PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 915–920 (1975)

NaCl Intake and Preference Threshold of Spontaneously Hypertensive Rats[1] (38926)

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Rats made hypertensive either by renal encapsulation with latex envelopes or following administration of large doses of desoxycorticosterone acetate have an aversion to NaCl solution when allowed to choose between it and water to drink (1–6). Wolf *et al.* (7) also reported that rats with a genetic predisposition to hypertension ingested less NaCl solution than did those from a strain genetically resistant to developing hypertension. Recently, Catalanotto *et al.* (8) and McConnell and Henkin (9) reported a NaCl appetite in spontaneously hypertensive (SH) rats derived from the Okimoto and Aoki strain. The purpose of the study described below was to confirm the report of a salt appetite in spontaneously hypertensive rats, to determine whether preference threshold for NaCl solution was altered and to study the effect of acute intraperitoneal loading of NaCl solutions on excretion of sodium and potassium.

Methods. Experiment 1. Nine male adult Wistar/Kyoto normotensive and seven spontaneously hypertensive (SH) Wistar/Kyoto rats[2] bred at the University of Florida were used. The rats were caged individually in hanging wire mesh cages, given finely powdered Purina Laboratory Chow as food, and choice between distilled water and 0.15 *M* NaCl solution to drink. Food was provided in the spill-resistant feeders described by Fregly (10). Fluid containers consisted of infant nursing bottles with cast aluminum fountains as described by Lazarow (11).

The rats were allowed 3 days to become familiar with their surroundings, after which food and fluid intakes and body weight were measured daily by weight difference. Measurements were carried out over a 5-day period.

At the end of the period of measurement, the bottle containing NaCl solution was removed from the cages and distilled water was the sole source of drinking fluid. One week later, the rats were subjected to a diuresis study. Each rat was required to breath ether for a few moments at the beginning of the experiment to initiate reflex emptying of the bladder. This urine was discarded. The rat was then weighed and administered intraperitoneally 0.15 *M* NaCl solution at a load of 3% of body weight. The loading solution was warmed to 37° prior to administration. Each rat was placed in an individual stainless steel metabolism cage beneath which was a funnel which directed urine into graduated cylinders containing a small volume of light mineral oil. Standard fecal separators prevented contamination of urine by feces. At the end of a 5-hr period, each rat was again required to breath ether to initiate reflex emptying of the bladder. This urine was added to that voided during the 5-hr period. Each cage and funnel was rinsed with 10.0 ml of distilled water to assure that any residual urine was collected. The urine was analyzed for sodium and potassium by flame photometry using lithium as the internal standard.

One week later, a second experiment was carried out in an identical fashion excepting that the loading solution was 0.075 *M* NaCl solution.

Experiment 2. Nine female rats, born and raised at the University of Florida, were used. Five of the rats were hypertensive and four were normotensive. Blood pressures of unanesthetized rats were measured from the tail by means of a Transducer-Monitor-Coupler (Narco-Bio Systems) coupled to a Sanborn polygraph. Technical details of this

[1] Supported by Grant HE 14526-03 from the National Institutes of Health.

[2] A breeding stock of Wistar/Kyoto normotensive and hypertensive rats was kindly supplied by Dr. Carl Hansen, Veterinary Resources Branch, Division of Research Services, National Institutes of Health.

procedure have been published (12). All rats were caged individually and were given finely powdered Purina Laboratory Chow to eat and choice between distilled water and 0.25 *M* NaCl solution to drink. Food and fluid containers and other experimental conditions were as described in Expt 1. Intakes of food and fluids and body weight were measured daily for 5 days.

Experiment 3. Ten female rats were used. Half of this group was normotensive and half hypertensive. The rats were caged individually and given finely powdered Purina Laboratory Chow to eat. At the beginning of the experiment each rat was given a choice between two bottles each containing distilled water. The fluid and food containers were the same as those described in Expt 1. One week was allowed for adjustment to the cage and bottles. During this time, intakes were measured daily to ascertain that each rat drank roughly equal amounts from each bottle. Positions of the two bottles on each cage were interchanged daily to avoid habit formation in selection of drinking fluid. At the end of this time, 2-day test periods were begun. During the first test period, each rat was offered a choice between two bottles of distilled water. During the second test period a choice was offered between distilled water and 0.006 *M* NaCl solution. All NaCl solutions used were made from a concentrated stock NaCl solution by dilution. Each dilution was checked for accuracy by determination of the sodium concentration by flame photometry. During subsequent 2-day periods, each rat was given a choice between distilled water and the following molar NaCl concentrations in chronological sequence: 0.001, 0.003, 0.006, 0.009, 0.012, 0.015, 0.020, 0.025, 0.030, 0.050, 0.075, 0.100, 0.150, and 0.200. Daily intakes of water, NaCl solution, and food were measured.

