). While the present evidence does not permit to distinguish among those or other mechanisms, it supports the view that intrarenal redistributions of SNGFR are not induced by variations in dietary sodium intake and do not play a role in the chronic regulation of urinary sodium excretion.

Summary. In a previous study, the distribution of nephron filtration rates was not affected by variations in dietary sodium, but anesthesia may have blunted a physiological response. Therefore, unanesthetized littermate rats placed on high (7 mEq/day) or low (0.4 mEq/day) sodium intake were studied. Daily urinary sodium excretion was measured in metabolic cages. One hour after recovery from ether anesthesia and surgery, the inulin clearance and urinary sodium excretion were measured. ^{14}C-ferrocyanide was then infused during 12 sec and the left kidney pedicle tied off. Arterial pressure did not change. A rapidly fatal dose of pentobarbital preceded removal of the kidney. Urinary sodium excretion rate was reduced relative to mean daily cage measurements, to the same extent in conscious as in anesthetized rats on high sodium intake. Separate night and day urine collections revealed a marked circadian rhythm of sodium excretion rate in all rats, with lower rates during the day sufficient to account for those observed during the experiment. The superficial/juxtamedullary nephron filtration ratio was 0.90 ± 0.04 (SE) in six unanesthetized rats on low sodium and 0.84 ± 0.06 on high sodium ($p > 0.10$). Paired analysis of five littermate pairs also showed no difference (−0.069 ± 0.050) in spite of a significant difference in urinary sodium excretion (2.20 ± 0.38 μEq/min, $p < 0.005$). This study supports the view that dietary sodium does not affect the intrarenal distribution of nephron filtration rates.

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