Low-dose furosemide modulates taste responses in the nucleus of the solitary tract of the rat

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Low-dose furosemide modulates taste responses in the nucleus of the solitary tract of the rat. Am J Physiol Regul Integr Comp Physiol 287: R706–R714, 2004. First published May 20, 2004; 10.1152/ajpregu.00090.2004.—Taste-evoked neural responses in the nucleus of the solitary tract (NST) are subject to both excitatory and inhibitory modulation by physiological conditions that influence ingestion. Treatments that induce sodium appetite predominantly reduce NST gustatory responsiveness to sodium stimuli. When sodium appetite is aroused with 10 mg of the diuretic furosemide (Furo), however, NST gustatory neurons exhibit an enhanced responsiveness to NaCl. In addition to inducing a sodium appetite, 10 mg Furo supports a conditioned taste aversion (CTA). A lower, 2-mg dose of Furo induces an equivalent sodium appetite, but not a CTA. To determine whether the anomalous electrophysiological results reflected the adverse effects of the 10-mg dose, we replicated the original experiment but instead used 2 mg of Furo. In chronically prepared, lightly anesthetized rats, the responses of 49 single NST neurons to 12 taste stimuli were recorded after subcutaneous injections of either 2 mg Furo or saline. There was no effect of treatment on NST neural responses to the four standard taste stimuli. In the NaCl concentration series, however, 2 mg Furo evoked significantly higher responses to the two highest concentrations of NaCl. There was no effect of treatment in the sucrose concentration series. Thus, unlike other methods that induce a sodium appetite, Furo increases NST neural responsiveness to NaCl. At least as far as the first central relay, sodium appetite apparently does not depend on specific changes in the sensory neural code for taste.

sodium appetite; single-unit activity

THE SINGLE BEHAVIORAL CHARACTERISTIC of salt appetite is that animals seek out and ingest sodium even at high concentrations that are normally avoided (30). Richter (22) originally suggested that the threshold for detection of environmental sodium might decrease during this motivational state, but the early electrophysiological tests found little support for this hypothesis (16, 20). While recording from rats fed a sodium-free diet, Contreras (5) confirmed the absence of a threshold change in the response of chorda tympani axons to rapid NaCl, but did detect a reduction in the magnitude of their response. Several subsequent studies replicated this observation using a variety of protocols for raising a sodium appetite (2, 5, 6, 12). This reduced magnitude of response to rapid NaCl has been replicated in taste neurons recorded from the nucleus of the solitary tract (NST) and, in one instance, from the parabrachial nuclei also using a variety of protocols (11, 15, 17, 27, 32, 33).

There has been one clear exception to these observations. When a sodium appetite was raised with the diuretic furosemide (Furo) and overnight exposure to an Na-free diet, a

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American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings.”

Surgery. The rats were anesthetized (Nembutal, 50 mg/kg ip) for the surgery, which was performed under aseptic conditions, and mounted in a stereotaxic instrument. Six stainless steel screws (1–72 × \(\frac{1}{8}\) in.) were set in the skull, and a pair of “fake” ear bars was positioned above the skull. A cap of dental acrylic was then molded around the skull screws and the conical tips of the fake ear bars (Lang Dental Manufacturing). When the real ear bars are displaced laterally, the fake ones can be fitted into the acrylic cap, thus painlessly securing the animal’s head in the stereotaxic device during recording trials. After surgery, the rats were housed individually in metabolism cages for ~1 wk.

Localization of gustatory NST. Seven to ten days after surgery, each rat was reanesthetized (Nembutal, 50 mg/kg ip) and mounted in the fake ear bars to localize the gustatory NST electrophysiologically. A 2.0-mm-diameter hole was drilled through the acrylic cap and the underlying interparietal bone, centered ~12.0 mm posterior to bregma and 1.6 mm lateral to the midline. After the dura was removed, a glass-coated tungsten microelectrode (2.0–5.0 MΩ at 1 kHz) was lowered through the hole using a hydraulic microdrive (FHC). To locate the gustatory area in the NST, when the electrode penetrated to the surface of the medulla, 0.1 M NaCl solution was applied to the anterior tongue every 50–100 μm. Typically, taste-evoked neural activity was encountered ~7.0 mm below the brain surface. After locating a taste response, the electrode was removed and the exposed brain covered with neomycin-hydrocortisone ointment (Upjohn). The skull hole was then covered by a small amount of bone wax (Ethicone) and two-component epoxy (ITW).