The criterion of preference threshold used was similar to that of Richter (13); namely, the concentration of NaCl solution at and above which simultaneous mean volume taken from the NaCl bottle exceeded significantly ($P < 0.05$) that taken from the water bottle. The significance of the difference between means was tested by the *t* test for the 95% confidence limit (14).

Results. Experiment 1. Hypertensive male rats had a spontaneous appetite for 0.15 *M* NaCl solution in that they ingested significantly ($P < 0.01$) more of it than did their controls (Table I). The increased NaCl intake was accompanied by a significant ($P < 0.01$) reduction in simultaneous water intake. The ratio of NaCl intake to total fluid intake was significantly increased in hypertensive rats. Food intake during this period was unchanged from controls. Mean body weight of the hypertensive group was significantly ($P < 0.01$) less than that of controls.

Intraperitoneal loading of male hypertensive rats with 0.075 *M* NaCl solution increased significantly their sodium output above control level during the 5-hr period (Table II). There were also significant increases in Na/K ratio and percentage of the fluid and sodium load excreted in 5 hr by hypertensive rats. When loaded with iso-

TABLE I. THE EFFECT OF SPONTANEOUS HYPERTENSION ON INTAKES OF WATER, 0.15 *M* NaCl SOLUTION AND FOOD BY MALE SH RATS[a]

Group	No. of rats	Mean body wt (g)	Mean intakes (ml or g/100 g body wt/day) of			NaCl/ total fluid (%)
			Water	0.15 *M* NaCl solution	Food	
Experiment 1						
Control	9	448	7.0	4.4	4.9	34.1
		±21[b]	±0.7	±1.4	±0.1	±8.0
Hypertensive	7	314	3.2	22.6	4.5	85.1
		±6[c]	±0.8[c]	±3.3[c]	±0.2	±5.4[c]

[a] Intakes in both experiments were measured for 5 days.
[b] One standard error of mean.
[c] Significantly different from control group ($P < 0.01$).

TABLE II. EFFECT OF INTRAPERITONEAL LOADING (3.0% OF BODY WT) OF NaCl SOLUTIONS ON EXCRETION OF URINE, SODIUM, AND POTASSIUM DURING A 5-HR PERIOD

Group	No. of rats	Mean body wt (g)	Urine output (ml/kg/5 hr)	Na output (mEq/kg/5 hr)	K output (mEq/kg/5 hr)	Na/K	% of Fluid load excreted in 5 hr	% of Na load excreted in 5 hr
0.075 M NaCl solution load								
Control	9	518	13.4	0.66	1.11	0.57	37.5	29.2
		±17[a]	±2.5	±0.11	±0.09	±0.06	±3.7	±4.7
Hypertensive	7	336	19.2	1.22	1.20	1.07	63.8	54.0
		±5[b]	±2.4	±0.15[b]	±0.18	±0.15[b]	±7.8[b]	±6.5[b]
0.15 M NaCl solution load								
Control	9	506	10.4	1.91	1.48	1.24	46.1	42.3
		±17	±1.9	±0.29	±0.14	±0.11	±10.6	±6.4
Hypertensive	7	325	10.2	1.84	1.12	1.83	34.1	46.6
		±7[b]	±2.0	±0.31	±0.31	±0.27	±6.6	±4.5

[a] One standard error of mean.
[b] Significantly different from control ($P < 0.01$).

TABLE III. EFFECT OF SPONTANEOUS HYPERTENSION ON INTAKES OF WATER, 0.25 *M* NaCl SOLUTION, AND FOOD BY FEMALE RATS[a]

Treatment	No. of rats	Mean body wt (g)	Mean systolic blood pressure (mmHg)	Mean Intakes (ml or g/100 g body wt/day) of			NaCl total fluid (%)
				Water	0.25 *M* NaCl soln	Food	
Control	4	280 ± 6[b]	144 ± 4	9.9 ± 1.0	1.0 ± 0.2	6.1 ± 0.2	9.1 ± 1.3
Hypertensive	5	235 ± 5[c]	179 ± 1[c]	8.4 ± 0.7	10.9 ± 1.6[c]	6.2 ± 0.1	55.4 ± 6.5[c]

[a] Intakes were measured for 5 days.
[b] One standard error of mean.
[c] Significantly different from control group ($P < 0.01$).

tonic NaCl solution, no significant differences occurred between groups.

Experiment 2. Hypertensive rats in this study showed a vigorous appetite for 0.25 *M* NaCl solution compared to control rats (Table III). No differences in water or food intakes were observed between groups. Again, total fluid intake and ratio of NaCl/total fluid ingested by the hypertensive group were significantly greater than that of controls. Mean systolic blood pressure of the hypertensive group was significantly elevated, and body weight significantly reduced, compared to that of the controls.

