Salt Level in Weaning Diet Affects Saline Preference and Fluid Intake in Dahl Rats

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SUMMARY Weanling Dahl salt-sensitive (DS) and salt-resistant (DR) rats were used to compare effects of feeding high or low NaCl diets on taste preference for, and intake of, a wide range of saline concentrations. The DS and DR were fed either 8.0 or 0.4% dietary NaCl for 4 weeks. Then, with all animals fed the 0.4% NaCl diet, their taste preferences for 0.0001 to 0.56 M saline were assessed using three 24-hour two-bottle preference tests of each solution versus distilled deionized water. Saline preference and intake were influenced by concentration and its interaction with genotype, with DS exhibiting higher preferences than DR for hypotonic saline. The DS preexposed to 8.0% dietary NaCl showed elevated consumption levels of water and total fluid (saline + water) that persisted throughout the 5-week test period, despite transfer to the 0.4% NaCl diet before the initiation of preference testing. Findings indicate that genotype, dietary NaCl levels in weaning diet, and saline concentration of preference test solutions interact to influence saline preference and saline and water intake in Dahl rats. (Hypertension 8: 1021-1026, 1986)

KEY WORDS • salt preference • sodium chloride • fluid regulation • taste • Dahl rat • hypertension • early experience

The relationship between NaCl level ingested in the diet and taste preference for salt is poorly understood. Salt intake in Americans is high, and children may receive greater exposure than adults to dietary salt. Children tend to adopt the eating habits of their parents, but the extent to which their salt consumption patterns early in life influence their longterm preference for salty foods is not known.

In humans and rats with a genetic predisposition to hypertension, high levels of dietary salt can precipitate the condition; thus, it is important to investigate possible influences of early salt exposure on later preference and intake in susceptible persons and animal models. Young Dahl salt-sensitive rats (DS) fed low or normal levels of dietary NaCl have higher blood pressures than their Dahl salt-resistant (DR) counterparts. Even in the absence of high NaCl feeding, blood pressures in the salt-sensitive strain increase with age until they eventually reach hypertensive levels. Feeding a high NaCl diet accelerates the rise in blood pressure in DS, and effects occur more rapidly and are more pronounced if the diet is introduced at weaning. These observations suggest that some mechanisms mediating hypertension in DS are independent of NaCl intake, others require high dietary salt for their expression, and a critical developmental period may exist during which the salt-sensitive strain is most vulnerable to the effects of high salt feeding. Blood pressures of DR remain within the normotensive range throughout life and are unaffected by high NaCl feeding. Thus, these two strains, whose characteristics have been reviewed in detail, are potential models for study of the interaction of genotype and early salt exposure in the development of salt preference.

The present study was conducted to compare saline preference and saline and water intake in DS and DR and to determine whether the two strains are affected, either similarly or differently, by high dietary NaCl levels consumed immediately after weaning.

Materials and Methods

Eighteen weanling male DS and 20 male DR were supplied by Brookhaven National Laboratories (Upton, NY, USA). The animals were shipped at 22 days of age and assigned to treatment groups at 23 days of age. Throughout the study animals were housed indi-
individually in hanging wire bottom stainless steel cages in a light-controlled (12-hour light, 12-hour dark) animal room maintained at 23°C. Animal care and use followed guidelines of the National Research Council.

Diets

Rodent Laboratory Chow 5001 (Ralston Purina, St. Louis, MO, USA), a constant formula rodent diet differing only in level of NaCl, was used. Ten rats of each genotype, designated as DS-High and DR-High, were assigned to the high salt condition and were fed a diet containing 8.0% NaCl. Ten DR and eight DS (DS-Low and DR-Low) were assigned to the low salt condition and were fed a diet containing 0.4% NaCl. Two DS escaped when crate damage occurred during shipment, accounting for the reduced number of rats in the DS-Low treatment group.

Design

Upon arrival, animals within each genotype were weighed and assigned either to the high salt or low salt diet. Initial body weights (± 1 SEM) were 82.4 ± 1.2 g and 85.0 ± 1.2 g for DS and DR, respectively, and were not significantly different among the four treatment groups. Each rat was fed its assigned diet for 4 weeks and provided with ad libitum access to distilled deionized water. Rats were weighed and their water intake was measured every 2 days. After 4 weeks, all rats were fed the low salt diet for the remainder of the study while taste preference testing for NaCl solutions was conducted. We purposely did not measure blood pressures of animals before preference testing in an attempt to avoid confounding our behavioral results by transporting the already stressed animals. The DS and DR obtained at the same time and subjected to identical dietary treatments preceding gustatory nerve recordings in parallel studies exhibited blood pressures of 108 and 103 mm Hg for DR-Low and DR-High and of 127 and 197 mm Hg for DS-Low and DS-High, respectively.

