Longitudinal Study of Salt Preferences in Normotensive and Hypertensive Rats

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SUMMARY To determine whether age-related changes in salt preferences occur over the lifespans of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY), the same animals of each genotype were tested as juveniles, and as young and older adults. Taste preference ratios for NaCl and KCl, at concentrations from 0.001 to 1.0 M, were calculated using 24-hour, two-bottle preference tests of each salt versus distilled water. Genotype exerted a significant effect on preference for both NaCl and KCl (p < 0.0005). At each age and across concentrations, SHR had consistently higher preferences than did WKY. Few marked, age-related changes in overall preference for NaCl were noted within either strain, but juvenile and older adult SHR and WKY exhibited stronger preferences than did young adults for the higher concentrations of NaCl below the rejection threshold (p < 0.001). Statistical age by concentration preference trends for KCl were similar to those for NaCl in SHR. Young adult WKY, however, had a significantly lower rejection threshold for that compound than did juveniles and older adults (p < 0.001). These results indicate that genotype, age, salt type, and salt concentration can interact to influence salt preference in hypertension.

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KEY WORDS • taste preference • spontaneous hypertension • sodium chloride • potassium chloride

THE relationship between gustatory sensitivity to salt, taste preference for salt, and hypertension is not clearly understood. Early researchers suggested that hypertensive humans cannot detect the taste of salt as well as can individuals with normal blood pressure.1-2 Taste threshold studies, however, have produced discrepant results, with some laboratories reporting elevated salt thresholds in hypertensive subjects and others reporting normal values. Two recent reviews of the evidence for salt taste changes with hypertension3-4 attribute ambiguities in part to lack of procedural uniformity across experiments. Additionally, it has been shown that taste threshold values for detection and for recognition of NaCl cannot predict the intensity perception and hedonic responses of hypertensive subjects to salt at suprathreshold concentrations representative of the "real taste world."13 Present evidence is suggestive that normotensive and hypertensive subjects are similar in salt detection threshold (i.e., the lowest concentration that can be detected but not identified), while recognition threshold (the lowest concentration that can be correctly identified) may be elevated in persons with hypertension. Both taste sensitivity and taste preferences for suprathreshold salt concentrations appear to be unaltered by hypertension. For a comprehensive evaluation of this evidence, see the 1984 review by Mattes.4

In humans the nature of the temporal relationship between the onset of hypertension and alterations in salt taste is unclear, perhaps because the onset of the disease is gradual and is thought to occur over many years. Taste perception has been reported to change, however, during the course of several other diseases.6-7 Age per se is also a factor reported to affect the ability to detect salt; small but reliable decreases in salt taste acuity have been observed with advancing age.8,9 Thus, the factors of age and disease state may affect salt taste in individuals with high blood pressure, and the long-term results might be alterations in salt intake that could affect the eventual course of the disease.

In contrast to findings reported in many human studies to date, adult spontaneously hypertensive rats (SHR) exhibit higher preferences than do age-matched normotensive Wistar-Kyoto rats (WKY) for NaCl10-17 and other sodium salts,11 as well as for KCl.11,12,14-16 Whereas high sodium loads exacerbate the spontaneous hypertension already present in SHR, severe sodium restriction results in failure to grow and premature mortality.18-21 Thus, it has been speculated that the
enhanced NaCl preference observed in SHR is a consequence of physiological need. Their high preference for KCl also might represent an adaptive mechanism, as the addition of 4% KCl to a 4% dietary NaCl load can reduce the magnitude of rise in the blood pressure of SHR.

Two studies have examined age effects on salt preference strength. McConnell and Henkin found that preference for 0.3 M NaCl over distilled water increased in SHR, but not in WKY, as the animals matured from the weanling stage to 19 weeks of age. In a preliminary report using the same testing procedure, reported that juvenile SHR and WKY both exhibited slightly higher preferences for a number of supra-threshold concentrations of NaCl than when restested as young adults, but noted no age differences in the preference of SHR for 0.32 M NaCl, the concentration most closely approximating that used by McConnell and Henkin.

