

Gestational and early postnatal dietary NaCl levels affect NaCl intake, but not stimulated water intake, by adult rats

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Curtis, Kathleen S., Eric G. Krause, Donna L. Wong, and Robert J. Contreras. Gestational and early postnatal dietary NaCl levels affect NaCl intake, but not stimulated water intake, by adult rats. *Am J Physiol Regul Integr Comp Physiol* 286: R1043–R1050, 2004. First published February 5, 2004; 10.1152/ajpregu.00582.2003.—We examined body fluid regulation by weanling (21–25 days) and adult (>60 days) male rats that were offspring of dams fed chow containing either 0.1, 1, or 3% NaCl throughout gestation and lactation. Weanling rats were maintained on the test diets until postnatal day 30 and on standard 1% NaCl chow thereafter. Ad libitum water intake by weanlings was highest in those fed 3% NaCl and lowest in those fed 0.1% NaCl. Adult rats maintained on standard NaCl chow consumed similar amounts of water after overnight water deprivation or intravenous hypertonic NaCl (HS) infusion regardless of early NaCl condition. Moreover, baseline and HS-stimulated plasma Na⁺ concentrations also were similar for the three groups. Nonetheless, adult rats in the early 3% NaCl group consumed more of 0.5 M NaCl after 10 days of dietary Na⁺ deprivation than did rats in either the 1% or 0.1% NaCl group. Interestingly, whether NaCl was consumed in a concentrated solution in short-term, two-bottle tests after dietary Na⁺ deprivation or in chow during ad libitum feeding, adult rats in the 3% NaCl group drank less water for each unit of NaCl consumed, whereas rats in the 0.1% NaCl group drank more water for each unit of NaCl consumed. Thus gestational and early postnatal dietary NaCl levels do not affect stimulated water intake or long-term body fluid regulation. Together with our previous studies, these results suggest that persistent changes in NaCl intake and in water intake associated with NaCl ingestion reflect short-term behavioral effects that may be attributable to differences in NaCl taste processing.

hypernatremia; thirst; development; taste; hypertension

UNDERSTANDING of the neurobiological bases of behavior and physiology has benefited immensely from the rich history of the study of early influences. Clearly, the intrauterine environment, and thereby fetal development, may be affected by the availability of nutrients, prevailing hormone levels, or pharmacological agents. Similar factors also may affect development during the early postnatal period. A critical period for the normal development of behavioral and physiological function has been implicated in systems ranging from sex-specific reproductive behaviors to sensation and perception (see Ref. 19 for review).

In species including human, sheep, and rat, challenges to body fluid regulation that occur during gestation and/or the early postnatal period have profound and long-lasting effects on behavior and physiology (2, 7, 8, 11, 13, 14, 18, 20, 27, 28, 33, 36–38). In this regard, manipulations of the NaCl content of chow consumed by female rats during pregnancy and lactation affect NaCl intake by their adult offspring, even after the offspring have been maintained on standard NaCl chow (3,

8–10, 26). After such early dietary NaCl manipulations, adult rats that were given high (3%) NaCl chow until postnatal day (PD) 30 have a greater preference for NaCl solutions than do adult offspring that were given standard (1%) or low (0.1%) NaCl chow until PD30 (3, 8–10). In addition, dietary Na⁺ deprivation elicits greater intake of concentrated NaCl solution by adult rats in the early 3% NaCl group compared with that by rats in the early 0.1% NaCl group (10). In contrast, maintenance on high NaCl chow after weaning does not have a long-term influence on NaCl intake by adult rats (23, 30, 39). Thus, unlike temporary dietary exposure to high or low NaCl as adults (23, 30, 39), manipulations of dietary NaCl levels during an early sensitive period lead to persistent increases in spontaneous, need-free, and stimulated intake of NaCl solutions by adult rats.

Early dietary NaCl manipulations may affect NaCl intake by adults via long-term alterations in gustatory processing of NaCl taste information. Consistent with this idea, electrophysiological responses of the chorda tympani nerve to lingual NaCl stimulation are affected by early exposure to an Na⁺-deficient diet (18). In addition, a recent study from our laboratory (28) showed that chorda tympani nerve responses in adult rats are affected by early dietary exposure to 3% NaCl, even after maintenance on standard NaCl chow. Nonetheless, changes in NaCl intake also may constitute a compensatory behavioral response to changes in renal function or to alterations of body fluid balance, and in particular of body Na⁺ balance, resulting from dietary NaCl conditions during an early sensitive period. The goal of this study was to determine whether the long-lasting effect of early dietary NaCl manipulations on NaCl ingestion by adult rats is attributable to a general influence on behavioral or physiological mechanisms that subservise body fluid regulation. We examined water intake stimulated by water deprivation or a systemic NaCl load in adult rats after early dietary NaCl manipulations. We also examined the effect of early dietary NaCl conditions on plasma Na⁺ concentration (pNa), hematocrit (Hct), and plasma protein concentration (pPro) in newborn and adult rats, along with the effect of a systemic NaCl load on pNa, Hct, and pPro in adults. Finally, we evaluated the effect of early dietary NaCl manipulations on the relationship between food and water intake during ad libitum feeding in weanling and adult rats.