Experimental

After the NST taste-responsive region was localized, data were collected over an 8-wk period with one recording trial per rat per week. The weekly injections of Furo (American Regent Labs) and saline were alternated, but the initial choice was random. A Day 1 recording day. On the pre-recording day, the rat was injected with either Furo (2 mg/0.2 ml sc) or isotonic saline (0.2 ml sc) at 10:00 AM and was given sodium-deficient powdered diet (ICN no. 960363) and distilled water. After saline injections, 50 mg NaCl was added to 100 g of sodium-deficient diet (0.05% NaCl) to maintain a neutral sodium balance. Urine volume was recorded 3 and 24 h after the injections, and the samples were frozen. Subsequently, the concentration of Na+ was measured from these samples.

Recording day. The next morning, the rat was lightly anesthetized with Nembutal (35 mg/kg ip), repositioned in the fake ear bars, and single neurons recorded from the NST for ~4 h. To maintain a constant level of anesthesia, small supplements of Nembutal (2–3 mg/kg ip) were given as needed. Rectal temperature was monitored and maintained between 35.5 and 36.5°C with a heating pad. After a recording session, the rat was returned to its metabolic cage with access to sodium-deficient diet and distilled water. Two experimenters participated in the recording sessions; both were blind to the animal’s condition. The taste responses obtained by these two investigators did not differ significantly from each other (\(F_{(1,45)} = 0.001, P = 0.97\)), whereas there was a significant influence of stimulus (\(F_{(2,540)} = 41.39, P < 0.001\)). As there was no interaction between experimenter and stimulus (\(F_{(2,540)} = 0.23, P = 0.997\)) or Na state (\(F_{(1,45)} = 1.79, P = 0.19\)), we combined their data in this report.

Post-recording day. Sodium appetite was tested on the day after the recording session, 48 h after injections of either Furo or saline. After overnight food and water measurement, 3% NaCl and fresh water were returned to the rat. Consumption of both was measured at 0.25, 0.5, 1, 2, and 24 h. After the 2 h-measurement, the Na-free powdered diet was removed and replaced with standard chow pellets.

Control days. On the remaining 4 days of the week, distilled water, 3% NaCl, and food intake were recorded at the same time each morning.

Data Acquisition

Extracellular recording. After the epoxy cap was removed, a glass-insulated, tungsten microelectrode (2.0–5.0 MΩ at 1 kHz) was advanced into the brain using the coordinates derived during the localization procedure. Single taste neurons were initially located using 0.1 M NaCl applied to the anterior tongue and then confirmed with a battery of other taste stimuli. Action potentials were amplified conventionally (A-M System) and archived on magnetic tape for subsequent offline analysis using a CED Power 1401 interface board and Spike2 software (Cambridge Electronic Design).

Taste stimulation. The standard stimuli were 0.1 M NaCl, 0.3 M sucrose, 0.01 M citric acid, and 0.01 quinine HCl (QHCl). A concentration series of NaCl and sucrose (0.01, 0.03, 0.1, 0.3, and 1.0 M for each) also was included in the stimulus sequence. Each of these 12 taste stimuli was applied at least once to the anterior tongue using a water-stimulus-water sequence with a minimum 20-s interval between the first water rinse and the stimulus and 10-s between the stimulus and the second water rinse. At least 2 min elapsed between each taste trial. The taste solutions and distilled water were contained in 1-ml syringes and an ~100-μl aliquot of fluid was delivered from them through a polyethylene tube (PE-10) positioned over the anterior tongue with a guide tube. The steel guide tube was connected to a TTL generator, thus marking the moment that the fluid contacted the tongue. Between trials, the tongue was rinsed with 3–4 ml of distilled water.

Data Analysis

Description of neuronal response. As previously mentioned, the experimenters were blinded to the sodium state of the animals during data collection. After completion of that phase, the code was broken and the neuronal responses were grouped depending on the animal’s sodium balance, either replete or depleted. Within those two categories, the cells were further characterized by their best stimulus, i.e., which one of the four standard stimuli (at the standard concentrations) produced the greatest response. These categories are NaCl best (N-best), sucrose best (S-best), citric acid best (C-best), and QHCl best (Q-best).