Preference Testing

Solutions employed in taste preference tests were made from reagent grade NaCl dissolved in distilled deionized water and presented in ascending concentrations from 0.0001 to 0.56 M (−4.0 to −0.25 log M). Saline concentrations were increased in one-half log steps between 0.0001 and 0.032 M and in one-quarter log steps at concentrations from 0.032 to 0.56 M, the range within which the highest preferences, followed by subsequent rejection, were predicted to occur based on earlier observations of spontaneously hypertensive and normotensive Wistar-Kyoto rats and of DS and DR.

Before the introduction of saline solutions in actual preference tests, all animals were given two 24-hour pretests in which they were presented with two bottles on their cage fronts, each containing distilled deionized water. This procedure conditioned the animals to the presence of two bottles from which to choose and, in the case of the two treatment groups making the dietary change from 8.0 to 0.4% NaCl, it offered a 48-hour transition period before the initiation of preference tests employing water and saline solutions. Three 24-hour two-bottle preference tests were conducted at each NaCl concentration. During each test, an animal had access to two drinking bottles, one containing the saline solution, the other containing distilled deionized water. The daily placement of the test bottle on the cage front was changed in a double alternation (right-left-right-left) sequence in an attempt to counterbalance effects of possible position preferences. Each rat’s preference score (percent preference) for the saline solution during a 24-hour test session was computed by dividing the amount of saline consumed (in grams) by total amount of fluid consumed (saline + water) and multiplying the quotient by 100. Whereas individual preference scores depend on the absolute intakes of both saline and water, they represent a ratio of saline to total fluid consumed. Thus, those scores alone lack the ability to reflect the absolute amount of saline and water consumed and total fluid consumption. Differential and total fluid consumption throughout preference testing was also examined.

Statistical Analyses

Effects of genotype and diet on body weight were examined on Experimental Day 28 only, after 4 weeks of feeding the assigned diets, using a simple two-way analysis of variance. Because of unequal variances, Ln transformations were performed on water, saline, and total fluid intake scores, which were averaged over 4-day intervals during the 4-week prepreference testing period. They were analyzed by a three-factor analysis of variance with repeated measures over the seven 4-day time intervals. Taste preference scores represent proportions that may vary between 0 and 1. The distribution of such proportions is skewed at each tail. Accordingly, individual taste preference scores were first normalized using the logit transformation in which logit preference $L = \ln(p/1-p)$, where $p$ is the ratio of test fluid consumed to total fluid consumed. Effects of genotype, diet, and their interaction on saline preference and on saline intake, water intake, and total fluid intake over the preference testing period were analyzed by three-factor analyses of variance with repeated measures over the 11 NaCl concentrations.

Results

Body Weights

Mean body weight gain on Experimental Day 28 (Figure 1) was influenced by genotype ($F_{1,34} = 15.23$, $p < 0.0005$) and diet ($F_{1,34} = 20.38$, $p < 0.0005$), with no significant genotype × diet interaction. Inspection of the data indicates that DR weighed more than DS regardless of dietary NaCl level and that, within each strain, animals fed 0.4% NaCl weighed more than those consuming 8.0% NaCl.
SALT PREFERENCE AND FLUID INTAKE IN DAHL RATS/Ferrell et al.

Prepreference Fluid Intake

Mean fluid intake for the four groups of rats during the 4-week period preceding preference testing (expressed per 100 g body weight) is shown in Figure 2. Fluid consumption, averaged over seven 4-day time intervals, was affected by genotype ($F_{1,34} = 30.05, p < 0.005$) and diet ($F_{1,34} = 1.887.5, p < 0.0005$). There was also a significant genotype x diet interaction ($F_{1,34} = 40.86, p < 0.0005$). During each 4-day interval preceding preference testing, DS fed the high NaCl diet drank significantly more water per 100 g body weight than did DR consuming the same diet ($p < 0.05$). The DS-High and DR-High animals drank approximately four times and three times, respectively, the amounts of water consumed by their DS-Low and DR-Low counterparts. When fed the low salt diet, the two genotypes differed significantly in water consumption during the initial 4-day period and again during the last two 4-day periods ($p < 0.05$), with DR exhibiting higher fluid intakes.

