Repeated sodium depletion affects gustatory neural responses in the nucleus of the solitary tract of rats

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Tamura, Ryo, and Ralph Norgren. Repeated sodium depletion affects gustatory neural responses in the nucleus of the solitary tract of rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1381–R1391, 1997.—Furosemide sodium depletions were induced repeatedly to determine the effects on gustatory neural responses in the nucleus of the solitary tract (NST) of chronically prepared, but lightly anesthetized, rats. Sodium-replete and sodium-deplete conditions were alternated four times in each rat. When rats were under depleted conditions, the responses to NaCl were significantly greater than in sodium-replete conditions. This effect was attributable primarily to an increase in the magnitude of response of those neurons that responded better to NaCl than to the other standard stimuli (sucrose, citric acid, and quinine hydrochloride). In addition, the largest change in responsiveness of the NaCl-best neurons occurred during the third and fourth sodium depletions. These results are essentially opposite to those reported for NST neurons when sodium appetite is induced by dietary sodium restriction. This suggests that the coding of intensity in the gustatory system is dependent not only on the animal’s deprivation condition, but also the method through which the deprivation is produced.

sodium appetite; single-unit activity; sodium-deficient diet; furosemide

In many species, depletion of body sodium stores produces an appetite during which normally rejected concentrations of sodium salts are ingested avidly (5). To account for the increased salt ingestion that accompanies the sodium depletion produced by adrenalectomy, Richter (22) proposed that changes in the taste buds gave rise to an enhanced ability to discriminate salt from other substances. Two subsequent studies, however, found no differences between either adrenalectomized or dietary sodium-deprived rats and controls in the responses of the chorda tympani (CT) nerve to sapid sodium (15, 19). In fact, dietary sodium deprivation or bilateral removal of the adrenals actually led to a decrease in CT responsiveness to superthreshold NaCl solutions (2–4, 13). These results suggested that the increased NaCl intake seen in sodium-depleted rats might be due, in part, to a reduction of the gustatory neural signal and thus a reduction in the perceived taste intensity of a sodium solution.

In the nucleus of the solitary tract (NST), dietary sodium restriction that was sufficient to induce a sodium appetite also reduced the responsiveness of gustatory neurons to sapid NaCl (12, 16). Although these two studies observed effects on sodium responsiveness similar to those reported for CT axons, the gustatory response profiles differed in other characteristics both from the peripheral data and from one another.

Another study of whole nerve CT responses used acute depletion of body sodium produced with the diuretic natriuretic furosemide. In this case, there was a modest reduction in the response to sapid sodium, but only at the highest concentration tested (1). These results tended to confirm the prior observations, but did introduce some inconsistencies as well. In behavioral studies, diuretic induction is the most common paradigm for raising a sodium appetite (at least in rodents), but its effects on gustatory neural responses have been examined only in this one study that measured whole nerve responses from the CT. After the first depletion, sodium repletion brings spontaneous intake almost back to predepletion levels, but subsequent depletions raise both need-induced and need-free NaCl intake (23, 24). There are no electrophysiological studies that test the influence of repeated sodium depletions on changes in the neural responses to taste stimuli. The increased need-free intake that follows multiple inductions, however, would be consistent with a decrease in overall responsiveness to NaCl and that would, in turn, decrease the perceived intensity of the salty taste. The present study examined the effects of both the first and subsequent acute sodium depletions produced by injections of furosemide on the gustatory neural responses of single neurons in the anterior NST.

METHODS

Animals

Eight male Sprague-Dawley rats (Charles River, 200–250 g at the outset) were housed individually in a room with 12 h light-dark cycle and constant temperature (22 ± 1.0°C). They had access ad libitum to distilled water, 3% NaCl solution (0.51 M), and standard rat pellets (Rodent Laboratory Chow 5001, Purina Mills). The fluids were presented on the front of the cages in graduated cylinders fitted with metal drinking spouts. Daily intake of water and 3% NaCl was recorded for the second week of a 2-wk acclimation period and, unless noted otherwise, throughout the remainder of the experiment. During this period, two rats consumed at least 5 ml of 3% NaCl on 2 consecutive days. This is the criterion that we use for excess baseline NaCl consumption, so these animals were dropped from the experiment. Therefore, six rats were prepared for recording; the behavioral and neural data derive from five of these animals.