METHODS

Subjects

Female rats [Sprague-Dawley, CrL:CD(SD)BR, Charles River Breeding Laboratories], 66 days old and non-littermates, were housed

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in clear plastic cages in a temperature-controlled room with a 12:12-h light-dark cycle. Rats were given ad libitum access to deionized water (dH₂O) and a pelleted test diet (Harlan Teklad, modifications of sodium-deficient diet TD 90228) consisting of either 3.0, 1.0, or 0.1% NaCl ($n = 26/\text{NaCl}$ condition) for 14 days. Each female then was housed with an adult nonsibling male for 14 days. Females were individually housed thereafter and remained on the NaCl test diets throughout gestation and lactation until pups were weaned. These methods incorporate several modifications of those used previously (3, 9, 10). First, we used diets containing starch, rather than sucrose, as the primary carbohydrate source throughout the experiments. Second, to reduce biases from common genetic background, intrauterine environment, or early postnatal experience, we used nonsibling rats as breeders and tested individual rats from multiple litters in each of the early dietary NaCl conditions in all experiments.

Pups were born as early as 21 days after males and females were paired. Within 24 h after birth (PD1), litters were culled to eight pups, retaining six to eight males/litter. Litters with fewer than eight pups were not included. Pups were weaned from their mothers at PD21, given ad libitum access to dH₂O, and maintained on the test diets for 9 additional days. At PD30, male pups were individually housed and given standard pelleted chow (Purina 5001; 1.0% NaCl). Except where noted, the offspring remained on dH₂O and standard chow for the duration of the study.

Some rats were tested in more than one experiment in *experiments 1–3*. In such cases, testing was separated by 1–3 wk. Each experiment included similar numbers of rats from each NaCl group that had been tested previously, and rats were not included in additional experiments after dietary Na⁺ deprivation.

Experiment 1: water intake after overnight water deprivation. Male rats ($\geq\text{PD60}$; $n = 11/\text{NaCl}$ condition) were randomly assigned to be deprived of water overnight (water bottles removed from the cages at ~ 1700 h) or to have ad libitum access to water. The next morning, dH₂O was given in graduated drinking tubes, and water intake (ml) was recorded after 5, 10, 15, 30, 45, 60, and 120 min. Two days later, rats were tested in the opposite condition.

Experiment 2a: water intake after 2 M NaCl infusion. Male rats ($\geq\text{PD60}$; $n = 5/\text{NaCl}$ condition) were anesthetized with pentobarbital sodium (0.5 ml/kg ip; Veterinary Laboratories, Lenexa, KS) and fitted with femoral venous and arterial catheters. The catheters were tunneled subcutaneously and exteriorized at the nape of the neck. Catheters were filled with heparinized 0.15 M NaCl and capped when not in use. Rats were permitted 48 h to recover and then were randomly assigned to receive 2 M NaCl [hypertonic NaCl (HS; 1 ml/h)] or 0.15 M NaCl [isotonic NaCl (Iso; 1 ml/h)]. On the test day, water bottles were removed from the cages and the venous catheters were connected to tubing attached to an infusion pump. Rats were permitted to acclimate for ~ 30 min before 1-h infusion with either Iso or HS. Deionized water then was given in graduated drinking tubes, and water intake (ml) was recorded after 5, 10, 15, 30, 45, 60, and 120 min. Two days later, rats were tested in the opposite condition.

Experiment 2b: pNa, Hct, and pPro. ADULT RATS. Two days after the behavioral tests in *experiment 2a*, rats were randomly assigned to receive HS or Iso infusion as described in *experiment 2a*. After the 1-h infusion, a 1-ml blood sample was withdrawn from the arterial catheter into a heparinized tube. A fraction of each sample was used to measure Hct (%) with a microcapillary reader (Damon/IEC, Needham Hts, MA), and the remainder was centrifuged. Plasma was removed for determination of pNa (mmol/l) using a Na⁺-sensitive electrode (Ciba-Corning 614; Ciba-Corning Diagnostics, Medfield, MA) and for pPro (g/dl) using a refractometer (AO Scientific Instruments; Leica, Northvale, NJ). Two days later, rats were tested in the opposite condition. Data were not obtained in both conditions from all rats due to catheter failure.

PD1 PUPS. Culled pups from a subset of the litters ($n = 6$ litters/NaCl condition) were decapitated, and trunk blood was collected into

heparinized tubes. Blood samples from each litter were pooled. Pooled blood samples consisted of 6–11 pups/litter, of which 40–100% were female.

Experiment 3: NaCl and water intake after dietary Na⁺ deprivation. Male rats ($\geq\text{PD60}$; 3% $n = 8$; 1% $n = 8$; 0.1% $n = 7$) were given 0.5 M NaCl in graduated drinking tubes, and 24-h intakes were recorded for 2–3 days. The 0.5 M NaCl then was removed, and rats were given Na⁺-deficient chow (0.0%, Harlan Teklad) in place of their regular chow for 10 days. Rats then were given 0.5 M NaCl and dH₂O in graduated drinking tubes. Intakes (ml) were recorded after 5, 10, 15, 30, 45, and 60 min, at 1-h intervals for the next 4 h, and then the following morning. The Na concentration of the ingested fluid was calculated $\{[\text{NaCl intake (ml)} \times 0.5]/[\text{total fluid intake (ml)}] \times 1,000\}$ for each hour during the 5-h test and for the overnight intakes.