NaCl Preferences and Fluid Intakes

Effects of genotype, diet, saline concentration, and their interactions on taste preference ratios and on saline, water, and total fluid intake are summarized in Table 1. Solution preference ratios over the 11 NaCl concentrations are shown separately for rats in the high NaCl and low NaCl dietary treatment groups in the left panels of Figures 3 and 4, respectively. Across concentrations, a marginal main effect of genotype on taste preference was observed, with DS tending to have higher saline preferences. A significant concentration effect and a concentration x genotype interaction were present. Preference for saline solutions increased with concentration to about 0.18 M and dropped dramatically as concentration was increased further. The DS in both diet groups exhibited higher preferences than did DR for those midrange concentrations.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>NaCl preference</th>
<th>Saline intake</th>
<th>Water intake</th>
<th>Total fluid intake (saline + water)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$F$</td>
<td>$p$</td>
<td>$F$</td>
<td>$p$</td>
</tr>
<tr>
<td>Genotype</td>
<td>1</td>
<td>3.59</td>
<td>0.067</td>
<td>0.94</td>
<td>0.340</td>
</tr>
<tr>
<td>Diet</td>
<td>1</td>
<td>1.20</td>
<td>0.282</td>
<td>1.45</td>
<td>0.237</td>
</tr>
<tr>
<td>Genotype x diet</td>
<td>1</td>
<td>0.16</td>
<td>0.689</td>
<td>2.44</td>
<td>0.127</td>
</tr>
<tr>
<td>Error</td>
<td>34</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration</td>
<td>10</td>
<td>68.62</td>
<td>0.0001</td>
<td>99.25</td>
<td>0.0001</td>
</tr>
<tr>
<td>Concentration x genotype</td>
<td>10</td>
<td>3.54</td>
<td>0.0002</td>
<td>4.79</td>
<td>0.0001</td>
</tr>
<tr>
<td>Concentration x diet</td>
<td>10</td>
<td>1.70</td>
<td>0.080</td>
<td>1.88</td>
<td>0.046</td>
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<tr>
<td>Concentration x genotype x diet</td>
<td>10</td>
<td>1.39</td>
<td>0.182</td>
<td>1.19</td>
<td>0.298</td>
</tr>
<tr>
<td>Error</td>
<td>340</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
No overall main effects of genotype, diet, or their interaction on saline intake were present. A significant concentration effect and concentration × genotype and concentration × diet interactions were present. NaCl intake per 100 g body weight increased with increases in concentration of the test fluid to 0.18 M and then decreased markedly. At concentrations through 0.1 M, DS-High ingested more saline than DS-Low, a diet effect not seen in DR. At concentrations through 0.18 M, DS-High ingested more saline than DR-High, whereas saline consumption of DS-Low and DR-Low was essentially the same.

Across concentrations, a significant main effect of diet and a genotype × diet interaction on water intake were present. The DS preexposed to the high NaCl diet subsequently consumed more water than did DS-Low and all DR. A significant concentration effect and a concentration × genotype interaction were present. The water intake of DS-High was highest compared with that of rats in the three other treatment groups when hypertonic saline concentrations were offered in the two-bottle preference tests.

Across concentrations, total fluid intake (saline + water) was significantly influenced by diet and by a diet × genotype interaction. The DS previously exposed to 8.0% dietary NaCl exhibited lasting elevations in total fluid intake, despite replacement of that diet with the 0.4% NaCl diet before the initiation of preference testing. The DS fed 0.4% NaCl throughout the course of the study resembled DR-High and DR-Low, which in turn showed similar total fluid consumptions. Total fluid intake during preference testing was also significantly affected by saline concentration, concentration × genotype, and concentration × genotype × diet interactions. Total fluid ingested increased in all four treatment groups until the test solution became hypertonic, after which it decreased. The DS preexposed to 8.0% NaCl diets consumed more total fluid than did DS-Low throughout preference testing. The total fluid consumption of the two groups of DR was similar until the test saline concentration reached 0.1 M, after which time DR-Low had slightly higher intakes.

**Discussion**

Our findings indicate that genotype, level of NaCl in weaning diet, saline concentration employed in the preference test solution, and their interactions influence saline preference ratios and actual intakes of saline, water, and total fluid. All significant effects exerted by genotype on saline preference and the various intake measures were dependent on the NaCl concentration being employed in the preference tests. Diet, in contrast, exerted significant main effects across concentrations on both water intake and total fluid intake. It also interacted with saline concentration in determining saline intake.

In our animals, obtained from the present day Brookhaven colony, we observed intake patterns somewhat different from those of the animals first selectively bred by Dahl and his colleagues over 20 years ago, whose saline intake differences were described by Wolf et al. in 1965. They reported that DS exhibited significantly lower absolute intakes of isotonic (0.15 M) saline and of 0.32 M hypertonic saline than did DR at 0.18 M. The saline intakes of our DS-Low and DR-Low were virtually identical and preference ratios of DS-Low were actually higher, because of their comparatively lower water intakes. At 0.32 M, our next ascending concentration, our DS-Low, like those of Wolf et al., had lower preference ratios and
sodium intakes than did DR. The observed differences may have developed over the years as a result of genetic drift.

