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RENAL CONCENTRATING ABILITY AND CALCIUM LOAD HANDLING IN THE SPONTANEOUSLY HYPERTENSIVE RAT[1]

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Abstract. The renal response to an oral load of calcium and the renal concentrating ability of spontaneously hypertensive (SH) and normotensive (WKY) rats were examined. The response to calcium loading was similar between SH and WKY animals in terms of urine volume, osmolality and calcium excretion. Compared to WKY controls, the ability of SH rats to concentrate urine during dehydration was decreased. The injection of vasopressin had no effect on the urine output of water-loaded SH rats, although a similar injection returned urine flow to control levels in WKY animals. It is suggested that the decreased ability of the SH rat to limit urine flow was due either to pressor effects of ADH in increasing perfusion pressure or to a decreased responsiveness of the renal tubule to ADH.

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Since the development of the spontaneously hypertensive (SH) rat (Okamoto and Aoki 1963) through the inbreeding of Wistar rats showing elevated blood pressure, this strain has been extensively utilized as an animal model for the study of the pathophysiology of essential hypertension in humans. The majority of research has centered on the cardiovascular system, and it has been shown that the increase in blood pressure results from a generalized increase in total peripheral resistance (Folkow *et al* 1970) while cardiac output (Iriuchyima 1973) and regional blood flow (Nishiyama *et al* 1976) are unchanged from control values. Reports of abnormal contractile responses in SH rat visceral smooth muscle (Altman *et al* 1977) further suggest that hypertensive-normotensive differences may not be confined to the cardiovascular system.

Morphological alterations of the kidney are not evident during the early stages of essential hypertension. On the other hand, a number of investigators have shown that humans with essential hypertension exhibit a more rapid sodium and water loss than normotensive controls following volume expansion (Farnsworth 1946, Krakoff *et al* 1970). Observations on the excretion of acutely administered saline in SH rats are not as well defined but generally indicate an increased rate of sodium loss with oral loading (Willis *et al* 1976). Modifications of renal function in hypertensive individuals may also occur in the response to dehydration and in calcium excretion. Khokhar and Slater (1976) observed increased ADH excretion in hypertensive patients and a tenfold increase in plasma vasopressin levels has been observed in experimental hypertension (Mohring *et al* 1977). In regard to the renal handling of calcium, Miss-Pages and Gairard (1975) reported a significant correlation between calcium excretion and arterial pressure at the onset of hypertension of deoxycorticosterone acetate treated rats.

To our knowledge, information is lacking concerning renal handling of calcium in the SH rat. In an effort to elucidate changes in renal function that may occur in hypertensive animals, urinary calcium excretion following oral loading was examined in SH rats. In addition, the urine concentrating ability during dehydration and the response to ADH challenge were examined in SH rats.

METHODS

Male spontaneously hypertensive (SH) rats of the strain developed by Okamoto and Aoki

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(1963) and parent strain Wistar-Kyoto rats (WKY) obtained from Harlan Laboratories were utilized throughout the study. Animals were housed at 23±2 °C with a 14-hour light 10-hour dark photoperiod. Water and Purina Rat Chow were available *ad libitum* unless indicated otherwise. Systolic blood pressure was determined under light (Stage III) sodium secobarbitol anesthesia (Lilly, 0.06 ml/100g) with the Friedman: Freed (1949) microphonic manometer indirect tail cuff 2 to 3 days prior to experimentation.

Electrolyte Loading. A total of 36 SH and 36 WKY rats were utilized for determination of the renal excretory response to calcium loading. Animals were accustomed to the handling procedures and the metabolism cages by sham runs for 4 consecutive days prior to data collection. Food was removed 6 to 8 hours before and water immediately prior to testing. The study was conducted on 2 consecutive days, with the animals receiving 2.0 ml of distilled water or 140 mg of calcium chloride in 2.0 ml of distilled water. The bladder was emptied by suprapubic massage and the animals placed 3 per metabolism cage for a period of 6 hours (1200 to 1800 hrs EST). Urine was collected under 4 cm of mineral oil to prevent evaporation. The volume excreted was measured in graduated cylinders, and the urine osmolality was determined with a Fiske OR osmometer. Urine calcium and creatinine were determined by standard atomic absorption and colorametric techniques.