General Procedure

After surgery to mount a cranioplastic appliance and subsequent localization of the target area, each rat had eight weekly data collection trials (four after furosemide administration (sodium deplete) and four after saline injections (sodium replete)) that included recording of NST gustatory neural responses and a sodium appetite test. The order of
sodium-replete and sodium-deplete trials was randomized, and the experimenter was blind to the animal’s condition.

Cranioplastic surgery. Surgery was performed under aseptic conditions. Rats were anesthetized (Nembutal, 50 mg/kg ip) and a cranioplastic cap was attached to the skull with stainless steel screws (1–72 X 1/8 in.). This cap was molded around the conical tips of “fake ear bars” that made it possible to fix the animal’s head in the stereotaxic device painlessly and thus permitted repeated recording trials using light doses of anesthesia (see Refs. 16 and 17 for details). After surgery, the rats were housed individually in metabolism cages in which distilled water, 3% NaCl, and normal chow pellets were freely available, unless otherwise noted.

Localization Seven to 10 days after surgery, each rat was reanesthetized (Nembutal, 50 mg/kg ip) and mounted in the stereotaxic device using the fake ear bars, and the NST was located electrophysiologically. A 2.0-mm/diameter hole was drilled through the cranioplastic and underlying interparietal bone centered –11.5 mm posterior to b and 2.0 mm lateral to the midline. Several penetrations were made with a recording electrode to locate the taste area exactly for the later single-unit recording sessions. Typically, the gustatory NST was located 11.6 mm posterior to b, 1.8 mm lateral to the midline, and 8.5 mm below the skull surface at b. Subsequently, the exposed brain was covered with hydrocortisone ointment (Neo-Cortef, Upjohn), and the hole was covered by a small piece of sterile plastic and dental acrylic to avoid infection.

Elicitation of sodium appetite. Sodium appetite was elicited by combining sodium-deficient diet with natriuresis produced by two injections of furosemide (5 mg, 0.5 ml/rat sc at 1400 and 1600) (34, 36). It has been reported that normal Sprague-Dawley rats exhibit neophobia to sodium-deficient diet (7, 1600) (34, 36). Therefore, to minimize this possible neophobic effect on neuronal activity, the sodium-deficient diet was given to together with normal chow pellets a few days before the first recording session. At the time of the first injection, the cage was replaced with a clean metabolism cage in which only sodium-deficient powdered diet (ICN no. 902903) and distilled water were available. On control trials, the rats received injections of 0.9% NaCl (0.5 ml sc) and the sodium-deficient diet to which 0.05% NaCl (wt/wt) was added to maintain a neutral sodium balance. Urine volume was recorded and 24 h after first injection; the concentration of Na⁺ and K⁺ was measured for 24-h urine collection. The recording of NST neural activity began after the 24-h collection (see Recording). After recording, the rat was returned to its metabolism cage, and only distilled water and the sodium-deficient diet (with or without 0.05% NaCl) were available. After overnight food and water intake was measured the following day, 3% NaCl and fresh water were returned, and consumption of both was measured at 0.25, 0.5, 1, 2, and 24 h (sodium-appetite test). After the 2-h measurement, the powdered diet was removed and replaced with standard chow pellets.

Recording

A rat was lightly anesthetized (Nembutal, 35 mg/kg ip) and mounted in the stereotaxic device with the fake ear bars. To maintain a constant level of anesthesia, small amounts of Nembutal (2–3 mg/kg ip) were given approximately every 10 min. Rectal temperature was monitored and maintained between 36.5 and 37.5°C with a heat pad. Glass-coated, tungsten microelectrodes with a ball-shaped tip (2.5–5.0 MΩ at 1 kHz) were positioned using coordinates derived during the localization procedure. After the acrylic cap was removed from the recording hole, the electrode was advanced through it into the vicinity of the NST with a micromanipulator (SM-20, Narishige) using the coordinates established earlier. Extracellular single-unit activity was recognized by its consistent waveform and amplitude; some spikes were recognized by the A-B break on the rising phase and their relatively long duration (usually >0.7 ms; Fig. 2, B and C. Ref. 11). Electrical activity was amplified conventionally, archived on magnetic tape, and counted with a window discriminator. Spike frequencies were computed on-line over 60 s with 2.0-ms bins using a microcomputer (IBM PC) equipped with a counter board (CIO-CTR5, Computer Boards) and displayed as peristimulus time histograms (250-ms bins).

