Increased Preference for Na^+ and K^+ Salts in Spontaneously Hypertensive (SH) Rats (37282)

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(Introduced by Frederic B. Bartter)

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Preference for NaCl in SH rats has been shown previously to be significantly higher than that of control normotensive rats (1). However, comparison of preference for other salts and for sour or bitter substances has not yet been undertaken. In order to define more clearly the preference behavior of SH rats, preference for various salt solutions (NaCl, KCl, $NaHCO_3$, $NaC_2H_3O_2$) and for quinine sulfate and HCl, was compared in SH and normotensive control rats.

Methods. Male SH rats (270 ± 10 g) were used as experimental animals and male, normotensive Wistar/NIH (WI/NIH) rats (273 ± 3 g), and male, normotensive Wistar/Kyoto (WI/KY) rats (315 ± 10 g), were used as controls. SH rats are a genetically hypertensive strain initially bred from a strain of Wistar rats in Japan, but bred at NIH since 1966. The characteristics of these rats have been previously described in detail (2). WI/NIH rats are a strain of normotensive Wistar rat, bred in the United States at NIH since 1950, and used as controls for SH rats in previous experiments by ourselves (1), and by other investigators (2, 3). WI/KY rats are a strain of normotensive Wistar rats derived from the same strain from which selective brother–sister inbreeding in Japan and later in the U.S. uniformly produced SH rats.

Each rat was housed individually, beginning 5 days prior to testing, in a specially designed stainless steel metabolism cage (4) to which metal feeding cups and two graduated drinking tubes (4) were attached. Humidity, temperature (24°), and light–dark cycles (5:30 PM–5:30 AM dark) were controlled. Food (pulverized Purina lab chow) and deionized distilled water were available *ad libitum*. Food intake was measured daily and did not differ between SH rats and either control group on any day regardless of the solutions presented for testing.

Measurement of preference behavior consisted of giving each rat a choice between water and a solution of solute in water over a 24-hr time period in a free-choice, two-bottle preference test (4, 5). A test period consisted of 7 consecutive days; during the first 4, rats were offered a choice between water and water plus solute, during the next 3, they were offered a choice between two bottles of water. On the third day of the test period the fluids in the bottles were switched to prevent position preference, each bottle being thoroughly washed with deionized, distilled water prior to the insertion of the alternate solution. All test solutions were prepared immediately prior to use with reagent-grade sodium chloride, sodium bicarbonate, sodium acetate, potassium chloride, hydrochloric acid and quinine sulfate (Matheson, Coleman and Bell, East Rutherford, NJ) and deionized, distilled water. Measurement of intake was recorded daily from 9:00 AM to 9:00 AM. Percent preference for the test solution was expressed as:

$$\frac{\text{ml test solution consumed/24 hr}}{\text{ml total fluid consumed/24 hr}} \times 100.$$

Data for the first 4 days of the test period were averaged for all animals under each condition, and the mean daily preference calculated. Total fluid intake (water plus test solution)/100 g of body weight and volume of test solution consumed/100 g of body weight,

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were also recorded. Body weight of each rat was measured weekly.

Weekly measurement of blood pressure using the arterial pulse in the tail was carried out with an inderect plethysmographic technique (6) with a physiograph and a small animal study unit attached (E. and M. Instrument Company, Houston, Texas, Physiograph "Four" with attachments 92-600-70, 94-800-78, 94-801-70, and 94-801-72 and a programmed electrosphygmomanometer). Prior to measurement of blood pressure, each rat was placed in a specially constructed heating box made by the Biomedical Engineering Instrumentation Branch, NIH. It was made of "Benelex" (compressed board) with two Chromolox strip heaters (325 W, 120 V), a thermometer and a Chromolox temperature control (50–250°F) attached. Prior to measurement of blood pressure, rats were kept in the box for 10 min at an ambient temperature of 37°.

Results. Figure 1 compares preference for the various salt solutions offered to SH rats, WI/NIH and WI/KY controls. SH rats exhibited increased preference with respect to controls for all concentrations of NaCl (0.15 *M*, 0.30 *M*, and 0.45 *M*), KCl (0.15 *M*), $NaHCO_3$ (0.15 *M* and 0.30 *M*) and $NaC_2H_3O_2$ (0.30 *M*), (*t* test, $p < 0.01$ for 0.30 *M* $NaHCO_3$, $p < 0.001$ for all others). No significant differences in preference were observed between SH rats and controls when presented with 0.45 *M* $NaHCO_3$, quinine sulfate (2.6×10^{-6} *M*), or HCl (0.002 *M* and 0.004 *M*).