Urine Concentrating Ability. A total of 18 SH and 18 WKY rats were accustomed to a daily schedule with food and water removed for 18 hours and available for 6 hours for 7 days prior to experimentation. On the 8th day, the animals were placed 3 per metabolism cage and left overnight for collection of control urine samples (1600 to 1000 hrs EST). During the succeeding 3 days, food was offered during the 6 hour interval, but water was withheld. Urines were collected under mineral oil for volume and osmolality determination. An additional 12 animals of each strain were divided into 2 groups for determination of plasma osmolality and blood hematocrit prior to water removal and after 66 hours of water deprivation. The animals were anesthetized with sodium secobarbitol, and 4.9 ml of blood was withdrawn from the heart into syringes containing 0.1 ml of sodium heparin (100 units/ml).

Renal Response to ADH Administration. The effect of ADH on urinary water excretion was examined in SH and WKY rats following 5 days on a 6-hour fasting/18-hour feeding schedule. The animals were then exposed to control water loading (distilled water via stomach tube, 5% body weight) or water loading plus ADH injection. Animals were placed individually into metabolism cages for urine collection from 1200 to 1800 hrs EST.

Differences among group means were analyzed by an analysis of variance, followed by t-tests if the analysis of variance indicated significance (Snedecor and Cochran 1967). Data are presented as the mean ± SE throughout the text.

TABLE 1

The body weights and blood pressure of SH and WKY groups utilized for electrolyte loading and renal concentrating ability determinations.

	N	AGE (weeks)	WEIGHT (g)	SystBP (Torr)
$CaCl_2$ Ingestion				
+SH	36	14–16	283±5*	172±6*
WKY	36	14–16	354±5	123±4
Dehydration				
SH	18	14–16	257±5†*	149±5*
WKY	18	12–15	312±4†	110±3
ADH Challenge				
SH	18	12–15	243±4*	161±4*
WKY	18	12–15	326±5	117±3

†At start of experiment, Mean±SE.
*Significant difference from WKY normotensive value.
+SH = Spontaneously hypertensive
WKY = normotensive rats.

RESULTS

Body weights were consistently lower and the sytolic blood pressure was higher

TABLE 2

The effect of calcium ingestion on urinary parameters in SH and WKY rats.†

	Control		Calcium Loaded	
	WKY	SH	WKY	SH
Urine Volume (ml/100g/6h)	0.92±0.04	0.82±0.05	1.00±0.07	0.84±0.10
Urine Osmolality (Osm/Kg)	1.70±0.10	1.97±0.05	1.47±0.07*	1.56±0.16*
Creatine Excretion (mg/100g/6hr)	1.30±0.05	1.16±0.06	1.27±0.10	1.11±0.11
Calcium Excretion (mg/100g/6hr)	0.16±0.02	0.11±0.02	0.27±0.03*	0.19±0.04*

*An asterisk indicates a significant difference of $P<0.05$ from the control value.
†SH = Spontaneously hypertensive, WKY = normotensive rats. Mean = SE.

in SH rats as compared to the WKY controls (table 1).

The urine volume and osmolality response to calcium ingestion were similar between SH and WKY groups, but both strains showed a significant decrease in urine osmolality with a slight but nonsignificant increase in urine volume. A significant increase in calcium excretion was noted in both the calcium-loaded SH (73%) and WKY (69%) animals. Creatinine excretion was not significantly affected in either group by the oral administration of calcium (see table 2).

The pattern of body weight loss in water-deprived animals was similar between WKY and SH groups (14–15%) and indicated a relatively severe dehydra-