Stimulation

Taste stimuli consisted of sodium salts, non-sodium salts, sugars, polyacrylates, and quinine hydrochloride (QHCl), all presented at room temperature. This article focuses on the responses to four standard taste stimuli (0.1 M NaCl, 0.3 M sucrose, 0.01 M citric acid, and 0.01 M QHCl) and a concentration series (0.01 M, 0.03 M, 0.1 M, 0.3 M, and 1.0 M) of NaCl and sucrose solutions. The first water and the taste stimuli were delivered in 50-µl aliquots to the ipsilateral side of the anterior tongue through a polyethylene tube (PE-10) positioned through a stainless steel guide tube to maintain constant spatial relations. A trial consisted of a water-stimulus-water sequence with 30-s minimal intervals between the first water rinse and the stimulus and 10 s between the stimulus and the second water rinse. After each taste stimulation, the tongue was rinsed with 3–4 ml distilled water at least three times. At least 2 min elapsed between taste stimulations. The moment of stimulus contact with the tongue was marked by a TTL sensor.

Data Analysis

All analyses were based on neuronal activity in 5-s samples, expressed as spikes per second. For water and stimulus trials, these samples began at the fluid-onset mark; for spontaneous activity, they began 5 s before the water-onset mark. Two different response measures were employed, the raw response (mean neuronal activity in 5 s) or a corrected response for water; the corrected response was the raw water response minus the mean spontaneous rate; for taste, it was the raw taste response minus the mean water response. In both cases, the response was considered significant if it deviated at least 2.5 SD from the base activity used in the calculation. Unless stated otherwise, the corrected responses are cited in the text. When a stimulus was repeated for a particular neuron, the response measure was the mean number of spikes per 5 s.

Appropriate analysis of variance tests were performed on each data set and statistically significant F-tests were evaluated further with Tukey’s Studentized Range Test (GLM Procedure, SAS User’s Group International). The Pearson product-moment correlation coefficients for all possible pairs of responses were calculated (the CORR Procedure, SAS User’s Group International) and used to conduct cluster analyses (average linkage method; the Cluster Procedure, SAS User’s Group International). Multidimensional scaling was done using both correlation coefficients (metric ratio model) and Euclidean distances (MDS Procedure, SAS User’s Group International).

Histology

After the recording sessions were completed (2 mo), the rats were reanesthetized and again placed in the stereotaxic apparatus. Several small electric lesions (10 µA +, 10 s) were made at the boundaries of the area from which taste-responsive neurons were isolated. The rats were then given a
further lethal dose of Nembutal (100 mg/kg ip) and perfused transcardially with physiological saline followed by 10% Formalin. The brain was removed, cut coronally in 50-µm sections, and mounted on slides, and alternate series of sections were stained with the cresyl Lecht violet and Weil procedures.

RESULTS

Sodium Appetite

Furosemide injections elicited a robust and reliable increase in 3% NaCl intake compared with saline injections (Fig. 1) [F(1,96) = 215.3, P < 0.0001]. In the sodium-deplete condition, there was a slight, but significant, trial effect on intake of 3% NaCl [F(3,48) = 3.30, P < 0.05], but not in the sodium-replete condition. Post hoc multiple comparison tests revealed significantly increased NaCl intake on the fourth depletion compared with the first two (P < 0.05). There also were significant differences in daily need-free 3% NaCl intake between trials [F(3,8) = 7.91, P < 0.01] (Table 1). Post hoc tests indicated that this change also occurred after the fourth trial compared with the first and second (P < 0.05). At 3 h, there were differences in urine volume between the sodium-deplete and sodium-replete conditions (23.9 ± 0.99, mean ± SE and 1.35 ± 0.21 ml, respectively), but no differences across trials. Similarly, there were obvious differences of sodium concentration in the subsequent 21-h urine collection between the two conditions (sodium deplete: 13 ± 2.64 µmol/ml; sodium replete: 130 ± 6.09 µmol/ml), but no significant trial effects (Table 1).

Neuronal Activity

Spontaneous activity and responses to standard taste stimuli. A total of 88 taste neurons were isolated and tested at least once with four standard taste stimuli and water. Of these, 42 were recorded while the rats were in the sodium-replete condition, and 46 were recorded while the rats were sodium depleted. Based on the recorded lesions made after all recording was completed, the cells were located in, or bordering on, the rostral NST at the level where the majority of CT axons terminate (9). An example of the taste responses of a neuron (unit 54) recorded in the sodium-replete condition appears in Fig. 2. This neuron responded briskly to 0.1 M NaCl (Fig. 2, A and Bb), less so to citric acid and QHCl (not shown), and not at all to sucrose (not shown) and water (Fig. 2, A and Ba).