On the basis of the neuronal activity in 5-s samples, the taste responses were expressed as spikes per second and reported as means ± SE. 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. Both a raw and a corrected response measure were employed. For water, the corrected response equaled the mean spontaneous rate subtracted from the raw water response; for taste, it was the raw taste response minus the mean of raw 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. When a stimulus was repeated for a particular neuron, the response measure was the average of those responses.

Statistics. Repeated-measures ANOVAs, the unequal N variant of Tukey’s honestly significantly different post hoc test, and t-tests were used to analyze the data: sodium intake, urine volume, Na+ concentration in the urine, and mean firing rates of neurons between Na-replete and -deplete groups and among taste stimuli (Statistica, StatSoft). The Pearson product-moment correlation coefficients for all possible pairs of responses were used to conduct cluster analyses (average linkage between groups) and multidimensional scaling (Euclidean distance model) using SPSS (SPSS). Using the excitatory component of the corrected response data, the breadth-of-responsive-ness metric was calculated from the formula of Smith and Travers (28). Comparisons of the number of neurons in each category were made using the \(\chi^2\) test.
Histology. It was not possible to confirm every recording site during the recording sessions (8 wk). The overall area of recording was confirmed after the test sessions were completed. 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 an overdose of Nembutal (100 mg/kg ip) and perfused transcardially with physiological saline followed by 10% formalin. The brain was removed, frozen, and cut coronally in 50-μm sections. Sections through the medulla were mounted on slides and alternate series stained with the cresyl leucht violet and Weil procedures. On the basis of these marking lesions, the recordings were made from neurons in the anterior NST, the area known to receive primary afferent gustatory projections from the anterior tongue (9).

RESULTS

Na Appetite

Once a week for 8 wk each animal received either Furo 2 mg or saline; a total of four injections of each. The Na-appetite test started the morning after the recording day, i.e., 48 h after the Furo or saline injection. Intake of water and 3% NaCl was measured five times from 15 min to 24 h during this test. An injection of Furo (2 mg) induced more intake of NaCl than did injections of saline (Na-replete).

Time (h)

3% NaCl intake (ml)

Fig. 1. Effect of 4 repeated saline (open symbols) and 2 mg furosemide (Furo; filled symbols) treatments on 3% NaCl intake (mean ± SE) during the 24-h sodium-appetite test. Injections of Furo 2 mg (sodium deplete) elicited greater intake of 3% NaCl than did injections of saline (sodium replete).

These differences were maintained across all four pairs of injections. Compared with the first two Furo tests, there was a tendency for the NaCl intake to increase during the third and fourth trials, but the difference was not significant (F3,18 = 0.70, P = 0.56).

Before the experimental session began, a baseline of 3% NaCl intake was determined by averaging consumption over 9 days for each rat. The mean baseline of the six rats was 1.22 ± 0.20 ml. This baseline was compared with the need-free NaCl intake on the days after the Na-appetite test after both saline (1.23 ± 0.23 ml) and Furo injections (0.95 ± 0.16 ml; Fig. 2A). These three measurements did not differ between themselves (F2,29 = 0.39, P = 0.68), across animals (F5,29 = 0.68, P = 0.70).

Fig. 2. Effects of saline (open bars) or 2 mg Furo (filled bars) injections on (A) posttest, need-free NaCl intake (interet test control days). B: urine volume 3 h after treatment with saline or Furo 2 mg. C: urinary sodium content 3 h after injections of saline or Furo 2 mg. D: sodium balance at 24 h postinjection was negative for both Furo- and saline-treated rats. Sodium deficit of Furo-injected rats was significantly greater than when they were treated with saline.
Table 1. Classification of taste neurons as a function of sodium state and best stimulus

