Sodium intake is increased by social stress and the Y chromosome and reduced by clonidine

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Ely, Daniel, Michael Herman, Lawrence Ely, Linda Barrett, and Amy Milsted. Sodium intake is increased by social stress and the Y chromosome and reduced by clonidine. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R407–R412, 2000—The objectives were to determine 1) if female rats have higher Na intake than males and if social stress increases Na intake, 2) if the sympathetic nervous system (SNS) mediates the stress effects and the gender effect, and 3) if the Y chromosome (Yc) from a hypertensive father increases Na intake. Four rat strains (n = 10/group) of both sexes were used: 1) Wistar Kyoto normotensive (WKY), 2) an F16 backcross with a Yc from a hypertensive father (SHR/y), 3) spontaneously hypertensive rat (SHR), and 4) an F16 backcross with a Yc from a normotensive father (SHR/a). Females showed greater baseline Na intake than males (hypertensive strains), intruder stress increased Na intake, and clonidine decreased Na intake, but not in WKY or SHR females. SHR/y males had higher baseline Na intake compared with WKY males. In conclusion, the higher Na intake in females during baseline and stress was partially mediated through the SNS in hypertensive strains and the SHR Yc was partially responsible for the increased Na intake in SHR/Y and SHR males compared with WKY.

Our laboratory is interested in the effects of sodium and stress on blood pressure (2, 7–9, 13) and the gender and genetic differences in terms of mechanisms of sodium appetite (18, 20). Previously, we showed that the sympathetic nervous system (SNS) was partially responsible for the stress-induced increase in sodium intake (2, 8). However, we did not examine females and, at that time, only had derived the F1 generation hybrid crosses [spontaneously hypertensive (SHR) × Wistar Kyoto (WKY)] to examine a Y chromosome effect. The objectives of the present study were to examine sodium appetite and preference in four strains of rats. Eight groups (n = 10/group) of male or female rats were studied from 4–6 mo of age: normotensive WKY rats, SHR, and F16 hybrid backcrosses between SHR female × WKY male (SHR/a), and the F16 reciprocal cross WKY female × SHR male (SHR/y) (7–9).

The rationale for these crosses was to observe the physiological effects of the SHR Y chromosome added to a WKY background (SHR/y) and removed from the SHR background (SHR/a). After 16 generations, 99.9% of the autosomes in SHR/y are from the WKY and the Y chromosome from SHR. Likewise, 99.9% of the autosomes in SHR/a are from SHR and the Y chromosome from a WKY. Therefore, the comparisons are between WKY vs. SHR/y, and SHR vs. SHR/a, because the only difference is the Y chromosome. Three or four animals were placed in a plastic cage (50 × 38 × 20 cm) with a stainless steel lid and a bedding of wood chips (P. J. Murphy Forest Products, Montville, NJ) that was changed weekly. Therefore, each strain of rat was housed in a total of three cages. Each strain was kept in a separate room with controlled humidity (40–50%) and temperature (25–27°C). Each group was given a four-bottle choice of NaCl solution ad libitum (0.0, 0.5, 1.0, and 1.5% NaCl) and were provided with rat chow (0.3% Na, Teklad, Madison, WI). The amount of sodium consumed in the food was ~50 mg/day. Total sodium consumed in the drinking water and saline concentration preference were determined daily by weighing the water bottles to the nearest 0.1 g and calculating fluid and sodium consumption as reported previously (2). To get an accurate baseline, Na intake and preference for three consecutive 14-day periods were measured to show if steady-state Na intake was consistent (6 wk total).

Intruder stress was done the following week by placing a same-sex King Holtzman rat in the test rat’s home cage for 2 h, and then 24 h Na and fluid intake were measured (2). The following week, clonidine (120 μg/20 g food = 0.4 mg/kg body wt) was administered in the food by purchasing powered food and mixing the clonidine into it. This dose was arrived at from dose-response curves, and a dose was selected that effectively lowered blood pressure at a consumption rate of 20 g of food per day (average) and produced clonidine blood levels of 5 ng/ml.