It is difficult to draw conclusions from the literature concerning possible developmental changes in salt preference of SHR and WKY because wide age ranges have not been employed and because the variety of salts used and number of stimulus concentrations tested have been limited. Thus, it is important to examine the stability of salt preference over a long period, including the early stage, for both SHR and WKY, to determine whether preference or rejection thresholds shift with time and whether changes occur in the absolute preference ratios and maximally preferred concentrations. If the magnitude of differences in purported cation preferences between SHR and WKY are small, they might be masked by the large within-strain variability that has been noted in some measurements in these animals (Fregly MJ, personal communication). To circumvent this potential problem, the most desirable protocol is a longitudinal one, testing the same individual animals at a number of intervals during their lifespans and using a wide range of stimulus concentrations. For that reason, we have devised the present study to obtain a more precise measure of preference behavior in the same groups of SHR and WKY at three different intervals (juvenile, young adult, and mature, or older, adult), examining preferences for both NaCl and KCl by presenting them over a wide concentration range with stimulus concentration increases progressing in small increments.

Methods and Materials

Ten male SHR and ten age-matched male normoten-sive WKY were supplied by Taconic Farms (Germantown, NY) and maintained in the rat colony. Throughout the 40-week study each animal was housed individually in a quiet, light-controlled (12 hr light/12 hr dark cycle) room maintained at 23°C, provided with distilled, deionized water, and fed ad libitum Rat Chow 5012 pellets (Ralston-Purina Co., St. Louis, MO). The diet is a complete life-cycle, constant formula rat diet containing (by weight) 0.28% sodium, 1.08% potassium, and 0.43% chloride.

Stimuli employed in the taste preference tests were reagent-grade NaCl and KCl dissolved in distilled, deionized water and presented at concentrations from −3.0 to 0.0 log M (0.001–1.0 M). Log molarity was used because perceived intensity over a wide range of stimulus concentrations tends to increase with the logarithm of the stimulus in many types of psychophysical measurements. Stimulus concentrations were increased in one-half log steps between −3.0 and −1.5 log M, and in one-quarter log steps at concentrations from −1.5 to 0.0 log M, the range within which highest preference scores, and subsequent rejection thresholds, were predicted to occur.

Taste preferences for the test stimuli were assessed in three separate experiments conducted over intervals during which the rats were ages 35 to 63 days (juvenile), 77 to 98 days (young adult), and 294 to 315 days (mature, or older, adult) respectively. Preferences were measured using the 24-hour, two-bottle choice technique, in which each animal had access to two drinking bottles during a 24-hour period, one containing the appropriate test solution, the other containing distilled, deionized water. Concentrations of each test compound were presented in ascending order, with the position of the test bottle on the cage counterbalanced in a right-left, left-right sequence. Because the order in which two different compounds are presented in a preference test situation can affect the outcome of the preference scores obtained, in each experiment SHR and WKY were randomly subdivided into two groups so that one-half of the members of each strain received NaCl preference tests first, followed by KCl preference tests; the remaining animals received their tests in the reverse order. For a 48-hour transition interval following completion of the concentration series with the first compound, and before initiation of tests with the second one, each animal was presented with distilled water in both of its drinking bottles. One week after the conclusion of the second series of taste preference tests during a period when the animals were receiving distilled, deionized water, the systolic blood pressure of each animal was measured by the indirect tail cuff method.

The preference score, or percent preference, exhibited by an individual subject for a test solution during a 24-hour session was computed by dividing the number of grams of that test solution consumed by the total grams of fluid consumed during the session (test solution + distilled, deionized water), then multiplying the quotient by 100. Individual taste preference scores were normalized using the logit transformation in which logit preference \( L = \ln \frac{p}{1-p} \), where \( p \) is the ratio of test fluid consumed to total fluid consumed. A repeated-measures analysis of variance was performed separately for NaCl and KCl. Because of the limitations of available computer programs in performing analyses involving the large number of salt concentrations that were used in this study, the analyses excluded the three lowest concentrations of each salt. Preference differences at specific salt concentrations and differences in systolic blood pressure were exam-
FIGURE 1. Preference for ascending concentrations of NaCl in SHR and normotensive WKY tested at three ages. Values are means ± SEM. ○ = p < 0.05; ● = p < 0.01; † = p < 0.001.