Experiment 4: food and water intake by weanling and adult rats. Weanlings remained on the test diets and were housed eight per cage; thus, for each day from PD21 to PD25, food and water intake (g) were calculated for each litter as intake/8 (3% $n = 5$ litters; 1% $n = 4$ litters; 0.1% $n = 8$ litters). Food and water intake by individual adult male rats being maintained on standard 1% NaCl chow were recorded from PD120 to PD125 ($n = 20/\text{NaCl}$ condition). For both weanling and adult rats, a water:food ratio was calculated for each day (water intake/food intake). Average daily food intake, water intake, and water:food ratio were calculated for each rat.

Statistics

Results are presented as group means \pm SE. Group differences were analyzed by appropriate ANOVA (3-factor ANOVA with repeated measures for stimulated water intake and for stimulated NaCl and water intake; 2-factor ANOVA with repeated measures for pNa, Hct, and pPro in adults; 3-factor ANOVA for ad libitum food and water intake; 2-factor ANOVA for water:food ratio; 1-factor ANOVA for pNa, Hct, and pPro in PD1 pups). Student-Newman-Keuls tests were used for pairwise comparisons of statistically significant ($P < 0.05$) main effects or interactions.

RESULTS

Experiment 1: Water Intake After Overnight Water Deprivation

Water intake was significantly increased after overnight water deprivation [Fig. 1A; $F(1,29) = 287.411$, $P < 0.001$] but was not affected by early NaCl condition. There was a significant interaction between treatment and time [$F(7,203) = 162.550$, $P < 0.001$]. Pairwise comparisons revealed that water intake after overnight deprivation was >0 by 5 min ($P < 0.001$). In contrast, water intake when not deprived did not increase at any time point. Thus water intake was different at each time point after the two treatments (P values < 0.001).

Experiment 2a: Water Intake After 2 M NaCl Infusion

Water intake also was significantly increased after intravenous infusion of HS [Fig. 1B; $F(1,12) = 38.025$, $P < 0.001$], and the pattern was similar to that after water deprivation. Water intake was not affected by early NaCl condition; however, there was a significant interaction between early NaCl condition, treatment, and time [$F(14,84) = 2.347$, $P < 0.01$]. Pairwise comparisons revealed that water intake after Iso did not differ at any time point. In contrast, after HS, water intake by rats in the 1% NaCl group was significantly greater than that by rats in the 3% and 0.1% NaCl groups at 120 min (P values < 0.001). Although there also were subtle differences in the latency to begin drinking after HS (water intake > 0 by 15

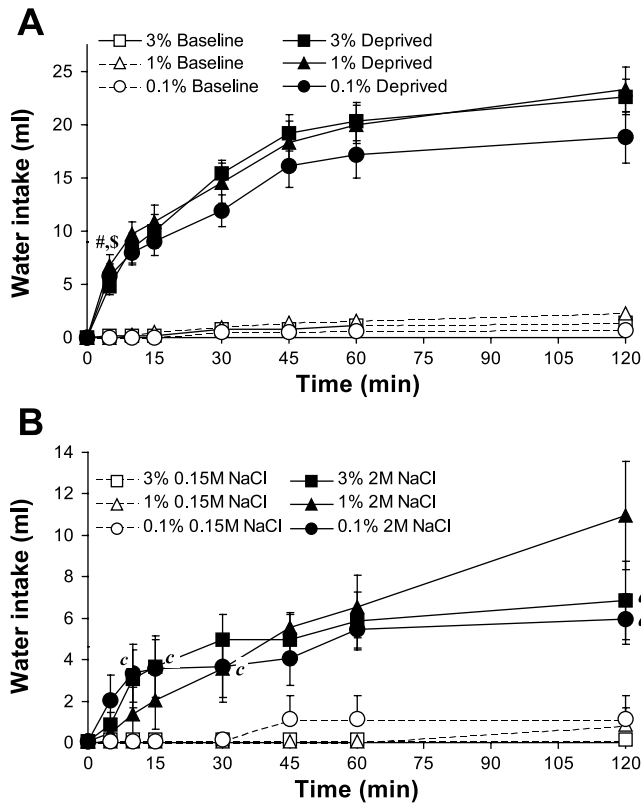


Fig. 1. Stimulated water intake (ml) by adult rats in the 3% (squares), 1% (triangles), and 0.1% (circles) NaCl groups. A: cumulative water intake in 2-h tests after overnight water deprivation (filled symbols) or when not deprived (open symbols). B: cumulative water intake in 2-h tests after 1-h iv infusion of 2 M NaCl (1 ml/h; filled symbols) or 0.15 M NaCl (1 ml/h; open symbols). #First time point > 0 (all groups); \$first time point greater than not deprived (all groups); ^adifferent from 1%; ^cfirst time point > 0 (individual groups).

min in rats in the 3% NaCl group; by 30 min in rats in the 1% NaCl group; by 10 min in rats in the 0.1% NaCl group; P values < 0.05), these differences were not obviously related to early NaCl condition.

Experiment 2b: pNa, Hct, and pPro

Adult rats. HS infusion significantly increased pNa [$F(1,5) = 62.410$, $P < 0.001$] and pPro [$F(1,5) = 15.764$, $P < 0.05$] but had no effect on Hct (Table 1). Early NaCl condition did not affect pNa, Hct, or pPro after Iso or after HS.