<table>
<thead>
<tr>
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<th>Na Replete (n = 27)</th>
<th>Na Deplete (n = 22)</th>
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<tr>
<td></td>
<td>Best stimulus</td>
<td>Second best</td>
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<tr>
<td>N best (18)</td>
<td>Nb (9)</td>
<td>N best (13)</td>
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<td></td>
<td>Nh &gt; S (3)</td>
<td>Nh &gt; S (2)</td>
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<td></td>
<td>Nb &gt; C (5)</td>
<td>Nb &gt; C (5)</td>
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<td>Nb &gt; Q (1)</td>
<td>Nb &gt; Q (3)</td>
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<td>S best (3)</td>
<td>Sb &gt; N (3)</td>
<td>S best (4)</td>
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<td>Sb &gt; N (3)</td>
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<td>Ch &gt; N (4)</td>
<td>Ch &gt; N (4)</td>
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<td>Q best (1)</td>
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Values in parentheses indicate no. of neurons in each category. N, S, C, and Q, significant responses to the standard concentration of NaCl, sucrose, citric acid, and QHCl, respectively; s, specific, b, best; >, second best.

0.64, or in their interaction ($F_{10.29} = 0.76, P = 0.67$). A separate analysis of need-free intake across trials also revealed no difference between means ($F_{1,33} = 0.51, P = 0.49$).

**Diuretic and Natriuretic Effects**

The diuretic and natriuretic effects of the Furo and saline injections were examined at 3 and 24 h on the prerecording day (Fig. 2, B and C). Compared with saline injections, Furo injections induced greater diuresis at both 3 and 24 h ($F_{1,44} = 20.97, P < 0.001$; Fig. 2B, 24 h data not shown). Urinary sodium content also was greater after Furo than saline ($F_{1,44} = 5.75, P < 0.01$; Fig. 2C). At 24 h postinjection, the body sodium balance was negative for both the saline and Furo rats, but significantly greater in the latter, deplete group ($F_{1,44} = 17.76, P < 0.001$; Fig. 2D). When the rats received a saline injection, 0.05% NaCl was added to the sodium-free diet overnight, but this apparently was insufficient to balance the sodium loss. This failure to compensate did not result from differential food intake overnight. The group given Furo and sodium-deficient diet ate 30.50 ± 0.25 g; those injected with saline and given the same diet with 0.05% NaCl added consumed 31.71 ± 0.75 g in the same period ($F_{1,9} = 0.066, P > 0.8$).

**Classification of Gustatory NST Neurons**

A total of 49 taste neurons was isolated and tested with taste stimulation—27 from animals after saline injections (Na-replete group) and 22 from rats after Furo (Na-deplete group). The majority of taste cells were identified as N-best; 18 cells in Na replete and 13 in Na deplete [$\chi^2 = 40.39$, degrees of freedom (df) = 3, $P < 0.001$]. Only one Q-best cell was recorded in each condition. The number of cells in the best-stimulus categories in the Na-deplete group did not differ from those in Na-replete group ($\chi^2 = 1.22$, df = 3, $P = 0.75$).

The corrected response profiles for each neuron in the Na-replete and -deplete groups are shown in Fig. 3. The cells are arranged in descending order within each best-stimulus category beginning with NaCl, sucrose, citric acid, and QHCl-best cells. The profile for each neuron can be read from top to bottom with the corrected water response and the spontaneous activity in the bottom two panels, respectively. The spontaneous activity was similar for cells in the Na-replete and -deplete conditions ($6.08 ± 1.35$ spikes/s; mean ± SE) and the Na-deplete conditions ($6.17 ± 1.54$; $t = 0.05$, df = 47, $P = 0.96$). The corrected water response also did not differ across the two groups ($t = 1.22$, df = 3, $P = 0.22$).
Responses to Four Standard Taste Stimuli

Although the standard stimuli produced responses that differed from one another \( (F_{3,141} = 25.25, P < 0.001) \), there was no average difference in those responses between the Na-replete and -deplete groups \( (F_{1,47} = 2.50, P = 0.12) \) and no significant interaction between Na state and taste \( (F_{3,141} = 0.39, P = 0.76; \) Fig. 4, first column). If the comparison was limited to N-best neurons, by far the largest category, then overall the responses in the Na-deplete group were significantly greater than those of the replete sample \( (F_{1,29} = 4.76, P < 0.05) \), but the interaction between sodium status and taste stimuli was not \( (F_{3,87} = 1.12, P = 0.35; \) Fig. 4, 2nd column). None of the planned post hoc comparisons between stimuli across state were significant. Sodium balance had no effect in either the S-best or the C-best cells \( (F_{1,5} = 0.86, P = 0.40 \) for S-best, \( F_{1,7} = 0.40, P = 0.55 \) for C-best).