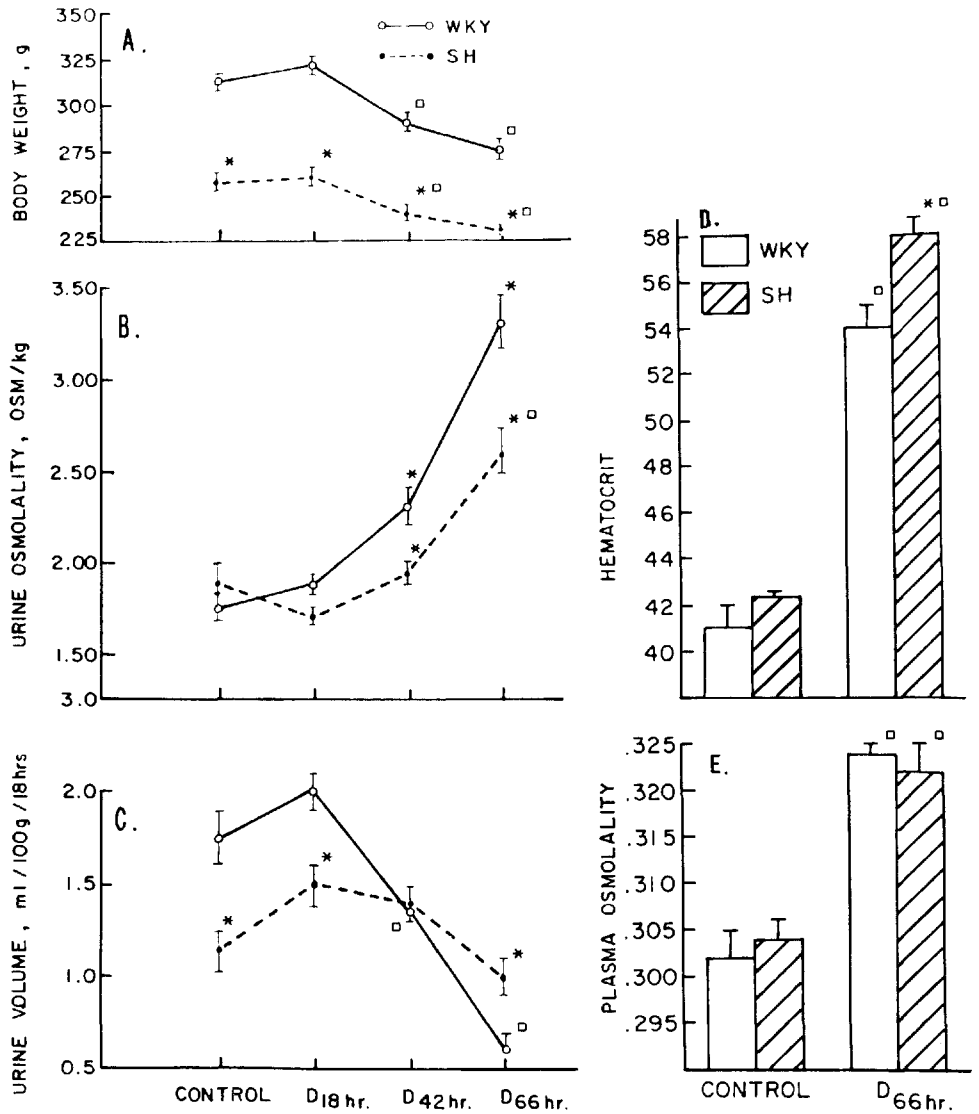

FIGURE 1. Changes observed in A. body weight, B. urine osmolality, C. urine volume, D. hematocrit, E. plasma osmolality during water deprivation in normotensive (WKY) and hypertensive (SH) rats. Data are expressed as means±SD (vertical bars). An asterisk indicates a significant difference between SH and WKY groups ($P<0.05$) and an open square a significant difference from control values.

tion by day 3 (fig. 1). The osmolality of WKY urine increased significantly from control values at 42 and 66 hours of water deprivation, while that of SH rats was increased only at the 66 hour determination. Urine osmolality of SH rats was significantly less than WKY values at both the 42 and 66 hour measurements. The volume of urine excreted by WKY rats was significantly decreased from the control value at 42 (23%) and 66 hours (66%) after water removal, but no significant change in urine volume from the control was observed for SH rats during the experiment. Plasma osmolality and blood hematocrit were markedly increased in both groups at 66 hours. The elevation in plasma osmolality was similar between groups; however, the hematocrit of SH rats was significantly increased above the WKY value (see fig. 1).

Water loading (5% body weight) was followed by a 65% and 54% increase in the 6 hr urine volume of WKY and SH rats, respectively (table 3). There was no change in plasma osmolality of either group at 3 hours after administration of the water load. The injection of ADH prior to water loading had no significant effect on plasma osmolality of WKY rats and returned urine volume and osmolality to near control levels. In contrast, the plasma osmolality of SH rats injected with ADH prior to water loading was increased above control and water-loaded levels, and the volume of urine excreted was not significantly different from water-loaded animals. Urine osmolality in the ADH water loaded SH rats, on the other hand, returned to near control values and was not different from that of the WKY rats.

DISCUSSION

The effect of the oral administration of calcium on the renal parameters examined was not significantly different between hypertensive and normotensive rats. There was a tendency, however, for decreased calcium excretion in SH animals during both the control and calcium-load measurements (table 1). This tendency may reflect slight alterations in the renal function of the SH rat that are not directly related to the tubular handling of calcium, as suggested by the tendency for decreased creatinine excretion in these animals. The reason for the decrease in urine osmolality, in the absence of changes in urine volume observed in both groups following calcium ingestion, is not clear. Itokawa *et al* (1974) have shown that marked systemic changes may be produced in the rat by varying the amount of calcium in the feed, suggesting that the alteration in the pattern of water and solute excretion observed was due to indirect effects of ingested calcium on renal function. In any case, the effect of the calcium load on urine volume and osmolality was similar in both SH and WKY rats.