PDI pups. Analysis of blood samples taken from culled pups on PD1 revealed a significant effect of early NaCl condition on pNa [$F(2,15) = 20.384$, $P < 0.001$]. Specifically, pNa in pups in the 0.1% NaCl group was significantly less than that in pups in the 1% and 3% NaCl groups (P values < 0.001), which were not different from each other (Table 1). In contrast, neither Hct nor pPro was affected by early NaCl condition (Table 1). It should be noted that there was no relationship between the percentage of females in the sample and pNa, pPro, or Hct.

Experiment 3: NaCl and Water Intake After Dietary Na⁺ Deprivation

Figure 2 shows intake of water and 0.5 M NaCl by adult rats in the 3%, 1%, and 0.1% NaCl groups in 5-h tests after 10 days

of dietary Na⁺ deprivation. Intakes were affected by solution [$F(1,20) = 16.280$, $P < 0.001$] and time [$F(10,200) = 36.584$, $P < 0.001$] but not by early NaCl condition. However, there were significant interactions between early NaCl condition and solution [$F(2,20) = 4.737$, $P < 0.05$] and between early NaCl condition, solution, and time [$F(20,200) = 2.482$, $P < 0.001$]. Pairwise comparisons revealed that NaCl intake was significantly greater than 0 by 10 min in rats in the 3% NaCl group (Fig. 2A; $P < 0.01$), by 15 min in rats in the 1% NaCl group (Fig. 2B; $P < 0.05$), and by 30 min in rats in the 0.1% NaCl group (Fig. 2C; $P < 0.01$). Water intake was delayed relative to NaCl intake but was significantly greater than 0 by 45 min in rats in both the 0.1% ($P < 0.05$) and 1% ($P < 0.01$) NaCl groups. Interestingly, water intake by rats in the 3% NaCl group was not greater than 0 until 240 min ($P < 0.01$). In fact, NaCl intake by rats in the 3% NaCl group was significantly greater than water intake at each time point after 10 min (P values < 0.01, 0.001). In contrast, NaCl intake by rats in the 1% NaCl group was greater than water intake only at 15 and 30 min (P values < 0.05), and NaCl intake by rats in the 0.1% NaCl group did not differ from water intake at any time point. In addition, water intake by rats in the 3% NaCl group was significantly less than that by rats in the 1% NaCl group at 45, 60, 120, and 180 min (P values < 0.05, 0.01, 0.001) and was less than that by rats in the 0.1% NaCl group at 60 min and thereafter (P values < 0.05, 0.001). NaCl intake by rats in the 3% NaCl group was significantly greater than that by rats in the 0.1% NaCl group at 15 and 30 min (P values < 0.05). Neither NaCl nor water intake differed between rats in the 1% and 0.1% NaCl groups at any time point.

As would be expected given the differences in ingestion of NaCl and water, the concentration of the fluid ingested during the 5-h test (Table 2) was significantly affected by early NaCl condition [$F(2,20) = 4.72$, $P < 0.05$]. Pairwise comparisons revealed that the concentration of the fluid ingested by rats in the 3% NaCl group was significantly greater than that ingested by rats in the 0.1% NaCl group ($P < 0.05$) and tended to be

Table 1. Plasma Na⁺ concentration, plasma protein concentration, and hematocrit in neonate and adult rats in the 3%, 1%, or 0.1% NaCl groups

	PD1	Adult	
		0.15 M NaCl	2 M NaCl
Plasma Na ⁺ concentration, mmol/l			*
3% NaCl	134.5 ± 1.3 [†]	143.8 ± 0.3	151.0 ± 0.4
1% NaCl	133.3 ± 0.9 [†]	141.0 ± 2.0	149.3 ± 0.7
0.1% NaCl	125.8 ± 0.8	142.7 ± 0.3	149.7 ± 2.3
Plasma protein concentration, g/dl			
3% NaCl	2.63 ± 0.03	5.88 ± 0.10	6.04 ± 0.21
1% NaCl	2.53 ± 0.11	5.83 ± 0.13	6.22 ± 0.21
0.1% NaCl	2.47 ± 0.08	5.78 ± 0.29	6.18 ± 0.24
Hematocrit, %			
3% NaCl	31.2 ± 0.5	40.4 ± 1.7	38.9 ± 2.4
1% NaCl	33.2 ± 1.9	43.8 ± 4.8	40.5 ± 2.4
0.1% NaCl	32.7 ± 1.4	37.2 ± 1.9	37.3 ± 3.5

Values are means ± SE. Baseline plasma Na⁺ concentration, plasma protein concentration, and hematocrit in neonatal rats were evaluated on postnatal day 1 (PD1). Plasma Na⁺ concentration, plasma protein concentration, and hematocrit in adult rats were evaluated after 1-h iv infusion of 2 M NaCl or 0.15 M NaCl at a rate of 1 ml/h. *Significantly different from 0.15 M NaCl; [†]significantly different from 0.1%.