Results

Taste Preference for NaCl

Longitudinal trends in taste preference for various concentrations of NaCl are shown for SHR and WKY in Figure 1. Significant main effects were observed for genotype and NaCl concentration (p < 0.0005), but not for age. Significant concentration by genotype (p < 0.001) and age by concentration interactions also were present (p < 0.001; Table 1). Juvenile and older SHR tended to show stronger preferences than did young adults for the three highest concentrations of NaCl before the rejection threshold was reached (rejection threshold is the lowest salt concentration that is rejected in preference to distilled, deionized water; i.e., where preference falls below the 50% level). Differences were significant (p < 0.01) only at -0.75 log M when examined by t test, with Bonferroni’s adjustment for multiple comparisons. The rejection threshold was 0.25 log M for each age group. In WKY, no significant age-related differences in NaCl preference occurred; however, similarly to SHR, juvenile and older WKY showed slightly stronger preferences than did young adults for the two highest concentrations before the rejection threshold was reached.

Taste Preference for KCl

Longitudinal preferences for concentrations of KCl are shown for SHR and WKY in Figure 2. Similarly to findings for NaCl preference, significant main effects were present for genotype and stimulus concentration (p < 0.0005). Unlike results of NaCl preference tests, a main effect of age was observed (p < 0.0005) when KCl was the taste solution employed. Significant interactions were found for age by genotype (p < 0.05) concentration by genotype (p < 0.0005), age by concentration (p < 0.0005), and age by concentration by genotype (p < 0.001; Table 2). Similarly to their responses to NaCl solutions, juvenile and older SHR exhibited slightly stronger preferences than did young adults for KCl at the two highest concentrations before the rejection threshold was reached. All three age groups of SHR had the same KCl rejection threshold of -0.5 log M. The most noteworthy developmental difference observed in either strain for either salt was in the acceptance by WKY of higher concentrations of KCl. Compared with their juvenile and older counterparts, young adult WKY exhibited a rejection threshold for KCl that was one-half log unit lower. The rejection threshold was -0.75 log M for juvenile and

<p>| Table 1. Summary of Analysis of Variance for Effects of Genotype, Age, and NaCl Concentration on Preference for NaCl in Spontaneously Hypertensive and Wistar-Kyoto Rats |</p>
<table>
<thead>
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<th>Source</th>
<th>df</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>1</td>
<td>65.10</td>
<td>51.47†</td>
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<tr>
<td>Error (within animal)</td>
<td>15</td>
<td>1.26</td>
<td></td>
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<tr>
<td>Age</td>
<td>2</td>
<td>0.55</td>
<td>0.68</td>
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<tr>
<td>Age × genotype</td>
<td>2</td>
<td>0.10</td>
<td>0.13</td>
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<tr>
<td>Error (animal × age)</td>
<td>30</td>
<td>0.81</td>
<td></td>
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<tr>
<td>Concentration</td>
<td>6</td>
<td>153.97</td>
<td>171.92†</td>
</tr>
<tr>
<td>Concentration × genotype</td>
<td>6</td>
<td>5.27</td>
<td>5.88†</td>
</tr>
<tr>
<td>Error (animal × concentration)</td>
<td>90</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Age × concentration</td>
<td>12</td>
<td>1.92</td>
<td>2.89*</td>
</tr>
<tr>
<td>Age × concentration × genotype</td>
<td>12</td>
<td>0.82</td>
<td>1.24</td>
</tr>
<tr>
<td>Error (animal × age × concentration)</td>
<td>180</td>
<td>0.66</td>
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</table>

*p < 0.001.
†p < 0.0005.
old WKY and −1.25 log M for young adults. Examination by t tests with appropriate adjustments for multiple comparisons revealed that preferences in young adults were significantly lower (p < 0.05) at −1.25 and −1.0 log M. Within each strain, at each age tested, the rejection threshold was higher for NaCl than for KCl.