Concentration Response Functions for NaCl and Sucrose

Taste neurons in the Na-deplete set responded more vigorously to all five concentrations of NaCl than did the replete cells, but overall the group differences were not significant \( (F_{1,47} = 1.42, P = 0.24) \). Nevertheless, taste responses to NaCl did increase across concentrations \( (F_{4,188} = 52.18, P < 0.001) \) and the interaction between sodium state and concentration was significant \( (F_{4,188} = 2.92, P < 0.05) \). Post hoc tests revealed that the neural responses to the two higher concentrations of NaCl, 0.3 and 1.0 M, were greater in the Na-deplete than in the Na-replete cell group \( (P < 0.05; \) Fig. 5). A similar effect was found in the comparison just of the S-best cells. In this case, the interaction between sodium status and concentration only approached significance \( (F_{4,116} = 2.22, P = 0.07) \), but the responses to 0.3 and 1.0 M NaCl of Na-deplete group were significantly greater \( (P < 0.05) \). Although the responses to the NaCl concentration series for the S-best and C-best neurons also were greater in the sodium-deplete groups, neither comparison approached significance \( (S\text{-best: } F_{1,5} = 0.85, P = 0.40; \) C-best: \( F_{1,7} = 1.19, P = 0.31) \).

The sucrose concentration series also produced increasing responses in the total sample and in the S-best cells \( (F_{4,188} = 25.17, P < 0.001 \) and \( F_{4,20} = 10, P < 0.001, \) respectively). In neither case, however, did the difference between the Na-deplete and -replete groups approach significance \( (F_{1,47} = 1.56, P = 0.22 \) and \( F_{1,5} = 0.75, P = 0.43, \) respectively).

Breadth of Tuning

The breadth of responsiveness (or entropy) was obtained for each neuron using the excitatory components of responses to four standard taste stimuli. The means for each best-stimulus category and treatment are listed in Table 2. Although the entropy was higher in the Na-deplete group, the differences were not significant \( (F_{1,41} = 2.68, P = 0.11) \). Nevertheless, in the Na-replete group, nine of 18 N-best cells (50%) responded only to NaCl, whereas only three of 13 cells were that specific in the Na-deplete group.

Cluster Analysis and Multidimensional Scaling

The Pearson’s product-moment correlation coefficients (Pearson \( r \)) for all possible pairs of response profiles to the standard stimuli were calculated for neurons in both the Na-replete and -deplete groups. Calculated separately, these matrices were used to conduct cluster analyses; calculated together, they were used for multidimensional scaling. The cluster analyses (not shown) revealed a switch in the most different group of neurons as a function of body sodium state. Except for the lone Q-best cell, in the replete state, the most isolated group consisted of eight neurons that responded best or second-best to citric acid. Their overall response correlation with the other neurons in the sample (save the Q-best cell) was +0.26. In the deplete sample, the most different set was four S-best cells; their average response correlation was −0.23.

The multidimensional scaling used the Pearson \( r \) from all possible pairs of response profiles regardless of the rat’s Na state \( (49 \times 49) \). We calculated through four dimensions, but the first two account for 96% of the variance, so those relationships are depicted in Fig. 6. Placement within the space is primarily a function of a cell’s best stimulus, not of the rat’s sodium need. This implies that both samples were drawn from the same population of NST taste neurons.

DISCUSSION

The present experiment replicated a previous one \( (32) \). The major difference was the dose of diuretic used to induce a sodium appetite, 10 mg of Furo then vs. 2 mg now. The high
dose of Furo (10 mg) enhanced responses of NST gustatory neurons to sapid NaCl, whereas other salt appetite regimens had a more general inhibitory effect on taste responses, including a reduction in the activity elicited by NaCl (11, 12, 15, 17, 31). These regimens included dietary sodium deprivation, adrenalectomy, and intracerebroventricular renin, but in behavioral studies, the use of Furo is far more common (2, 8, 19, 23–26). Another recognized procedure, the subcutaneous injection of colloid (29), has not been employed in electrophysiological studies.