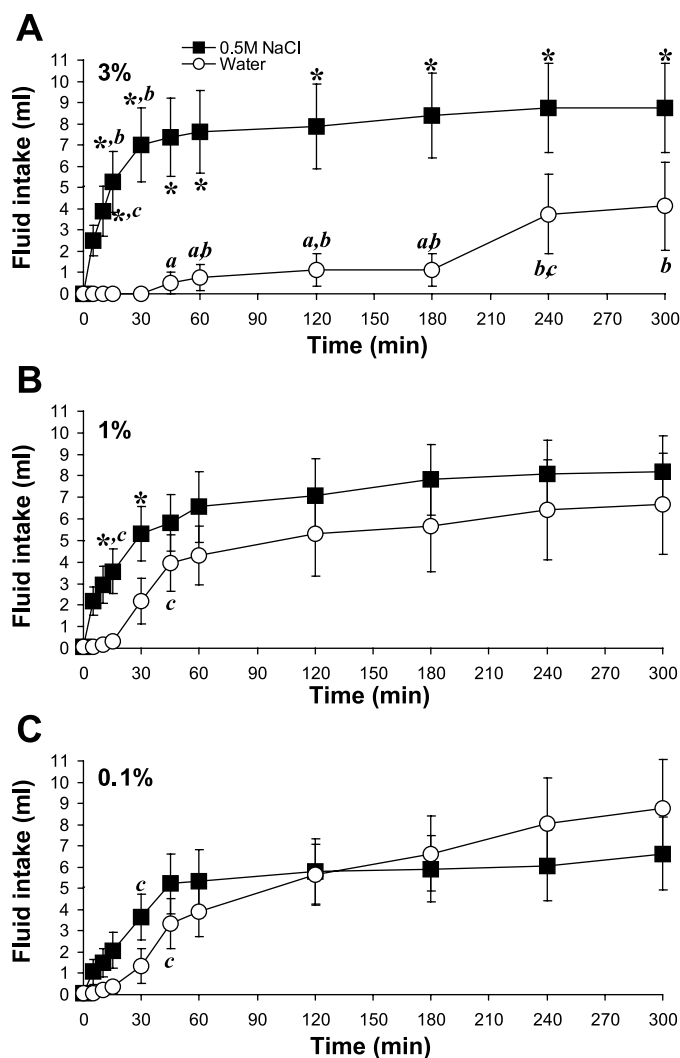


Fig. 2. Cumulative intake of 0.5 M NaCl (ml; filled squares) and water (ml; open circles) by adult rats in the 3% (A), 1% (B), and 0.1% (C) NaCl groups in 5-h tests after 10 days of dietary Na^+ deprivation. *Greater than water; ^adifferent from 1%; ^bdifferent from 0.1%; ^cfirst time point >0 .

greater than that ingested by rats in the 1% NaCl group ($P = 0.059$). The concentration of the fluid ingested by rats in the 1% NaCl and 0.1% NaCl groups were not different ($P = 0.320$). Neither time nor the interaction between early NaCl condition and time affected the concentration of the ingested fluid.

Table 2 also shows overnight intake of 0.5 M NaCl and water by adult rats in the 3%, 1%, and 0.1% NaCl groups after the 5-h test. Intakes of solutions were significantly different [$F(1,20) = 4.21$, $P < 0.001$; water greater than NaCl]; however, neither early NaCl condition nor the interaction between early NaCl condition and solution affected overnight intakes. As expected based on the lack of group differences in overnight NaCl and water intakes, there was no effect of early NaCl condition on the concentration of the ingested fluid (Table 2).

Experiment 4: Food and Water Intake by Weanling and Adult Rats

As expected, ingestion was significantly affected by age [$F(1,71) = 190.750$, $P < 0.001$] and the substance consumed

[$F(1,71) = 503.740$, $P < 0.001$]. Specifically, adult rats consumed more than did weanlings, and all rats ingested more water than chow. Ingestion also was affected by early NaCl condition [$F(2,71) = 8.693$, $P < 0.001$] and by the interaction between early NaCl condition, age, and the substance consumed [$F(2,71) = 7.082$, $P < 0.01$]. Pairwise comparisons revealed no group differences in chow intake at either age (Fig. 3A). Thus neither early NaCl condition nor the specific diet consumed (i.e., test diets by weanlings vs. standard 1% NaCl chow by adults) affected chow intake. In contrast, there were group differences in water intake (Fig. 3A). Rats in the 3% NaCl group consumed significantly more water than did rats in the 1% NaCl group, both as weanlings and as adults (P values < 0.001). Weanling rats in the 0.1% NaCl group consumed significantly less water than did weanlings in the 3% NaCl group ($P < 0.001$) but amounts comparable to those by weanlings in the 1% NaCl group. Interestingly then, adult rats in the 0.1% NaCl group consumed significantly more water than did adult rats in the 1% NaCl group ($P < 0.05$) but amounts comparable to those by adults in the 3% NaCl group.

The relationship between water intake and food intake, expressed as a ratio (water intake/food intake), is shown in Fig. 3B. The water:food ratio depended on age [$F(1,71) = 45.083$, $P < 0.001$] and on early NaCl condition [$F(2,71) = 11.957$, $P < 0.001$]. There also was a significant interaction between early NaCl condition and age [$F(2,71) = 14.732$, $P < 0.001$]. Pairwise comparisons revealed that, in weanling rats, the water:food ratio was significantly greater in the 3% NaCl group compared with those in the 1% and 0.1% NaCl groups (P values < 0.001), which were not different from each other. In adult rats, however, the ratio in rats in the 3% NaCl group did not differ from those in the other two groups, but the ratio was significantly greater in the 0.1% NaCl group compared with that in the 1% NaCl group ($P < 0.05$). Group comparisons of the water:food ratio in weanling vs. adult rats revealed that the ratio in adults did not differ from that in weanlings in the 3% NaCl group. In contrast, the water:food ratio in adults was