By testing solutions spanning nearly 4 log units, we have extended observations of Dahl rats' salt preferences to the hypertonic range, over which DS, relative to DR, tended to express higher saline concentrations, regardless of NaCl level fed during the 4-week period immediately preceding preference testing. It is noteworthy that while we and others have used the term low salt in reference to 0.4% NaCl diet as contrasted with high salt diet containing 8.0% NaCl, it is not a low salt diet in an absolute sense. Dietary NaCl levels of 0.22% give maximal body weight gain in weanling rats, and levels of 0.05% have been established as adequate for gestation and lactation.

The DS showed enhanced preferences for, and intakes of, a range of hypertonic saline concentrations despite the fact that high NaCl intake accelerates the age-related rise in blood pressure in that strain. Furthermore, DS previously fed 8.0% NaCl ingested more saline per 100 g body weight during preference tests than did rats maintained on 0.4% NaCl throughout the study. Rats have a hedonic liking for salt that is unrelated to physiological need. Taste is involved in the continued appetite for salt after deficiency states have been corrected. Sectioning of the gustatory nerve, bypassing the oral taste receptors through the esophageal fistula, and lesioning thalamic taste areas, reduce salt preferences. We are examining neural taste responses to NaCl concentration series in DS and DR fed 0.4% of 8.0% NaCl diet for three or more weeks. Preliminary data indicate that genotype differences in salt taste sensitivity exist between DS and DR and that feeding the high salt diet to either strain increases the magnitude of its neural taste response to NaCl. Thus, differing peripheral taste inputs might play a role in the preference and intake behaviors observed in this study. However, central nervous mechanisms, hormones, and postgestional effects mediated by other receptors are also involved in salt appetite.

Similarly to observations by Contreras and Kosten, for Sprague-Dawley rats, our animals fed high salt diets had lower body weight gain than rats fed low salt diets. The highest salt concentrations fed by those researchers was 3.0%, but their animals received both prenatal and neonatal exposure through the dams' diet. In the present study, DR-Low and DR-High had water intakes that were virtually identical across the 11 saline concentrations. The DS-High exhibited slightly higher water intakes than did DS-Low at saline test concentrations up to 0.056 M. When saline solutions exceeded that concentration, water intakes of DS-High were still higher relative to DS-Low. Contreras and Kosten, in contrast, observed decreased water consumption by offspring of their high NaCl–fed dams. Other researchers have reported that offspring of salt-deprived Sprague-Dawley dams have much higher water intakes than do normally reared pups. Those two studies suggest that concentration of sodium consumed prenatally and neonatally is inversely related to the degree of fluid intake as adults and that during the early developmental period the rat may be particularly sensitive to lasting alterations in dietary salt concentrations of set point levels for maintaining fluid balance. Our failure to find similar water intake patterns in our Dahl strains might arise from the fact that, although DS and DR were originally derived from Sprague-Dawley stock, they were selectively bred for elevated blood pressure response, or lack of it, with extremely high NaCl feeding. As DS become hypertensive, they also manifest progressive renal lesions. This kidney damage may necessitate higher water consumption to excrete solute loads. The Dahl salt-resistant rat cannot be considered an "average" normotensive rat like the Sprague-Dawley, for it is unusually hardy to withstand chronic 8.0% NaCl loads with no elevation in blood pressure. It might represent an extreme end of the normal distribution of normotensive rats. Possibly, in selecting for resistance to hypertension in the presence of a high NaCl diet, Dahl and his colleagues incidentally selected for altered salt taste characteristics in that strain. Indeed, the salt taste preference function reported here for DS closely resembles that for Sprague-Dawley rats, and both strains exhibit overall higher preferences than do DR.

A chief concern is possible adverse effects of early NaCl exposure on subsequent water intake and fluid balance in the DS, a model for low renin salt-sensitive human hypertension. The hypertension is more severe and is produced more quickly and reliably when a high NaCl diet is introduced immediately after weaning rather than postponed until the animal is several months old. Early introduction of salt into the diet means that it acts on a less mature and perhaps more malleable organism, with cumulative exposure at all subsequent periods. Thus, water intake and the regulation of fluid balance in the infant may be influenced by early exposure to and perception of saltiness. Results of the present study support that idea, as a 4-week preexposure of weanling DS to 8.0% dietary NaCl brought about lasting elevations in water intake, despite replacement of that diet by one containing only 0.4% NaCl.

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