As with the previous 10-mg dose study, in rats treated with 2.0 mg of Furo, NST taste neurons responded more to sapid NaCl than did cells from the same animals after they had been treated with the vehicle. This finding refutes our hypothesis that the increased responsiveness of NST neurons observed after the higher Furo dose reflected some aversive consequence of such an injection. Although both doses can produce equivalent intake of 3% NaCl, 10 mg of Furo supports robust taste aversion learning, but 2 mg does not (13, 14). Thus we are left with the original conundrum, why does a diuretic-induced sodium appetite increase gustatory neural responsiveness to NaCl when other treatments, which produce the same behavioral effect, reduce the magnitude of neural responses to sapid stimuli? This question is embedded in the larger issue of how this motivational state, salt appetite, alters the sensory neural activity produced by its consummatory stimulus.

| Table 2. Breadth of responsiveness (entropy) for each best stimulus category of taste neurons |
|-----------------|-----------------|
|                | Na Replete      | Na Deplete      |
| N best         | 0.65±0.25       | 0.78±0.14       |
| S best         | 0.69±0.09       | 0.79±0.12       |
| C best         | 0.82±0.07       | 0.89±0.05       |
| Q best         | 0.58            | 0.83            |
| Total          | 0.68±0.22       | 0.80±0.12       |

Values are means ± SE.
Pharmacology

One possible reason for the different effects of Furo compared with other methods of raising a sodium appetite is pharmacological. In this scenario, the increased responsiveness of NST gustatory neurons occurred because of Furo effects that are unrelated to body sodium loss. Given the present data this possibility cannot be refuted entirely, but several factors militate against it. The half-life of furosemide is ~1.5 h (10) and, because the animals received their injections 24 h before the recording sessions, the direct effect of circulating Furo is unlikely to be an issue. Truly long-term or permanent effects of Furo are also ruled out because, as in the prior experiment (31), these animals were used as their own controls, i.e., they received Furo 1 wk, saline the next.

Furosemide: 2 vs. 10 mg

Electrophysiology. Although treatment with 2 or 10 mg of Furo both enhanced taste responses, some differences exist between the two data sets. The higher dose of Furo increased taste responses to the standard stimuli significantly overall; with the lower dose, a significant difference was observed only in the N-best category. In parallel, post hoc tests revealed that, in the 10-mg dose study, the significant increase could be ascribed to the NaCl response, both for the entire sample and for the N-best cells. In the 2-mg dose tests, however, post hoc comparisons between neural responses to the same stimuli across diuretic and control treatments failed to reveal any significant difference. Thus, although the trends were similar across the two doses, using the standard stimuli, 2 mg Furo influenced only the N-best cells and those only nonspecifically.

Whereas only one concentration of citric acid and QHCl was tested, the influence of 2.0 mg of Furo was examined with five concentrations of NaCl and sucrose. The responses to NaCl solutions increased with increasing concentration in both the Na-replete and -deplete rats but, in cells recorded after Furo, they were consistently larger. Again, parallel results were found in Tamura and Norgren (32), with some differences in detail. In the prior study, the high dose of Furo raised the responses to each of the five NaCl concentrations an equivalent amount. Thus the overall difference between repelte and deplete states was significant, but the interaction between Na balance and NaCl concentration was not, i.e., the two response functions were parallel. In contrast, in the current, low-dose data, the overall group differences were not significant but the interaction across NaCl concentration was. This interaction was evident in the increasing difference between the Furo and the control responses as a function of stimulus concentration (±13.2% at 0.01 M NaCl to 39.5% at 1.0 M). Among these differences, the changes in responses to hypertonic solutions (0.3 and 1.0 M) were significant. Similar results were found among N-best neurons; responses to 0.3 and 1.0 M NaCl were significantly greater after the injection of 2 mg Furo. In comparison, 10-mg Furo injections produced significant increases in the responses only to the lower three concentrations of NaCl, 0.01, 0.03, and 0.1 M, in the N-best category.

As is common in rats when stimuli are restricted to the anterior tongue, the responses to sucrose were meager at best. They did increase with concentration, but without any differences between the Furo and control groups. In the 10-mg Furo study, there was a tendency for the S-best cells in replete rats to respond more to both sucrose and NaCl, but statistically there was no difference between groups.