Between-Strain Differences in Salt Preference
For ease of comparison, preferences of SHR and WKY shown by age and for each salt in Figures 1 and 2 have been replotted on the same graphs in Figure 3. Differences were present between SHR and WKY in preference for both NaCl (top) and KCl (bottom). At each age tested, for each salt concentration, whenever significant differences occurred, SHR preferred that

<table>
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<th>MS</th>
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<tbody>
<tr>
<td>Genotype</td>
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<td>74.11</td>
<td>110.37§</td>
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<tr>
<td>Error (within animal)</td>
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<tr>
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<td>2</td>
<td>11.41</td>
<td>17.28§</td>
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<td>Age × genotype</td>
<td>2</td>
<td>2.76</td>
<td>4.18*</td>
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<tr>
<td>Error (animal × age)</td>
<td>24</td>
<td>0.66</td>
<td></td>
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<tr>
<td>Concentration</td>
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<td>52.25</td>
<td>71.17§</td>
</tr>
<tr>
<td>Concentration × genotype</td>
<td>6</td>
<td>10.71</td>
<td>14.59§</td>
</tr>
<tr>
<td>Error (animal × concentration)</td>
<td>72</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Age × concentration</td>
<td>12</td>
<td>1.45</td>
<td>2.74†</td>
</tr>
<tr>
<td>Age × concentration × genotype</td>
<td>12</td>
<td>1.61</td>
<td>3.04‡</td>
</tr>
<tr>
<td>Error (animal × age × concentration)</td>
<td>144</td>
<td>0.53</td>
<td></td>
</tr>
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* p < 0.05.
† p < 0.005.
‡ p < 0.001.
§ p < 0.0005.

Concentration of NaCl or KCl more than did WKY. Rejection thresholds for both salts occurred at higher concentrations for SHR at each age than for comparable WKY.

![Figure 2. Preference for ascending concentrations of KCl in SHR and WKY tested at three ages. Values are means ± SEM.](image1)

0 = p < 0.05; † = p < 0.005.

![Figure 3. Comparison between SHR and WKY in preference for NaCl (top) and KCl (bottom). At each age, SHR demonstrated stronger preferences and higher rejection thresholds for each salt than did age-matched WKY. Values are means ± SEM. Significance levels indicated are for two-tailed Student's t tests.](image2)

0 = p < 0.05; † = p < 0.005.
Blood Pressure

Systolic blood pressures were taken at the end of the second series of preference tests when the animals were approximately 3.5 months old. The average systolic pressures (± 1 SEM) in SHR and WKY were 179 ± 7 mm Hg and 133 ± 5 mm Hg respectively (p < 0.001).

Discussion

In general, our studies confirm those of earlier investigators, who reported increased preferences for NaCl and KCl in SHR, and extend them by demonstrating that the enhanced preferences for those compounds persist long after the animals reach maturity and that the hypertensive state is of long standing. In present-day stock from Taconic Farms (the source of the animals used in this study) and in SHR reared within our own breeding colony, blood pressure begins to plateau around the twelfth to thirteenth week of age, 30 so the initial measurements were made in the early hypertensive (rising pressure) phase, the second measures when the pressure was leveling off, and the third ones when the pressure had stabilized.

In the case of NaCl preference, while no significant overall main effect of age nor age by genotype interaction was found, both age by concentration and genotype by concentration interactions were present. At every concentration for which significant differences existed, juvenile and older rats preferred the solution to a greater extent than did young adults. The tendency for younger subjects to prefer stronger concentrations of salt than older subjects has been noted in humans 27 and other species, 28 although reasons for these differences are not known.

Age exerted a significant main effect, and age by genotype interaction was significant in influencing KCl preference. Those effects, as well as the various other significant interactions, were due largely to the markedly lower rejection threshold that was unique to young adult WKY. We have no good explanation for why the strong avoidance of -1.25 and -1.0 log M KCl was limited to that genotype at one developmental age. As rats of both strains were housed together and treated identically throughout the entire study, the failure of young adult SHR to display a similar avoidance pattern suggests that it represents a strain difference peculiar to WKY or random variation for those 10 particular WKY, rather than environmental variables specific to the test situation.