On the basis of their largest response to the four standard taste stimuli, the neurons were classified as follows: NaCl best, sucrose best, citric acid best, or QHCl best. In Fig. 3, the spontaneous activity and the response profiles of each neuron recorded in the sodium-replete (A) and sodium-deplete (B) conditions are arranged in descending order of response magnitude, beginning with the NaCl-best cells on the left, followed by the sucrose-best, then the citric acid-best, and the QHCl-best neurons. The number of cells in each best-stimulus category appears in Table 2; the mean spontaneous firing rate and response profiles appear in Fig. 4. Spontaneous firing rates did not differ between the two conditions, either within categories or across the entire sample, nor were there any significant responses to water.

When all cells and the four standard taste stimuli are considered, the responses were significantly higher in the sodium-deplete condition than in the sodium-replete condition [12.6 ± 1.32, mean ± SE, spikes/s vs. 8.19 ± 0.93, respectively; F(1,350) = 6.83, P < 0.01]. Nevertheless, the effects differed by stimulus and by best-stimulus category. Specifically, the responses to NaCl were significantly higher when the rats were sodium deplete than when they were replete [F(1,86) = 5.46, P < 0.05], but the other standard stimuli did not differ as a function of diet condition. This specificity extended to the NaCl-best neurons. In the deplete condition, these cells responded more to NaCl than in the replete state [F(1, 55) = 6.89, P < 0.05], but their responses to the other standard stimuli did not differ. The responses of cells in the other best-stimulus categories did not differ across condition for any of the stimuli (Fig. 4).

Breadth of responsiveness. The entropy measure for breadth of response (29) was calculated for the neurons in each best-stimulus category as a function of depletion condition using the excitatory components of the

<table>
<thead>
<tr>
<th>Volume of 3-h Urine Collection, ml</th>
<th>Sodium Content in 21-h Urine Collection, µmol</th>
<th>Need-Free 3% NaCl Intake, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>341 ± 38.5</td>
<td>1.07 ± 0.24</td>
</tr>
<tr>
<td>Second</td>
<td>349 ± 37.1</td>
<td>1.40 ± 0.20</td>
</tr>
<tr>
<td>Third</td>
<td>241 ± 42.1</td>
<td>2.53 ± 0.44</td>
</tr>
<tr>
<td>Fourth</td>
<td>242 ± 70.4</td>
<td>3.53 ± 0.59</td>
</tr>
</tbody>
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Values are means ± SE.
responses to the four standard taste stimuli. As evident in Table 3, there were no significant differences between the two conditions.

Responses to NaCl and sucrose concentrations. Sixty-five neurons were tested with the NaCl concentration series, 48 with sucrose. For NaCl, both depletion $[F(1,315) = 12.5, P < 0.001]$ and concentration $[F(4,315) = 10.8, P < 0.0001]$ influenced the responsiveness of the NST neurons (Fig. 5Aa), but there was no significant interaction between the two factors. When only NaCl-best neurons were considered, the responses to the NaCl series were clearly higher during the deplete condition $[F(1,195) = 19.1, P < 0.0001$, Fig. 5Ab], but the responses of sucrose-best neurons to the NaCl series were slightly, but significantly, lower $[F(1,60) = 4.72, P < 0.05$, Fig. 5Ac]. Specifically, during the deplete condition, the NaCl-best neurons responded more at the three lower NaCl concentrations (0.01, 0.03, and 0.1 M, $P < 0.05$), whereas, for the sucrose-best cells, the two higher concentrations (0.3 and 1.0 M) tended to be more effective in the replete condition ($P < 0.075$). No significant effects of condition occurred in response to the sucrose concentration series, but there was a tendency for the NaCl-best cells in deprived rats to respond more to the 1.0 M sucrose $[F(1,145) = 3.87, P < 0.075$, Fig. 5Bb] and for the sucrose-best neurons to respond less $[F(1,53) = 3.36, P < 0.075$, Fig. 5Bc].