Finally, another obvious, if more prosaic, reason for the somewhat more robust effect with 10 mg of Furo was the sample size. Eighty-eight single neurons were tested by Tamura and Norgren (32) and 49 in the current low-dose replication.

Behavior. As with the electrophysiological results, the behavior of the rats given 2 mg of Furo differed somewhat from those given 10 mg. These behavioral differences did not contribute directly to the electrophysiological variability in the two experiments, because they occurred between recording sessions, but they may reflect central processes that did. After vehicle injections, the 3% NaCl consumption was slightly higher during the current low-dose experiment (4.0–5.5 ml in 24 h) than in the original 10-mg dose experiment (3.0–5.0 ml). Because the NaCl intake after Furo differed in the opposite direction, the relative difference in sodium consumption between the two doses was increased compared with their absolute values. Another difference appeared in the need-free intake of 3% NaCl. Between the low-dose trials, need-free intake dropped to between 0.8 and 1.1 ml/day. Similar intake occurred after the first two trials in the high-dose experiment, but after trials 3 and 4, when the majority of the neural response differences appeared, need-free intake averaged 3.0 ml/day.

The smaller injection did induce an Na appetite; rats drank significantly more 3% NaCl solution than when they received saline injections. Nevertheless, the amount of NaCl ingested after 2 mg Furo was smaller than that after 10 mg in the Tamura and Norgren study (32). Depending on the trial, in the present study, the Na-deplete rats drank 7.0–10.0 ml of 3% NaCl in 24 h. During the first three trials, the rats in the 10-mg dose experiment ingested 12.0–14.0 ml of the same stimulus and, on the fourth trial, almost twice that much. In a parametric study (13), a 2.0-mg dose was tested three times. With the first set of rats, the 2.0-mg dose produced an intake of 3% NaCl equivalent to the 10-mg dose and in the same range as in the Tamura and Norgren study (32). In the second experiment of this series, 2.0 mg was given to two separate squads of rats, one in a single dose, the other divided. In this case, intake of 3% NaCl ranged between 8 and 12 ml in 24 h, i.e., it overlapped both the low- and the high-dose electrophysiological experiments (32). On the basis of these observations, the post-Furo NaCl intake in the current, electrophysiological study was within the normal range, but smaller than in the similar 10-mg experiment. This behavioral difference might reflect the same underlying state that produced similar, but smaller, NST gustatory response changes in the current low-dose experiment compared with the previous high-dose one.

Furo vs. Other Regimens

Although the details differed, the overall effect of a low or a high dose of Furo was similar—NST gustatory responses to NaCl increased. In similar experiments that used other regimens for inducing an appetite, gustatory neurons at three different levels of the taste system decreased the magnitude of response to the same sapid stimulus. After 10 days on an Na-free diet or after adrenalectomy, gustatory responses in the
chorda tympani (CT) nerve to sapid NaCl were reduced (5, 6, 12), but without a change in threshold (6, 16, 20).

In the NST, a reduction in response to NaCl was the common result regardless of whether the appetite was induced by Na-deficient diet or intracerebroventricular injections of renin. After sodium-deficient diet, a reduction in response to sapid stimuli was observed in the NST of both anesthetized (11) and awake, behaving rats (17). In both experiments, the reduced responsiveness was more general across stimuli than in the Furo studies, but still greatest for NaCl in N-best neurons. The major difference in the results was in the responses of S-best neurons to NaCl (see Ref. 17 for a full discussion). One other study reported that the responses of NST neurons from rats fed a sodium-deficient diet did not change (33). In this latter investigation, a possible key difference was the use of a sodium-deficient (0.03% NaCl) rather than a sodium-free diet (see Ref. 32).

An intracerebroventricular injection of renin can be used to induce an Na-appetite rapidly and without a negative sodium balance (1, 21). With this paradigm, the responses of NST gustatory neurons to sapid NaCl were reduced, but only at hypertonic concentrations (15, 31). A similar reduction in hypertonic NaCl responses (0.3 and 0.5 M) was observed in pontine parabrachial gustatory responses after injections of 14 mg Furo (7 mg × 2; Ref. 27).