The concentration of urine and reduction of urine flow by SH rats during water deprivation was less marked than that recorded for WKY rats. During the period of water removal, SH urine osmolality increased by 44%, whereas a 103% increase was noted in WKY controls. Concurrently, the 18 hr urine

TABLE 3

Plasma and urine parameters in water loaded and ADH injected WKY and SH rats.†

	Control		Water Load		Water Load+ADH	
	WKY	SH	WKY	SH	WKY	SH
Urine Volume (ml/100g/6hr)	0.75±0.02	0.63±0.04	1.24±0.11*	0.97±0.12*	0.83±0.08†	0.90±0.10*
Urine Osmolality (Osm/Kg)	1.20±0.05	122±0.08	0.67±0.04*	0.65±0.04*	1.12±0.07†	1.13±0.12†
Plasma Osmolality (mOsm/Kg)	302±3	304±2	303±3	302±2	305±1	308±1*†

*An asterisk indicates significant difference from control ($P<0.05$).

†Significant difference from water load values ($P<0.05$).

SH = Spontaneously hypertensive, WKY = normotensive rats. Mean±S.E.

volume of WKY animals decreased 66%, while the 13% drop in SH urine volume was not significantly different from the value obtained prior to water removal. In control measurements obtained from animals held in metabolism cages for 6 hrs without water (tables 2, 3), the urine volume of SH rats was slightly reduced compared to WKY rats; whereas, a highly significant reduction was observed for SH rats during the 18 hr determinations of the dehydration experiment. Wright *et al* (1977) have shown that the total body water of SH rats is decreased compared to normotensive controls, suggesting that these animals may normally be slightly dehydrated. A possible explanation of the reduction in urine volume by SH rats during the control and 18 hr intervals of the dehyration experiment may be that even mild (18 hr) water restriction elicits a substantial effort for water conservation in these animals. In fact, the failure of the SH animal to reduce urine volume further during extended water deprivation suggests that the reduction in urine flow induced at 18 hours was near maximal for the animal, indicating that the maximum level of reduction of urine output is decreased in SH rats compared to normotensive WKY animals.

An early and exaggerated response to dehydration may explain the similarity in body weight loss, plasma osmolality, and slight increase in hematocrit of SH rats as compared to WKY animals following 66 hrs of water removal. Although the renal capacity for water conservation was reduced in SH animals, the reduction of urine flow to near minimal output during the first few hours of water removal served to limit the dehydration of the animal to a level similar to controls (see fig. 1).

The nature of the mechanism underlying the decreased ability of the SH rat to concentrate urine is not known. Stumpe and co-workers (1970) showed a decrease in the transit time along the loop of Henle in hypertensive rats, suggesting that this phenomenon is due to "pressure diuresis" resulting from the elevation in perfusion pressure of the kidney and a washout of the medullary osmotic gradient (Selkurt *et al* 1965). Bierwalters and Arendshorst (1978) showed that glomerular filtration rate and renal plasma flow in conscious SH rats does not differ from WKY values, suggesting that wash out of the medullary gradient is unlikely under normal conditions. It is conceivable, however, that the pressor effect of excess endogenous ADH release in SH rats during dehydration could result in the elevation of perfusion pressure and initiate the mechanism proposed by Selkurt *et al* (1965), thereby decreasing the renal concentrating ability of the rat.

Our results indicate a negligible effect of ADH administration in reducing urine output in water-loaded SH rats, which was in marked contrast to the WKY group that returned to pre-load levels. Although the urine volume of water-loaded SH rats receiving ADH remained elevated at levels comparable to those recorded after water loading only, the urine osmolality increased to near control levels following the water load and ADH. In addition, the plasma osmolality of SH animals receiving a water load+ADH was increased at 3 hrs after the treatment. These findings may reflect a pressor effect of ADH on the hyper-responsive vasculature of the SH rat, resulting in water and solute diuresis through an increase in perfusion pressure and extravasation of fluid into the extracellular space. An additional possibility is that the renal tubule of the SH rat is less responsive to ADH, resulting in decreased ability to limit urine flow. In either case, the renal response to dehydration and ADH injection was shown to differ between SH and WKY animals in a manner indicating a reduction in the ability of the SH rat to reduce urinary water loss.

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