A two-way ANOVA was used to test significance between the various strains, genders, and treatments for salt preference and salt intake, and Newman-Keuls t-tests were used where appropriate to compare between two groups. Significance was assumed when P < 0.05. Experiments were approved by the institutional animal care and use committee in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals published by the US Department of Health and Human Services (NIH Pub. #86–23).

Results

Figure 1 shows the 14-day baseline Na intake averages over the three time periods (each 2 wk; A, B, C) by

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gender and strain. There was a significant strain effect in all three periods (F = 143, P < 0.001; F = 36, P < 0.001; F = 11.32, P < 0.01, respectively). Also, there was a significant gender effect (females higher Na intake) in all three time periods (F = 3.64, P < 0.05; F = 6.61, P < 0.01; F = 2.82, P < 0.05, respectively). Females in all strains had significantly (P < 0.001) higher Na intake than males.

After 6 wk of baseline data, intruders were placed in the home cage for 2 h and then 24 h Na and fluid intake were measured to observe the effect of acute stress. Figure 2 shows the stress-induced increase in Na intake by strain and gender based on percentage increase over the last baseline measurement for each group. In general, the males had a larger percentage increase in Na intake than females in all strains, although females still had the larger absolute Na intake after stress. The greatest stress-induced change by strain was in the SHR/y males (138%) compared with WKY males (28%, P < 0.001). There were no significant strain differences by gender between SHR vs. SHR/a, but males had the higher percentage increase compared with females (P < 0.001).

Figure 3 shows the effect of clonidine on stress-induced Na intake expressed as a percentage decrease compared with stress alone (Fig. 2). The SHR/y females had greater decreases than the WKY female counterparts (P < 0.001 in females, not significant in males), SHR/a males had significantly less reduction than SHR males (P < 0.001), and there was a significant difference between SHR/a males vs. SHR/a females (P < 0.001).
For the salt preference analysis (% of each NaCl concentration consumed), the last baseline period was used because there was little variation (nonsignificant) across time. With regards to baseline salt preference in females, there was a strong strain Na preference effect ($F_{5,530}, P < 0.001$). The SHR females had significantly higher preference (80%) for 0.5% NaCl than the SHR/a group (54%; $P < 0.05$); however, the SHR/a females had a higher preference (20%) for 1.0% NaCl than SHR (4%; $P < 0.05$; Fig. 4A). There were no significant differences in preferences between the WKY and SHR/y females, with the 0.5% NaCl the most preferred (74 and 65%, respectively). Stress did not significantly alter preference in the females, and there was still a preference for the 0.5% NaCl ($F_{5,191}, P < 0.001$); however, there was an increase across all strains of the 1.0 and 1.5% NaCl from 8 and 2.4% to 13 and 9%, respectively. The 0% NaCl increased from 20% during baseline to 30% during stress (averaged across all strains), and the 0.5% NaCl decreased from 70 to 45% across strains (Fig. 4B). There were no significant strain differences or interactions. The clonidine treatment combined with stress tended to increase the preference for higher percentage NaCl solutions in all strains; however, there were no statistical strain or preference differences (ANOVA; Fig. 4C). The increase in the 1.5% NaCl for each strain was as follows: SHR/a = 10 to 15%, WKY = 7 to 16%, and SHR/y = 17 to 45%. With regards to the SHR/a vs. SHR/y female comparisons, the SHR/a females had less preference for 0.5% NaCl ($P < 0.05$) than for 1.0% NaCl ($P < 0.05$) during baseline (Fig. 4A). During the stress period, there were no significant female SHR/a vs. SHR/y differences. During the clonidine period, the SHR/a females preferred the 1.0% NaCl over the 1.5% NaCl compared with the SHR/y females ($P < 0.05$). With regards to the same comparison in males, the SHR/a males preferred 0.5% NaCl and 1.0% NaCl more than the SHR/y males ($P < 0.05$, Fig. 5A); however, during stress, the SHR/a males drank less 0.5% NaCl and more 0% NaCl than SHR/y males ($P < 0.05$, Fig. 5B). During the clonidine treatment, the SHR/a males drank more 0% NaCl than SHR/y males ($P < 0.05$; Fig. 5C).