Our data from older rats fail to provide evidence for changes in preference or rejection thresholds, which might be interpreted to mean that animals of either strain had distorted or reduced taste acuity when tested as older (mature) animals. Preference thresholds are not necessarily predictive of preferences for supra-threshold concentrations, however. Also, there is no simple relationship between taste acuity, a sensitivity measure, and taste preference, a hedonic measure. Additionally, it is noteworthy that while our rats in that group were comparatively older than animals of previous investigators, 10, 12, 15 they were not extremely old rats in an absolute sense (<1 year old). Because the average lifespan of SHR is shorter than that of WKY, 18 months versus 24 months, 29 one cannot wait until the animals are in the aged category to complete a longitudinal study because some of the rats will have been lost by that time. The only taste sensitivity study of extremely aged rats (up to 32 months) employed neural taste recordings in Fisher 344 rats, a strain in which no substantial deficits in response magnitude with age occurred. 30 Our use of the same animals at three stages in their development proved worth the time and expense required for longitudinal testing, as the extremely small mean square values (see Tables 1 and 2) for overall animal effect and for every animal interaction effect indicate a great deal of within-animal consistency in our study. This finding is very important, as a frequent problem encountered by other researchers with SHR and WKY has been large within-strain variability, which has the potential for masking small but real between-strain differences.

The effect of hypertensive genotype on preferences was profound at each age. In all three age groups, SHR exhibited higher salt preferences than did WKY. Some physiological reasons hypothesized for the enhanced salt preference of SHR have been salivary Na/K ratio, aldosterone levels in the blood, and central nervous system mechanisms. 13 Measurement of plasma, salivary, and urinary electrolyte levels accompanying salt intake and preference behavior are needed to elucidate possible mechanisms, although in a practical sense, such an undertaking presents methodological difficulties. Taste preference measures are exquisitely sensitive to extraneous environmental manipulations such as blood sampling and blood pressure measurement. Thus, in attempting to make too many types of measurements in such a study, one risks compromising the validity of the taste preference scores. An additional complication in interpreting salivary electrolyte concentrations is their dependence on salivary flow rate. 31

Genotype differences in salt taste sensitivity probably contributed to the observed preference differences. We have recorded chorda tympani taste nerve responses of SHR and WKY ranging in age from 18 days to more than 20 months. 10 Across ages, KCl elicited less neural activity relative to NaCl in SHR than in WKY. This altered neural activity might provide information to the central nervous system of SHR to signal the need to alter ingestion of NaCl, KCl, or both. More recently, in tests using Dahl S and R rats we found that the two strains differed in taste nerve response to chloride salts of sodium, potassium, and calcium, and dietary NaCl loading within each strain changed the magnitude of increase in neural response to a given increase in salt stimulus concentration. 32 Other supporting evidence that altered gustatory nerve firing patterns are related to changes in NaCl intake or preferences comes from Contreras, 33 who found that the chorda tympani nerves of normotensive sodium-deprived rats were less reactive to NaCl solutions than were the nerves of sodium-replete controls and that
individual ‘sodium-best’ taste nerve fibers were most affected. Contreras proposed that the specific reduction in peripheral nerve responsiveness to sodium might be related to the increased salt intake of the sodium-deprived animals.

It is still unclear whether the increased NaCl appetite in SHR precedes the onset of hypertension or whether it is secondary to the disease. Mogenson and Morris conducted preference tests and measured arterial blood pressure with the indirect tail cuff method in SHR and WKY. In that study blood pressures for SHR and WKY were similar at 5 weeks of age (the time of the first pressure measurements) and did not become significantly different until 9 weeks after birth. On the other hand, salt taste preference was already significantly higher in SHR when first tested at 5 weeks of age and they concluded that the increased salt appetite was apparently not the consequence of the elevated blood pressure. Their conclusion rests on the fact that they failed to find any increase in blood pressure before 5 weeks of age, but others have detected a significant early increase in arterial blood pressure in SHR — some as early as the day of birth — by using several different measurement techniques. Gray has reported that pressure rapidly increases throughout the early developmental period in SHR and plateaus during the early adult stage. Thus, results of those studies do not support the contention that high NaCl preference necessarily precedes the onset of hypertension.

Preference tests have not been conducted in neonatal SHR. The limited number of investigations into the ontogeny of taste preferences in neonatal normotensive rats, however, suggests that in the early developmental stage they reject NaCl solutions. Thus, at the present time, firm conclusions cannot be drawn concerning the temporal relationship between increased sodium preference and hypertension in SHR, but the possibility exists that they develop concurrently and very early in the developmental period.

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AGE-RELATED SALT PREFERENCES IN WKY AND SHR

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