Table 2. Concentration of fluid ingested by adult rats in 3%, 1%, and 0.1% NaCl groups during 5-h tests after 10 days of dietary Na^+ deprivation; overnight 0.5 M NaCl intake, water intake, and concentration of fluid ingested by adult rats in 3%, 1%, and 0.1% NaCl groups during overnight access after 5-h tests following 10 days of dietary Na^+ deprivation

	NaCl Group		
	3%	1%	0.1%
Na concentration of ingested fluid, mmol/l	*		
60 min	475.9 ± 17.3	304.3 ± 41.7	316.1 ± 40.9
120 min	462.7 ± 24.1	321.6 ± 45.3	270.4 ± 50.0
180 min	468.1 ± 20.9	332.1 ± 41.7	257.5 ± 51.4
240 min	419.8 ± 39.3	324.7 ± 44.4	238.5 ± 50.7
300 min	415.8 ± 41.3	322.0 ± 45.3	245.7 ± 48.8
Overnight			
NaCl intake, ml	11.3 ± 4.1	9.3 ± 2.5	6.7 ± 1.8
Water intake, ml	47.8 ± 8.2	34.2 ± 3.7	38.6 ± 4.3
Na concentration of ingested fluid, mmol/l	76.3 ± 19.0	93.6 ± 16.1	70.4 ± 14.2

Values are means ± SE. *Significantly different from 0.1% NaCl.

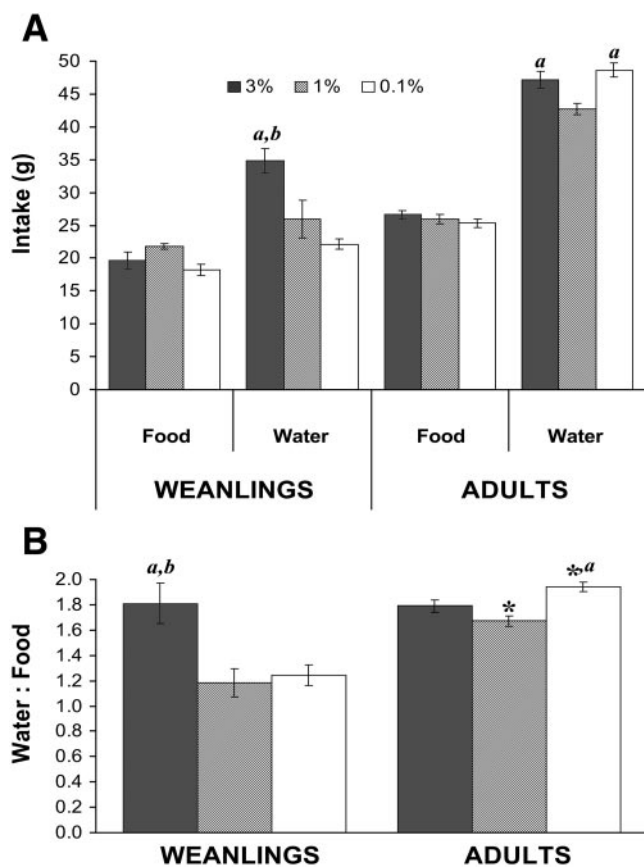


Fig. 3. A: ad libitum intake of food and water (in g) by weanling and adult rats in the 3% (dark gray bars), 1% (light gray bars), and 0.1% (open bars) NaCl groups. B: water intake-to-food intake ratio (Water:Food) during ad libitum consumption of food and water by weanling and adult rats in the 3% (dark gray bars), 1% (light gray bars), or 0.1% (open bars) NaCl groups. *Different from weanlings; ^adifferent from 1%; ^bdifferent from 0.1%.

significantly greater than that in weanlings in both the 1% and 0.1% groups (P values < 0.001).

DISCUSSION

Previous studies have shown that manipulations of dietary NaCl levels during gestation and the early postnatal period lead to persistent changes in need-free and stimulated NaCl intake by adult rats (3, 8–10, 26, 36, 37). This study sought to determine whether early dietary NaCl conditions have selective effects on NaCl ingestion or whether changes in NaCl intake reflect long-lasting, generalized changes in the behavioral and physiological mechanisms that subservise body fluid homeostasis.

The present results confirm and extend studies (3, 8–10) establishing a relationship between early dietary NaCl levels and NaCl ingestion during adulthood. Previous studies typically used long-term intake tests, and, although there were group differences in NaCl intake (3, 8–10), the effects often were subtle. In the present study, adult rats reared either on 3%, 1%, or 0.1% NaCl exhibited striking differences in the temporal patterns of water and 0.5 M NaCl intake in short-term tests after dietary Na⁺ deprivation (Fig. 2). Specifically, rats in the 3% NaCl group consumed primarily NaCl and very little water. Rats in the 1% NaCl group consumed somewhat less NaCl and

substantial amounts of water, although less water than NaCl. Finally, rats in the 0.1% NaCl group consumed even less NaCl and greater amounts of water. These group differences were apparent throughout the 5-h test but were especially noticeable in the first several hours. Thus early dietary NaCl conditions can have profound effects on NaCl intake, particularly on stimulated NaCl intake, and the effects are pronounced in short-term tests.