With regards to baseline salt preference in males, all strains preferred the 0.5% NaCl ($F = 191, P < 0.001$) with no significant strain effect. The SHR/a group preferred 1.5% NaCl more than SHRs ($P < 0.05$; Fig. 5A). Similar to the females, stress did not significantly change Na preference from the 0.5% NaCl ($F = 47, P < 0.001$) and there was not a significant strain effect...
study, the females of the hypertensive strains had higher baseline Na intake than males, which was consistent across time and supports the idea that the four-water bottle preference methodology can accurately measure sodium intake, which we previously reported (2). The increased Na intake in females compared with males was mainly due to more consumption of 0.5% NaCl rather than increased preference for a higher concentration. One explanation for this result is higher SNS activity in the females, which may be driving the Na appetite. Indeed, clonidine significantly reduced (50–60%) the stress-induced increase in Na intake in all female hypertensive strains (SHR, SHR/a, SHR/y). Because stress did not increase the female WKY Na intake, it is not surprising that clonidine did not further reduce the intake. In SHR rats and in some forms of human hypertension, there was elevated SNS activity (1, 11–13), which could lead to increased Na appetite. Also stress elevates SNS sodium intake (8). It does not appear that blood pressure elevation itself can account for the increased Na intake, because chronic treatment with a vasodilator in SHR normalized blood pressure but did not attenuate salt appetite (15). A similar conclusion was arrived at in a cosegregation analysis of blood pressure and salt intake between SHR and WKY crosses (6, 33). Blood pressure was intermediate in the F1 and F2 crosses between SHR and WKY as expected, but there was no relationship between blood pressure and salt appetite in these crosses (33). It appears that the increased salt appetite in SHR starts very early (3 wk of age; Ref. 3). Stress decreased sodium excretion, and renal SNS denervation abolished this response in borderline hypertensive rats (27). Increased SNS activity in SHR can also increase intestinal absorption of Na (29), which may be a compensation for the enhanced fecal loss of Na in SHR (22). Females may require more Na intake than males for normal growth and pregnancy.

With regard to strain differences in Na intake, the SHR/y males and females had higher Na intake than their WKY counterparts. This is not surprising for the SHR/y males because they have increased SNS activity (7) compared with WKY, which could explain the large stress-induced Na intake; however, it does not explain the elevated SHR/y female Na intake, which will be discussed later. Yamori et al. (32) also found a large increased 0.2% saline preference in stroke-prone and stroke-resistant SHR with elevated SNS activity. SHR/y males are compared with WKY males because they differ only in their Y chromosome source, and SHR are compared with SHR/a for the same reason; however, a comparison of SHR/y to SHR/a examines the differences of the SHR Y chromosome and the autosomes. Comparison of Na intake in Fig. 1 shows no male or female significant differences between SHR/a vs. SHR/y in any of the three periods; however, rats with a different form of hypertension not associated with elevated SNS (renovascular hypertension produced by aortic ligation) did not show an increased Na preference (14). Other mechanisms, such as the renin-angiotensin system (4, 17, 31) and other hormonal

Fig. 5. Male sodium preference based on percentage by strain (means ± SE) for last baseline period (A), *P < 0.05 SHR/y vs. WKY and SHR/a vs. SHR for respective Na concentration, †P < 0.05, ††P < 0.01, †††P < 0.001 for 0.5% NaCl vs. 0% NaCl by strain; stress period (B), †††P < 0.01 SHR/y vs. WKY for 0.5% NaCl, †††P < 0.001 for 0.5% NaCl vs. 0% in SHR/y; stress and clonidine (C), *P < 0.05 SHR/y vs. WKY for respective Na concentration, †P < 0.05 for 0.5% vs. 0% NaCl by strain.
pathways (30), are also involved in thirst and Na appetite as well as specific brain regulatory areas (30). There may also be uterine or fetal influences on salt appetite; however, cross suckling SHR rats to a normotensive Sprague-Dawley mother did not lower the exaggerated saline preference of SHR at 3 or 6 mo (5). The SHR fetus is exposed to higher osmolarity and Na concentration than that of WKY, which may play a role in establishing salt appetite (10), and milk factors may also influence salt appetite because SHR has higher Na in the milk (23).