Experiment 3. Control rats, as a group, first detected the difference between distilled water and NaCl solution when the concentration of the latter was 0.030 *M* (30 mEq/liter). Thus, the preference threshold of the control group lay between 0.025 and 0.030 *M*. Hypertensive rats detected the difference between distilled water and NaCl solution when the latter was 0.012 *M* (12 mEq/liter). The preference threshold of the hypertensive group lay between 0.010 and 0.012 *M*. Thus, hypertensive rats can detect NaCl at a concentration 2.5 times lower than controls (Fig. 1).

It should also be noted in Fig. 1 that the volume of NaCl solution ingested by hypertensive rats was greater than that of controls at all concentrations tested above threshold, including hypertonic concentrations. Maximal intake of NaCl solution occurred when 0.100 *M* was given to controls and 0.150 *M* was given to hypertensives. Mean body weight of the hypertensive rats was significantly less than that of controls throughout the experiment. Initial mean body weights of control and hypertensive groups were 333 ± 7

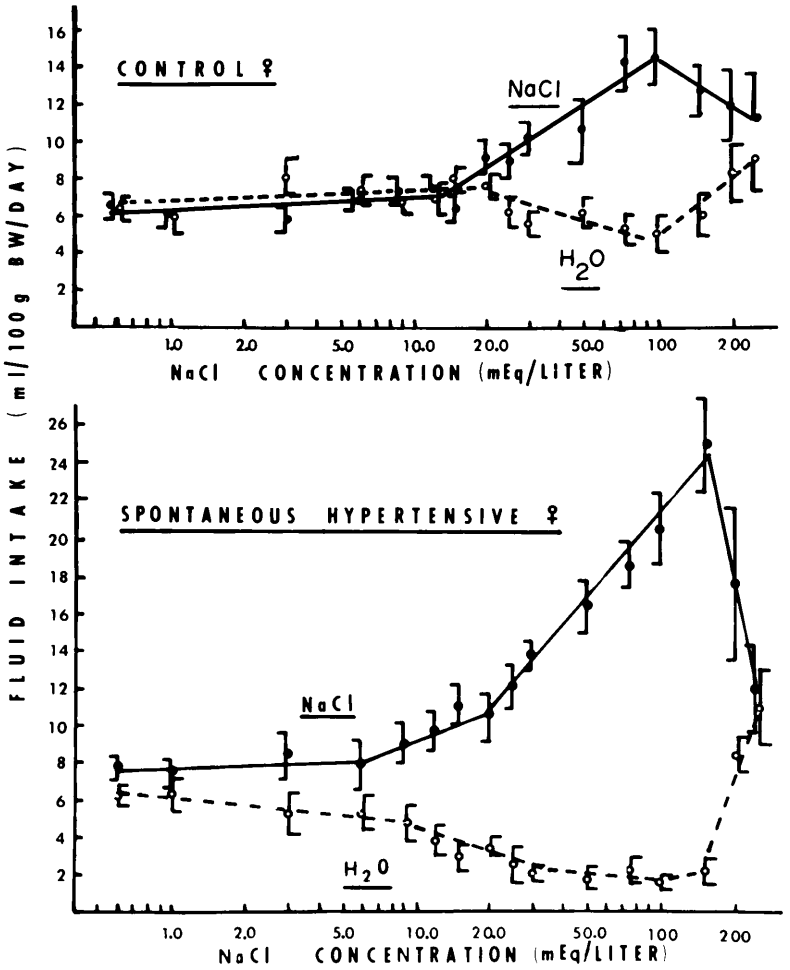

FIG. 1. Spontaneous intakes of NaCl solution (solid line) and distilled water (dashed line) are shown at each concentration of NaCl solution offered to control rats (upper panel) and spontaneous hypertensive (lower panel) rats. One standard error is set off at each mean.

(SE) and 236 ± 3 g, respectively. Final mean body weights were 353 ± 10 and 237 ± 10 g, respectively.

Discussion. The results of these studies verify and extend the recent reports of Catalanotto *et al.* (8) and McConnell and Henkin (9) that SH rats have an appetite for NaCl solutions. The salt appetite is present in both males and females and appears even when a choice is offered between water and a hypertonic NaCl solution (0.025 *M*). The salt appetite is accompanied by a reduction in preference threshold for NaCl solution to a value approximately one-third that of controls (Fig. 1). The preference threshold concentration of control rats (0.030 *M*) was similar to values reported earlier from this laboratory (0.029, 0.023, 0.030) (15–17) but was higher than that reported by Weiner and Steller (0.009 *M*) (18) and Richter (0.0094) (19), both of whom used different strains of rats. The preference threshold concentration of the SH group was similar to that observed earlier for rats treated with propylthiouracil (16), hydrochlorothiazide (15), or Enovid (17). It is of interest that all the experimental procedures thus far tested inducing a chronic appetite for NaCl solution, viz., adrenalectomy, hypothyroidism, and administration of certain hormones and drugs, including des-

oxycorticosterone, propylthiouracil, hydrochlorothiazide, and Enovid, are also accompanied by a reduced preference threshold for NaCl solution (15–17, 20, 21).