Effects of repeated sodium depletions on neuronal responses to NaCl. As mentioned in the introduction, repeated sodium depletions with furosemide typically increase both need-free and need-induced salt intake (23, 24). Since NST neurons, especially the NaCl-best ones, respond more to sapid sodium in the deplete condition, we analyzed these effects across the repeated cycles of sodium depletion and repletion that were used in these semi-chronic preparations. Although a trend seemed apparent, the average differences in response to NaCl across individual trials did not reach significance either for the entire sample or for the NaCl-best
neurons (data not shown). When the data were combined to increase and balance the sample size, both the early (trials 1 and 2) and later (trials 3 and 4) NaCl-best samples exhibited increased responsiveness to sapid sodium when the rats were depleted $F(3,53) = 4.27, P < 0.01$; Fig. 6B]. Post hoc comparisons revealed that the increased responsiveness was accounted for by neurons from depleted rats in the later trials (tested against data from both the early and late sodium-replete trials; $P < 0.05$). When compared with the same data, the responses from the early sodium-deplete trials were not significantly different. In the sodium-replete condition, the mean responsiveness in the early period (18.5 ± 6.41 spikes/s) was virtually the same as that in the late period (17.9 ± 5.01 spikes/s). The variation of response intensity among NaCl-best neurons in the latter two sodium-deplete sessions tended to be larger than that in other groups. The number of neurons in each group is shown in parentheses in Fig. 6.
Hierarchical cluster analysis and multidimensional scaling. The relationships apparent from the best-stimulus categories were checked using less arbitrary analyses based on correlation coefficients. All possible Pearson product-moment correlation coefficients were generated from the response profiles produced by the standard taste stimuli for the 42 neurons in the sodium-replete (861 pairs, 42 × 41.5) and 46 in the sodium-deplete condition (1,035 pairs, 46 × 45.5). Using these matrices, a hierarchical cluster analysis for neurons recorded in each condition was derived through the average linkage method and represented as a dendrogram (Fig. 7, A and B). The level at which two neurons or two groups of neurons join together in the dendrogram indicates their overall correlation and is referred to as cluster similarity. Each neuron’s number and response category based on its response to the standard taste stimuli are listed on the right.

In both conditions, the two largest clusters are dominated by NaCl-best and sucrose-best neurons. In fact, in the deplete condition, the first four clusters, each of which has internal correlations greater than 0.5, separate the sample into best-stimulus categories almost perfectly. Only two cells (80 and 43) that responded best to NaCl are segregated with the seven that are acid best. In the replete condition, the cluster analyses does not track the best-stimulus categorization quite as straightforwardly. Nevertheless, the cluster analysis confirms two related points. First, as noted in a prior publication (17), cluster analysis and best-stimulus categorization organize neuronal responsiveness in similar ways. Second, depleting body sodium with a natriuretic does not drastically alter the relative response profiles of NST gustatory cells.

Multidimensional scaling illustrates this second point more dramatically (Fig. 8). In this two-dimensional display, all the neurons from both the replete and deplete conditions were analyzed using Euclidean distances and labeled by their best-stimulus category. The two samples are distributed in the space in an essentially identical manner. This also was true when the analyses were done separately for each sample and when the three-dimensional solutions were plotted (not shown). Thus it appears that the major effect of repeated acute treatment with natriuretics on NST gustatory neurons is to increase the magnitude of response of the NaCl-best cells to sapid sodium.

**DISCUSSION**

The major observation of this study is that acute sodium depletion produced by injections of the natriuretic furosemide significantly increased the responsiveness of NST gustatory neurons to sapid sodium without substantially altering their responsiveness to other stimuli. The effect was observed across a range of NaCl concentrations and was more pronounced after multiple depletions. McCaughey et al. (14) reported a trend toward increased responsiveness to NaCl in NST taste neurons after recovery from a diet-induced appetite. When examined during a diet-induced appetite, however, previous studies, including one from this laboratory, reported exactly the opposite effect of sapid NaCl: a reduction in gustatory neural responsiveness.