We have previously shown that the Y chromosome from SHR has an influence on blood pressure (9) and it elevates indexes of the SNS (7), which could explain the elevated Na appetite in male SHR and SHR/y as previously described; however, this does not explain the elevated SHR/y female Na appetite. SHR/y females should theoretically be genetically identical to the female WKY because they do not have a Y chromosome and they were backcrossed for 16 generations to the same mother strain, WKY. Earlier we reported a potential X chromosome effect on salt intake and not a Y chromosome effect (2). This discrepancy could be due to the generation difference in the earlier report, which was based on the first F1 cross that had 50% WKY and 50% SHR genes contributing to the offspring. Even though we saw blood pressure differences in this study, there was not a Y effect on Na intake; however, with more backcross generations (F16 = 99.9% WKY genes), the SHR Y male effect was contrasted more on a WKY background and the effect became pronounced, but the apparent X chromosome effect in females was still present. Also, the SHR/y females in this study showed a significant clonidine-induced reduction in Na intake similar to that of the SHR/y males and unlike the WKY females, who did not show a clonidine response. This type of result showing an SHR/y female effect different from that of a WKY female has occurred before in our laboratory with regard to blood pressure (25) and renal renin angiotensinogen mRNA (26). Therefore, we think there is something genetically and uniquely important occurring in the females in our backcrosses that influences several phenotypes in addition to salt intake that we are measuring. Females in general appear to have a higher Na intake partially due to increased SNS, but the SHR/y females may have an additional mechanism driving Na appetite. One possibility to explain this is that of genomic imprinting. This phenomenon, where the paternal and maternal alleles are expressed differently in somatic cells, has been suggested in cancer and other diseases (28). Some imprinted genes only exhibit in selected tissues and others at different developmental times (21). Because imprinting is sex specific, it is not unusual that different X chromosomes might affect imprinted genes differently. As predicted, the SHR vs. SHR/a strains did not show any differences in Na intake or stress responses that suggest that the WKY chromosomes that contributed to the SHR/a strain did not modify the Na intake. Because the SHR/a strain has the same autosomes as SHR, it is not surprising that the autosomal component or components can compensate for any lack of Y effect. The only significant difference between SHR and SHR/a strains was the lack of clonidine response in the SHR/a males. Even the WKY males showed a response with clonidine, so the SHR/a lack of response is difficult to explain. The clonidine response in the SHR/y males was more similar to that of SHR than either WKY or SHR/a, which implies a Y chromosome effect.

In conclusion, SHR/y males with the SHR Y chromosome have higher baseline and stress-induced sodium intake than WKY males, which was partially blocked by clonidine, which reduced central SNS outflow. Preference for specific NaCl concentrations did not appear to be influenced by genetic background in males but was altered in the SHR vs. the SHR/a females.

Perspectives

Results of this study, taken together with our previous work, indicated that SHR/y females share a number of phenotypic similarities with SHR rather than WKY. Our original hypothesis was that SHR/y females would not differ phenotypically from WKY females, because the SHR/y females, derived from SHR fathers but backcrossed 16 generations to WKY mothers, have all their autosomes and both X chromosomes from WKY. We propose that genomic imprinting involving the renin-angiotensin system may be responsible for some of the SHR/y female differences in Na intake and preference observed in the present study. Although it is unlikely that either the renin or angiotensinogen genes are themselves imprinted, our evidence suggests that they are coregulated by another gene that may be imprinted in SHR/y but not in WKY females. This third imprinted gene, or the product of another imprinting gene, may well participate in regulating a family of genes, possibly including those responsible for the observed phenotypic differences between SHR/y and WKY females. Furthermore, because differences in blood pressure are one of these phenotypic differences, we suggest a role for imprinting in regulation of blood pressure. Further studies by Dr. Milsted in our laboratory are examining the proposed imprinting in these rats.

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