Of critical importance, the present study also shows that early NaCl manipulations did not affect behavioral responses to treatments that stimulate water intake. Regardless of early NaCl condition, both water deprivation and HS infusion stimulated comparable water intake by adult rats (Fig. 1). Moreover, pNa in adult rats was not different during baseline conditions and increased comparably after HS infusion (Table 1), suggesting that the water consumed after HS was appropriate for water and electrolyte balance. Although we did not measure urinary Na⁺ excretion or plasma hormone levels, the observation that HS-stimulated increases in pNa were comparable also suggests that hormonal and renal responses to hypernatremia were not affected by early NaCl conditions. Collectively, these results indicate that early dietary NaCl manipulations have selective effects on NaCl ingestion that are not attributable to chronic changes in body fluid balance or to generalized changes in compensatory responses to body fluid challenges.

Surprisingly, then, water intake associated with NaCl ingestion was affected by early NaCl condition (Fig. 2). Adult rats in the 3% NaCl group drank proportionally less water compared with 0.5 M NaCl during 5-h tests after dietary Na⁺ deprivation, so the concentration of the ingested solution was greater (Table 2). In contrast, adult rats in the 0.1% NaCl group consumed proportionally more water, so the concentration of the ingested solution was lower (Table 2). It is possible that early NaCl conditions affect the ability to retain Na during dietary Na⁺ deprivation or the ability to excrete Na⁺ in urine during NaCl ingestion, thereby altering the water intake necessary for body Na⁺ regulation. However, early NaCl conditions also affect need-free ingestion of both NaCl and water (3, 8–10, 26). Moreover, neither water intake nor pNa differed among the groups after HS infusion (Fig. 1B; Table 1), suggesting that neither urinary Na⁺ retention nor excretion is affected by early NaCl conditions. An alternative explanation is that the hypernatremia that results from a systemic NaCl load is better able to elicit compensatory water intake. Consistent with this idea, after HS, both the volume of water consumed and the latency to begin drinking were similar in adult rats regardless of early NaCl condition (Fig. 1B). When water intake was associated with ingestion of a concentrated NaCl solution (Fig. 2), both the volume of water consumed and the latency depended on early NaCl condition, with smaller volumes and longer latencies in rats in the 3% NaCl group and greater volumes and shorter latencies in rats in the 0.1% NaCl group.

Interestingly, early NaCl condition affected fluid intake and concentration primarily during the initial part of the 5-h test (Table 2). In contrast, overnight NaCl intake, water intake, and the concentration of the ingested fluid were comparable in all groups (Table 2). NaCl consumption elicits behavioral and physiological responses that involve both early preabsorptive signals, such as taste, and later postabsorptive signals, such as

increased pNa (e.g., 25). Although we do not have measurements of pNa during the behavioral test, it seems likely that the delayed water intake by adult rats in the 3% NaCl group is attributable to the time necessary for absorption of the ingested NaCl and the subsequent increase in pNa. Thus together with observations that HS infusion produced comparable increases in water intake and in pNa in all rats regardless of early NaCl condition, these results suggest that compensatory responses elicited by postabsorptive signals such as increased pNa are unaffected by early NaCl conditions.

In addition to postabsorptive signals, ingestion is influenced by preabsorptive signals from the oral cavity (e.g., taste, swallowing) and the stomach (e.g., distension), as well as by local osmoreceptor signals arising from the liver. Numerous studies have focused on such preabsorptive signals associated with NaCl intake (1, 4, 15, 22, 25, 35). However, only a few studies have examined the effect of early dietary NaCl conditions on responses to preabsorptive signals, and those studies focused on taste. For example, early dietary Na⁺ deprivation affects NaCl taste responses by adult rats, as well as the activity and morphology of gustatory pathways (18, 20, 36, 38). Early dietary NaCl manipulations using NaCl levels similar to those consumed by humans and sufficient for normal reproduction in rats (3, 9, 10) also affect sensory neural responses. We recently reported that electrophysiological responses of the chorda tympani nerve to lingual NaCl stimulation were reduced in adult rats in the 3% NaCl group and were inhibited to a greater degree by amiloride (28), an Na⁺ channel blocker (17) that impairs the ability of rats to discriminate between Na⁺ and non-Na⁺ salts (34) but not to distinguish among NaCl concentrations (5). Taken together, these observations suggest that, rather than being secondary to altered body fluid regulation, differences in NaCl intake by adult rats after early NaCl manipulations may be attributable to changes in sensory coding related to NaCl taste.

Thus we suggest that manipulations of early NaCl levels alter the set point for what is perceived as normal NaCl taste, with specific behavioral consequences that persist after return to normal dietary NaCl levels. The gustatory system undergoes great structural and functional change during early development (16, 24), further supporting the idea that the NaCl level on which the rat was reared may have long-term consequences for the detection of and responses to NaCl taste. Consistent with this idea, during the initial part of 5-h tests after dietary Na⁺ deprivation, NaCl intake was greater in adult rats in the 3% NaCl group and less in rats in the 0.1% NaCl group (Fig. 2), even though all rats had previously been maintained on standard 1% NaCl chow for >30 days. Early dietary NaCl conditions also affected water intake stimulated by the taste of NaCl. Specifically, water intake secondary to NaCl ingestion was less in adult rats in the 3% NaCl group and greater in rats in the 0.1% NaCl group in these short-term tests (Fig. 2). Finally, early NaCl conditions also affected water intake associated with feeding even when rats had been maintained on the standard 1% NaCl chow for >3 mo (Fig. 3). In fact, regardless of whether NaCl was in solution or in chow, adult rats in the 3% NaCl group drank less water for each unit of NaCl consumed, whereas adult rats in the 0.1% NaCl group drank more water for each unit of NaCl consumed. It seems likely that differences in NaCl intake and in water intake associated

with NaCl ingestion reflect altered responses to preabsorptive signals associated with NaCl taste.