The apparent relationship between reduced preference threshold for NaCl solution and NaCl appetite has not been explained satisfactorily, although a number of possibilities exist and have been presented (22). For example, it has been suggested that these two phenomena may be related directly to the Na/K ratio of the saliva bathing taste receptors and indirectly to blood level of aldosterone which appears to control the ratio of salivary Na/K (22). Blood levels of aldosterone may be reduced in SH rats. Sokabe (23) has reported reduced renal renin content, release and angiotensin formation in SH rats and Koletsky *et al.* (24) reported reduced juxtaglomerular granulation. It is also possible that the salt appetite and reduced preference threshold are induced by central nervous mechanisms responding to either altered aldosterone or electrolyte concentrations of blood or to altered extracellular fluid volume. Changes in body sodium content induced by increased renal sodium loss (Table II) may also play a role.

SH rats appear to be relatively hypothyroid compared to control rats (25–27). The salt appetite observed in SH rats may be related to this fact since hypothyroidism induced by either surgical thyroidectomy or a number of different antithyroid drugs increases the appetite for NaCl solution and reduces NaCl preference threshold (22). It is difficult to state which, if any, of the suggestions mentioned above can explain the spontaneous appetite and reduced preference threshold for NaCl solution. Further studies will be required to separate these possibilities.

SH rats had an increased total fluid intake (NaCl plus water) compared to controls (Table I and III). An increased fluid intake is typical of rats with other types of experimentally induced hypertension and has been attributed, in part at least, to renal tubular hypoxia and damage (4, 6). The ability of the SH rat both to produce and respond to antidiuretic hormone was not tested but may be important in explaining the increased total fluid intake.

SH rats increased their urinary sodium output when loaded acutely with a hypotonic NaCl solution but not when loaded acutely to the same extent with isotonic saline solution (Table II). Reasons for the difference are difficult to state but may be related to changes in glomerular filtration rate as well as an altered renal tubular reabsorptive capacity for sodium. Further study will be necessary to verify and explain the differences in urinary sodium excretion under these conditions.

The reduced growth rate and body weight of SH rats has not been explained adequately. No differences in body weights between original SH rats and normotensive rats of the same strain were reported by Aoki (28). During successive generations other genetic changes may have occurred. For example, present evidence suggests that SH rats are hypothyroid relative to controls (25–27). This may account for the reduced rate of growth. Secretion of growth hormone by the anterior pituitary of SH rats awaits testing. Even if subsequent testing shows no deficiency of growth hormone in SH rats, it is well known that there is an interaction between growth hormone and thyroxine such that growth hormone may fail to manifest its full physiological effects if adequate thyroxine is absent (29). While relative food intake (g/100 g body wt/day) of the SH rats is similar to that of normotensive controls (Tables I and III), actual food intake (g/day) is less.

SH rats differ from rats with experimentally induced hypertensions as well as from the genetically induced type of hypertension in that they have an appetite and a reduced preference threshold for NaCl solution as contrasted to the NaCl aversion of the latter types (1–7). Further, renal hypertensive rats showed no change in their preference threshold for NaCl solution (30). It is thus obvious that an elevated blood pressure per se cannot account for either the salt appetite or salt aversion. The mechanism or mechanisms responsible for this difference in NaCl intake among the hypertensive rats remains for further study.

Summary. Both male and female spontaneously hypertensive (SH) rats have an appetite for NaCl solution. The appetite is

present when a choice is offered between distilled water and either isotonic or hypertonic (0.25 *M*) NaCl solution to drink. Total fluid intake (water plus NaCl solution) was greater for SH rats than for controls while food intakes (g/100 g body wt/day) of SH rats were not different from controls. Mean body weight of SH rats was always less than that of controls. The appetite for NaCl solution was accompanied by a significant reduction in preference (detection) threshold. SH rats could detect the difference between distilled water and NaCl solution when the concentration of the latter was 12 mEq/liter compared to a control threshold of 30 mEq/liter. The NaCl appetite and reduced NaCl preference threshold induced by spontaneous hypertension is in marked contrast to the NaCl aversion induced by other types of experimentally induced hypertension in rats. The mechanism or mechanisms responsible for these differences remain for further study.

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Received March 31, 1975. P.S.E.B.M., 1975, Vol. 149.