Thus treatment with Furo increases the responses of NST taste cells to sapid sodium, especially in those neurons that respond best to NaCl in the first place. Other treatments, which can produce a behaviorally equivalent Na appetite, have the opposite effect; they reduce the response of NST taste cells to sapid NaCl. Although the data are limited, it appears that even Furo treatment may reduce responses to NaCl at other levels of the gustatory system, namely on the periphery and in the second central relay, the pontine parabrachial nuclei.

How to explain these neurophysiological differences in the face of essentially identical behavior? In a prior publication, we came up with three possible mechanisms: the number of Na-appetite challenges, their time course, and their hormonal consequences (32). Hormonal differences do result depending on the treatment, but they did not track the neural response differences in any obvious fashion. In addition, intracerebroventricular renin induces an Na appetite in a matter of minutes (15). Although this rapid effect probably results from a massive intracerebral release of angiotensin II (1), it is unlikely that peripheral hormone status changes that rapidly and certainly precludes any genomic effects.

The other two possible mechanisms, the number of challenges and their time course, were discounted largely on circumstantial evidence. Subsequent data from two renin studies and the current experiment provide additional evidence relevant to these two mechanisms. Briefly, dietary deprivation of NaCl takes 7–10 days to raise an appetite, whereas Furo treatment has the same effect overnight. The substantial difference in induction times might underlay the opposing effects on coding. This logic is undermined by intracerebroventricular renin, however, that induces an appetite much more rapidly than Furo but produces neural changes resembling those after dietary deprivation (15, 31). In addition, unlike both diet and Furo, intracerebroventricular renin produces both its behavioral and neural effects without systemic sodium loss.

Dietary induction was tested only once, although in the chronic recording experiment the deprivation was maintained for up to 50 days (11, 17). In the NST Furo studies, four separate trials were used and, in each case, the rats were permitted to replenish their systemic sodium losses. In the high-dose study, the effects on neural responses did not become significant until the third and fourth trials (32). This allowed the speculation that the differential effects depended on multiple trials, systemic sodium replenishment, or both. With the use of the same paradigm, however, the present low-dose study found no effect of trial order on the neural response changes. This eliminates one version of this explanation, i.e., the effects of Furo do not begin until after multiple trials have occurred, but leaves a second in play, i.e., the effects accumulate during multiple trials with sodium replacement.

This explanation is bolstered by the most contrary observation to arise from these experiments. In the CT (2) and the parabrachial nuclei (PBN; 27), high-dose Furo reduced neural responses to sapid sodium but in the NST, which is synaptically between CT and PBN; the same treatment increased them (32 and the current data). Aside from the locus of recording, the major difference is that the CT and PBN experiments used only a single depletion per rat but the two NST studies used multiple treatments. It should be noted that in one of the intracerebroventricular renin experiments data were collected during two treatments in each rat and that the net result was a reduction in NST taste responses to sapid NaCl (31).

**Perspectives**

In the end, the influence of multiple trials with NaCl replacement cannot be resolved with the available data. This would require recording in the NST after multiple dietary depletions with replacement or a single depletion study with Furo or probably both. These further data might settle this essentially methodological conundrum, but they probably would not settle what the coding changes signify for the behavioral change that is the hallmark of this motivational state: the switch from active avoidance of strong salt to its avid ingestion.

Both changes in response have been interpreted behaviorally. A decreased magnitude of response, particularly at hypertonic stimulus concentrations, might mean reduced aversion (7). The behavioral change, however, is not reduced aversion, but a switch from avoidance to active seeking and ingestion. Conversely, an increased magnitude of response, particularly relative to other stimuli, might mean that environmental sodium would be easier to detect and thus more likely to garner attention (32). Whereas selective attention is characteristic of motivation, it does not by itself dictate a change, particularly a reversal, in the reward value of a stimulus.

Neurons in the first two levels of the gustatory system, the chorda tympani nerve and the nucleus of the solitary tract, change how they code sapid sodium after procedures that normally induce an Na appetite. The changes are not always consistent and, in any event, seem unlikely to account for the dramatic behavioral shifts in response to sapid sodium. Anatomical and lesion-behavioral evidence implicates the next synaptic level, pontine parabrachial nuclei, in this hedonic shift (18). It will require neurophysiological data, however, to determine if these third-order neurons encode this change them-
selves or merely pass on the gustatory sensory activity for interpretation by the limbic forebrain.

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GRANTS

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