Total fluid intake/100 g of body weight was significantly greater in SH rats as compared with WI/NIH controls for NaCl (0.15 *M* and 0.30 *M*) and $NaHCO_3$ (0.15 *M*) (*t* test, $p < 0.001$) and for 0.15 *M* KCl (*t* test, $p < 0.01$); it was significantly greater in SH rats than in WI/KY controls for 0.30 *M* NaCl and 0.30 *M* $NaC_2H_3O_2$ (*t* test, $p < 0.001$) and for quinine sulfate (2.6×10^{-6} *M*) and 0.004 *M* HCl (*t* test, $p < 0.005$) (Fig. 2). There were no significant differences in total fluid intake between SH rats and WI/KY controls for $NaHCO_3$ (0.30 *M* and 0.45 *M*) or 0.002 *M* HCl.

For the SH rats total fluid/mEqiv of Na^+ ingested (excluding that in food which accounted for 1.2 mEqiv Na^+/100 g of body weight per day) ranged from 4.9 ml/mEqiv Na^+/100 g of body weight for NaCl (0.30 *M*) to 6.9 ml/mEqiv Na^+/100 g of body

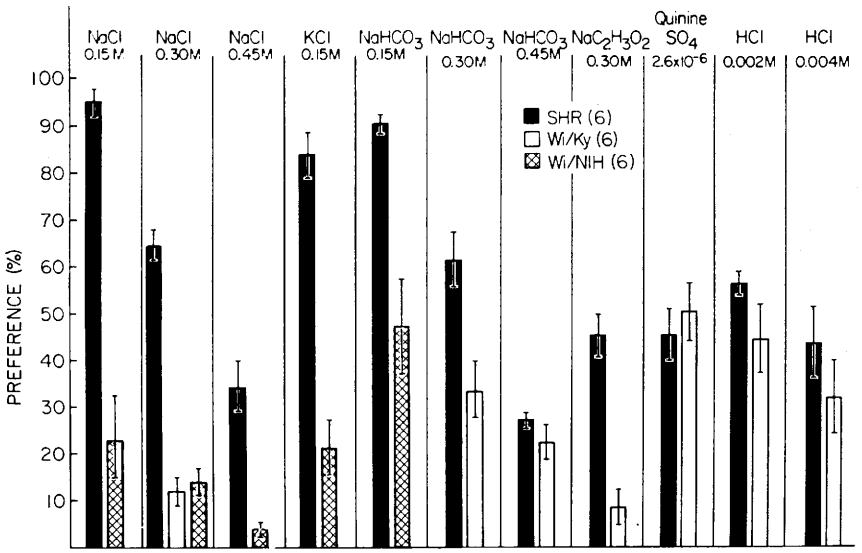

FIG. 1. Preference for various salt solutions and HCl in spontaneously hypertensive rats (SHR), a Wistar strain, and in normotensive control rats, Wistar/Kyoto (Wi/Ky) and Wistar/NIH (Wi/NIH). Vertical bars represent the mean daily preference for a four-day test period in each group of rats for each substance tested. Lines through the bars represent ± 1 SEM.

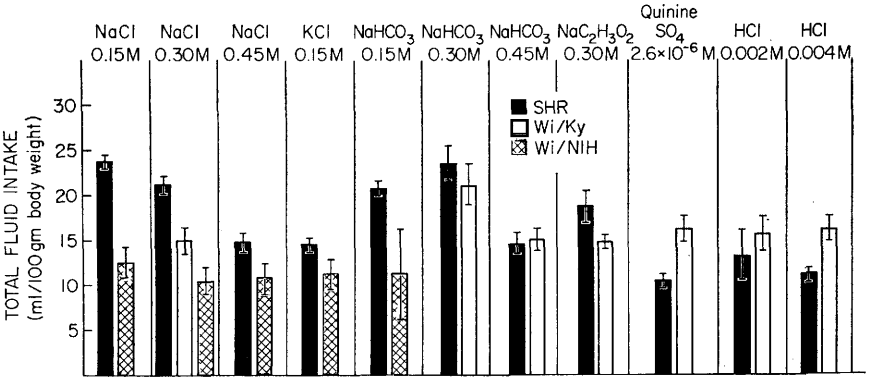

FIG. 2. Total fluid intake (water plus test solution) imbibed by SH, Wi/Ky and Wi/NIH rats during each four day test period. Vertical bars represent the mean daily intake calculated per 100 gm body weight for each group of rats for each substance tested. Lines through the bars represent ± 1 SEM.

weight for 0.45 *M* NaCl, 0.30 *M* $NaHCO_3$ and 0.30 *M* $NaC_2H_3O_2$. Total fluid intake/mEqiv of K^+ was 8.5 ml/mEqiv K^+/100 g of body weight for 0.15 *M* KCl. However, for the controls, fluid intake ranged from 7.8 ml/mEqiv Na^+/100 g of body weight for 0.30 *M* $NaHCO_3$ (WI/KY), to 210 ml/mEqiv Na^+/100 g of body weight for 0.45 *M* NaCl (WI/NIH). WI/NIH controls had a total fluid intake of 36 ml/mEqiv K^+/100 g of body weight with 0.15 *M* KCl.