Despite alterations in NaCl taste sensitivity, however, the present results suggesting that body Na⁺ regulation is unaffected by early NaCl conditions predict that, ultimately, increased pNa as a result of NaCl consumption will elicit appropriate behavioral and physiological responses. Moreover, the ability to respond to NaCl stimuli is not specific to adults, nor does it require being maintained on standard 1% NaCl chow for several months. Circulating levels of the Na⁺-conserving hormone aldosterone were elevated in weanling rats in the 0.1% NaCl group while being maintained on 0.1% NaCl chow (12). In the present study, ad libitum water intake by weanling rats being maintained on the test diets reflected the Na⁺ content of the chow (Fig. 3A). Caloric requirements are met by food consumption, which complicates interpretation of these results. However, examination of the relationship between water intake and food intake, as expressed by the water:food ratio (Fig. 3B), revealed that weanlings ingested more water for each gram of chow consumed when the NaCl content of the chow was high.

Finally, pNa in rats in the 3% NaCl group was normal as early as PD1, suggesting that prenatal development of physiological responses to increased pNa occurs normally. This observation is consistent with reports that hypernatremia produces appropriate behavioral, neuroendocrine, and neural responses in near-term sheep and rats (40, 41), and in neonatal rats (32). However, pregnant rats are hyponatremic and volume expanded, so the effect of the 3% NaCl diet on pNa in the dams, and thereby on the composition of fetal plasma or amniotic fluid, may be buffered, as indicated in previous studies (21, 29). Moreover, pNa on PD1 was decreased in rats in the 0.1% NaCl group, suggesting that manipulation of dietary NaCl levels during development in utero may affect the development of the ability to maintain normal pNa, at least in regard to early dietary exposure to low NaCl. Specific behavioral and physiological responses to hypernatremia develop postnatally (6), as do the neural connections associated with those responses (31). Thus it remains possible that early NaCl manipulations retard the development of Na⁺ regulation. Although the effect does not appear to be permanent, the time course of the development is unknown. Our water:food ratio data show that, at least for weanlings in the 3% NaCl group, there are some behavioral responses consistent with Na⁺ regulation. At present, other behavioral and physiological responses to hypernatremia have not been examined in weanlings or in neonates after early NaCl manipulations. Further investigation may provide additional information about the mechanism, critical period, and development of Na⁺ regulation and about the effect of early NaCl conditions.

In summary, the adult rats reared either on 3%, 1%, or 0.1% NaCl exhibited striking differences in the temporal patterns of 0.5 M NaCl intake after dietary Na⁺ deprivation that were especially noticeable in the first several hours of short-term tests. These group differences in NaCl intake are not attributable to impairments in behavioral or physiological mechanisms associated with Na⁺ regulation, as all rats had normal baseline pNa, consumed similar amounts of water in response to intravenous HS, and had comparable HS-induced increases in pNa. Moreover, all rats drank comparable amounts of water in conjunction with NaCl intake in the later part of behavioral

tests when postabsorptive signals influence ingestion. Nonetheless, early dietary NaCl conditions affected water intake stimulated by NaCl ingestion during the initial part of short-term tests, as well as water intake associated with feeding. Together with our previous studies (3, 8–10, 28), these observations support the idea that early dietary NaCl manipulations have selective consequences that occur despite unimpaired Na⁺ regulation: taste-driven alterations in NaCl intake and in the behavioral responses to preabsorptive taste signals associated with NaCl intake.

Perspectives

An important caveat to this conclusion involves another of the sequelae of high dietary NaCl-elevated blood pressure. Compared with adult rats in the 1% and 0.1% NaCl groups, rats in the 3% NaCl group have higher baseline blood pressure and enhanced pressor responses to peripheral administration of ANG II (7, 8, 11). It is unclear whether or how elevated NaCl intake and elevated blood pressure in this model may be related; however, changes in hormones or sympathetic outflow as a result of early high NaCl levels may underlie both effects. An alternative explanation is that the consequences of NaCl intake contribute to increased blood pressure. Consistent with this idea, compared with adult rats in the 1% and 0.1% NaCl groups, blood pressure increased even further in adult rats in the 3% NaCl group when given high NaCl diet as adults (11).

Regardless of the mechanism, the observations of elevated blood pressure are surprising. High NaCl intake tends to produce hypertension only in genetically susceptible individuals, and Sprague-Dawley rats do not exhibit this genetic bias. Therefore, the dual effects of early exposure to high dietary NaCl levels, i.e., increased NaCl intake and elevated blood pressure, have implications for human hypertension that are staggering: high blood pressure may occur as a consequence of pre- or early postnatal exposure to high NaCl, regardless of genetic predisposition or subsequent diet. Moreover, high NaCl consumption as adults may further increase blood pressure. Ironically, our data suggest that, at least in rats, those animals most susceptible to cardiovascular complications arising due to early exposure to high NaCl levels also are more likely to consume larger amounts of NaCl as adults, thereby exacerbating the hypertension.

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