In rats fed a sodium-free diet for at least 10 days, both CT axons and NST gustatory neurons responded less, rather than more, to sapid sodium stimulation (2–4, 12, 16). Other studies, which used different paradigms for inducing sodium appetite, reported similar, if less dramatic effects, or no changes in CT responsiveness to NaCl. Adrenalectomy reduced responsiveness of the CT nerve over a concentration range from hypotonic to isotonic NaCl (0.01–0.1 M), but, at hypertonic concentrations (0.3 M), the effect was blunted in the phasic response and reversed during tonic activity (13). Furosemide treatment produced a marginal reduction in responsiveness of the CT nerve, but only at the highest concentration of NaCl tested (0.5 M) (1).
Another replication of the experiment that used a low-sodium diet (0.03%), as opposed to a sodium-free one, found no difference in the NST response to NaCl (or other stimuli) between the sodium-deplete and sodium-replete conditions (33). If the animals had been reared on such a low-sodium diet since early in gestation, however, their NST taste neurons did respond less to NaCl. When allowed to recover from this formative experience, by living on a diet containing 1.0% NaCl, the same cell population became hyperresponsive to sodium. In sum, the current results, produced after systemic sodium depletion produced with furosemide, challenge a number of studies that induced an appetite using a sodium-free diet. When a sodium appetite was produced by other methods, such as adrenalectomy, a single furosemide depletion, or an intraventricular infusion of renin, the changes that occur in the responsiveness of gustatory neurons to sapid sodium tend to fill in the gap between those two extremes (1, 13, 32).

In addition to the different methods used to induce a sodium appetite, other factors might account for the discrepant changes in NaCl coding among the various studies. Some of these differences, such as the recording level (peripheral vs. central), the presence or absence of anesthesia, the method of sapid stimulation, or even the diet, have been dealt with in an earlier discussion of the issue (16). The present experiment used lightly anesthetized chronic preparations in which only the anterior tongue was stimulated while recording from single neurons in the NST. Similar conditions prevailed in at least one prior study that produced essentially opposite results (12). The diets and methods of stimulation did differ between these two studies. These factors are unlikely to account for the differences in responsiveness to NaCl, however, because a third study that used diets identical to those in the present experiment (and a third method for applying sapid stimuli) nevertheless found that NST cells were less, rather than more, responsive to NaCl in sodium-deprived, awake, behaving rats (16).

One important difference between the present experiment and all prior ones that deal with taste coding was the inclusion of multiple depletions and repletions. This is known to increase both the need-induced and the need-free sodium intake, as it did in our rats (23, 24). Not only did the later depletions enhance the increased responsiveness to sodium in NaCl-best neurons, but the effects were completely reversible, because the cells tested during the intervening need-free recording sessions failed to exhibit any change in response to sapid stimuli. This implies that the enhanced need-free intake of NaCl observed after mul-

Fig. 5. Comparison of averaged response profiles of total (a), NaCl-best (b), and sucrose-best (c) neurons to concentration series of NaCl (A) and sucrose (B) between the sodium-replete (open circles) and sodium-deplete (solid circles) conditions.
Multiple sodium depletions cannot be attributed to changes in gustatory coding. It also means that the changes in responsiveness to NaCl during need-induced sodium appetite were transitory (see Ref. 14 for contravening evidence). Nevertheless, when rats were maintained on sodium-deficient diet for more than 50 days, the decreased NST responsiveness to sapid sodium was maintained throughout. Although the change in coding differed between the two studies, taken together, the results indicate that the altered gustatory responsiveness to sapid NaCl stimulation was directly attributable to an ongoing state of relative body sodium deficit.

Despite their distinctiveness in the present design, multiple depletions alone seem unlikely to account for the differences in results between the present experiment and another one that used a diuretic to induce a sodium appetite. Bernstein and Taylor (1) recorded from the whole CT nerve in acute preparations that had a single furosemide treatment. They reported a modest reduction in the whole nerve response to sapid NaCl, but only at the highest concentration (0.5 M). We tested single cells in the anterior NST and observed an increased response across a range of NaCl concentrations. The differences between the two studies are not entirely contradictory, because 1) the sample of neurons obtained during our first depletion was small, 2) our effects reached statistical significance only at concentrations.
centrations of 0.1 M NaCl or less, and 3) we recorded single neurons in the NST.

The contradiction is starker in comparison with the studies that used diet to induce a sodium appetite (2, 3, 12, 16), because some of these recorded from the NST (12, 16) and all reported effects at similar concentrations. This leaves only the mechanism of sodium challenge, the number of challenges, and their apparent time course to account for the differing effects. Only a single period of dietary sodium restriction was used, albeit in one case it lasted more than 50 days. Dietary restriction induces a sodium appetite more slowly than does furosemide. In practice, the dietary experiments were conducted after at least 10 days on sodium-free chow, whereas our recording sessions began 24 h after the furosemide injections. As mentioned above, when an appetite was raised on a sodium-free diet, a prominent effect was a reduction in the magnitude of response of gustatory neurons to sapid sodium. In the present experiment, we cannot assert that an increase in responsiveness existed during the equivalent period, the first furosemide depletion, because the neuronal sample from each recording day was small. Nevertheless, the trend was there from the beginning, and certainly there was no evidence of a decrease in the response of NST taste neurons at any concentration during any of the four depletions.