Intake of Na^+ in fluid, expressed as mEqiv/100 g of body weight for 24 hr was significantly greater in SH rats compared to WI/NIH and WI/KY controls for all solutions for which an increased preference had been demonstrated. For the SH rats this ranged from 1.75 mEqiv/100 g of body weight for 0.45 *M* $NaHCO_3$ to 4.5 mEqiv/100 g of body weight for 0.30 *M* NaCl and $NaHCO_3$. Intake of sodium by WI/NIH and WI/KY controls never exceeded 0.8 mEqiv/100 g of body weight for any solution.

Systolic blood pressure of the SH rats was 195 ± 8 mm Hg (mean ± 1 SEM) while systolic blood pressure of the WI/NIH rats was 120 ± 8 mm Hg, that of the WI/KY rats 116 ± 6 mm Hg. No significant change in blood pressure occurred in any group of rats during the 13 weeks of the experiment.

Discussion. These findings demonstrate that SH rats prefer not only solutions of sodium salts more than controls but also a potassium salt as well. However, the absence of any difference in preference for quinine sulfate and HCl between SH rats and controls suggests that altered taste acuity in SH rats cannot account for their increased preference for salts. Indeed, these data are the first to demonstrate an increased preference for salts without changes in preference for sour or bitter substances in any rat.

Rats made hypertensive by latex encapsulation of the kidney demonstrated aversive behavior for a variety of solutions. This behavior was attributed to defective water metabolism and to concomitant development of increasingly elevated blood pressures (7, 8). Another group of rats with a genetic predisposition to develop hypertension exhibited both aversive behavior for NaCl and a lower-than-normal intake of salt and water (9). The present studies are also the first to demonstrate in a hypertensive rat an increased preference for not only sodium but also potassium-containing salts which is maintained throughout the adult life of the animal.

An inverse relationship was found between the concentration of the salt solution presented (NaCl or $NaHCO_3$) and preference for that solution; *i.e.*, as the concentration of salt increased, preference decreased. This concentration dependence was similar for

solutions of NaCl and $NaHCO_3$.

Increased salt intake is generally accompanied by increased fluid intake in both hypertensive and normotensive rats (7–10). Although exhibiting an increase in total fluid intake compared with controls, SH rats imbibed significantly less fluid per mEqiv of sodium than did the normotensive controls. The mechanism for this phenomenon is not readily apparent.

The SH rats demonstrated a marked avidity for NaCl imbibing 2–5 times the Na^+ imbibed by the normotensive controls. This increased intake of both Na^+ and fluid occurred as long as Na^+ was presented and the increased fluid intake ended as soon as the salt solution was replaced with water. Although SH rats ingested large quantities of Na^+ during 8 of the 13 weeks of the experiment there were no significant changes in their hypertension upon comparison of initial and final values. It is well known that hypertension in man is associated with increased salt and fluid intake and that one of the earliest successful treatments for essential hypertension was dietary restriction of salt and fluid (11–13). In the SH rat there is a clear-cut relationship between increased salt preference on the one hand and genetically determined hypertension on the other. However, the SH rat prefers both Na^+ and K^+ salts imbibing both at a significantly increased rate. This lack of intake specificity, which is independent of taste acuity, may be an important clue to the mechanism by which increased salt preference and subsequent hypertension may occur.

Summary. Preference for solutions of various salts and for quinine sulfate and hydrochloric acid was compared in spontaneously hypertensive (SH) male rats and in two groups of normotensive control rats by a two-bottle preference test. SH rats exhibited a significantly increased preference for salt solutions containing Na^+ and K^+, but for neither quinine nor hydrochloric acid. When given a choice between 0.30 *M* NaCl and water, SH rats imbibed 2–5 times the amount of Na^+ imbibed by the controls. SH rats imbibed significantly more total fluid (salt and water) than did controls as long as salts were presented. However, as soon as water alone was presented, total fluid intake returned to control levels.

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