If the experimental circumstances themselves fail to track the differing changes in response to NaCl, perhaps the altered hormonal milieu that mediate salt appetite provide a less ambiguous correlation with these seemingly contradictory electrophysiological results. Oxytocin inhibits sodium appetite, but its levels do not change drastically as a function of either dietary sodium restriction or furosemide depletion (30, J. Verbalis, personal communication). Angiotensin II levels rise with sodium depletion, in males almost by an order of magnitude, but the levels remain constant across repeated furosemide treatments (24). In male rats, aldosterone levels rise four- to fivefold after furosemide treatment, reestablish those levels during two subsequent depletions, but then almost double again during the fourth furosemide-induced depletion (24).

In vitro, aldosterone appears to increase the number of active amiloride-sensitive sodium channels in the apical membrane of taste cells (10) and thus provides at least a theoretical mechanism for an increase in responsiveness of NaCl-best taste neurons to sapid sodium. Appealing as such a scenario might be, it must contend with several points that fail to fit in. First, aldosterone levels also increase substantially during dietary sodium deprivation, in one determination by a factor of eight compared with controls fed the same diet to which NaCl was added (R. Sakai, personal communication).

In addition, the second increase in aldosterone levels did not occur until the last furosemide treatment, but our data exhibited an upward trend in response magnitude across all four depletions.

In conclusion, a diet that contains little if any sodium raises a strong salt appetite, but blunts the response of peripheral and central taste neurons to sapid sodium. Furosemide-induced sodium depletion produces an equivalent salt appetite (20), but enhances the response of taste neurons to sapid sodium. Although evidence exists to the contrary (12, 16), early on the blunted responsiveness occurred primarily in neurons that responded best to NaCl. Inasmuch as sodium is the appropriate goal for the appetite, this led to speculation that this effect, blunted response to sodium in NaCl-best neurons, somehow played a role in the behavioral avidity for salt that characterizes this motivational state (4). In the present study, the increased magnitude of response in depleted rats was specific to sapid sodium stimuli and to NaCl-best neurons. There was a small decrease in the response of sucrose-best cells to sapid NaCl, but in general the response profiles of NST taste neurons changed little as a function of body sodium balance. The methodological or hormonal differences between the studies failed to account for the diametrically opposed differences in response that accompanied the two different procedures for inducing the same motivational state. In fact, using a third procedure for producing a salt appetite, intracerebroventricular injection of renin, still another profile of NST taste neuron responses to sapid sodium was produced (32). Regardless of how a sodium appetite is raised, rats still ingest large amounts of strong salt that they otherwise would avoid. The various procedures for producing the appetite, however, appear to alter the gustatory code for NaCl in different ways. As pointed out in a prior publication (16), this has altered the question from “How does taste guide an animal with a sodium appetite?” to “How does taste avoid confusing a rat with a sodium appetite?”

Perspectives

Changes in coding of the NaCl message as a function of sodium appetite have been observed in peripheral
neurons of the CT nerve and in central taste cells in the NST. Additional processing could take place further centrally and, if it does, the parabrachial nuclei (PBN) are an obvious place to investigate. The PBN contain the second central gustatory relay in the rat, and they project to both the thalamocortical taste system and to the ventral forebrain (18). Destruction of either gustatory cortex or the thalamic taste relay fails to interfere with sodium appetite (6, 25, 35). Bilateral lesions of the ventral forebrain (18). Destruction of either gustatory cortex or the thalamic taste relay fails to interfere with sodium appetite (6, 25, 35). Bilateral lesions of the ventral forebrain (18). Destruction of either gustatory cortex or the thalamic taste relay fails to interfere with sodium appetite (6, 25, 35).

In fact, the change in avidity for salt that attends sodium appetite might depend entirely on the ventral forebrain that controls hydromineral balance (21). This study was supported by National Institute of Health Grants MH-43787 and DC-00240. R. Norgren is a recipient of a National Institute of Mental Health Research Scientist Award (MH-00653). Portions of these data have been published previously in abstract form (31).

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