Intracranial renin alters gustatory neural responses in the nucleus of the solitary tract of rats

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DEPLETING BODY SODIUM STORES triggers an innate appetite for sodium (12), during which high, normally rejected concentrations of sodium salts are avidly ingested. In fact, the specific hunger for salt represents an important instance in which only a gustatory stimulus can guide behavior that is appropriate for restoring the metabolic imbalance (25–27, 32).

The effects of sodium depletion on the responses of gustatory neurons vary with the circumstances and thus are difficult to interpret. Pfaffmann and Bare (34) and Nachman and Pfaffmann (28) found no differences in the responses of the chorda tympani (CT) nerve to NaCl stimulation of the tongue between experimental (adrenalectomized or dietary sodium deprived) and control rats. Contreras (8) and Contreras and Frank (9) observed that dietary sodium deprivation led to a decrease in whole nerve and single-unit CT responsiveness to suprathreshold NaCl solutions. Contreras and coworkers (11, 20) also found that bilateral removal of rat adrenals led to a significant decrease in taste responsiveness for suprathreshold concentrations of NaCl and LiCl. Bernstein and Taylor (4) found that acute sodium depletion by combining sodium-deficient diet with the natriuretic drug furosemide led to a significant decrease in whole nerve CT responsiveness to sapid NaCl. Even though the sodium appetite induced was comparable between the chronic (sodium-deficient diet alone) and acute (sodium-deficient diet with furosemide) conditions (10, 39, 51), the electrophysiological effects with furosemide were more modest and limited compared with the data of Contreras and coworkers (8, 9, 11, 20).

Jacobs et al. (19) found that dietary sodium deprivation led to a decrease in single-unit responses of the nucleus of the solitary tract (NST) to NaCl in anesthetized rats. The decreased responsiveness to NaCl was attributed mainly to salt-profile neurons. More recently, McCaughey and Scott (23) produced similar, if more limited, effects using intracerebroventricular infusions of renin. Conversely, responsiveness of sugar-profile neurons to NaCl was increased. Subsequently, Nakamura and Norgren (30) produced similar results for NaCl-best neurons while recording single-unit activity in the NST of awake, behaving rats during dietary sodium deprivation but failed to observe any changed responses in sucrose-best cells.

Vogt and Hill (48) reported that, when deprived of dietary NaCl as adults, NST neurons responded as in nondeprived rats. When deprived of NaCl early in development, however, they recorded highly attenuated average response frequencies to sodium salts in the adults that were on a normal (high) sodium diet (48). Furthermore, we (46) found that acute sodium depletion produced with furosemide and overnight sodium deprivation actually led to a significant increase in single-unit responses of the NST to NaCl in lightly anesthetized rats. This increased responsiveness to

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NaCl came primarily from the activity of NaCl-best neurons.

A substantial body of evidence indicates that renin, angiotensin, and aldosterone, which act on the kidney to promote sodium conservation, also act on the brain to produce salt appetite (2, 3, 5–7, 13–15, 24, 35, 37, 40, 50). Anatomic studies revealed the existence of a renin-angiotensin system in the brain (1, 10, 21, 22). Therefore, we tested the effects of directly activating this hormonal cascade by infusing intracerebroventricular renin on gustatory neural responses in the NST. A preliminary report has been presented (47).

METHODS

Animals

Ten male Sprague-Dawley rats (Charles River, 200–250 g at the start of the study) were housed individually in standard metabolism cages (Acme Metal Products, model H1276) in a room with a 12:12-h light-dark cycle and constant temperature (22 ± 1°C). Unless otherwise specified, they had access ad libitum to distilled water, 3% NaCl solution, and standard rat pellets (Rodent Laboratory Chow no. 5001, Purina Mills). Solutions were presented on the cage front in plastic cylinders, graduated to 1 ml, and the hole was covered with sterile bone wax and a plastic plate that was firmly fixed to the skull with the larger cap using dental acrylic.

Localization

After a recovery period of at least 2 wk, the right NST in each rat was located electrophysiologically. Rats were re-anesthetized (Nembutal, 50 mg/kg ip) and mounted in the stereotoxic device. A hole was drilled through the cranioplastic cap and the underlining interparietal bone over the intended recording site. Typically, taste responses were located 11.2–12.0 mm posterior to β, 1.6–2.0 mm lateral to the midline, and 8.2–8.8 mm below the skull surface at β. Several penetrations were made with a recording electrode to locate the taste area for the subsequent single-unit recording sessions. Once recording was finished, the exposed brain was covered with hydrocortisone ointment (Neo-Cortef, Upjohn), and the hole was covered with sterile bone wax and a plastic plate that was firmly fixed to cranioplastic cap by a screw.

Elicitation of Sodium Appetite

Sodium appetite was elicited by adapting a method used previously by one of us in a behavioral experiment (35). The day before an intracerebroventricular injection (9:00 AM), the rat was placed in a clean metabolism cage, the standard rat pellets were replaced with powdered diet, and the 3% NaCl bottle was removed. Distilled water was freely available. At 1:00 PM the next day, a 1.0-μl pulse intracerebroventricular injection of renin (fraction 1, 100 ng) or the vehicle, 2% bovine serum albumin (BSA), was administered. To control for the water intake elicited by renin, all rats were allowed to drink water for 1 h before being anesthetized. The water intake was measured, and then the total water load was made up to 15.0 ml by gavage. If the rat drank >15.0 ml, no further fluids were given. Although the amount of voluntary water intake was a good indication of a successful renin injection, we allowed the rat brief access to 3% NaCl (<1 min) to be certain of the presence or absence of a sodium appetite. Once licking of 3% NaCl occurred, the bottle was immediately removed from the cage. During renin injection trials, if the rat did not drink at least 5 ml of water or did not ingest 3% NaCl immediately on presentation, the injection was regarded as ineffective and the recording was aborted. Otherwise, immediately after briefly licking the 3% NaCl, the rat was anesthetized and the recording session began (see Recording).

Subsequently, the rat was returned to its metabolism cage, which contained distilled water and powdered diet. The following morning, a bottle of 3% NaCl was placed on the cage and the rat’s consumption of water and 3% NaCl was recorded at 0.25, 0.5, 1, 3, and 24 h (sodium appetite test). In addition, urine was collected for 24 h before, for 24 h after the intracerebroventricular injection, and again for the 24 h after the intracerebroventricular injection day. The urine volume and the concentration of Na+ and K+ was measured for each collection period. Each rat was given two injections of renin and two of BSA, at weekly intervals and in a counterbalanced order. The individual doing the recording was unaware of the order of the injections.
After all the recording sessions were finished, two additional intracerebroventricular injections (1 renin and 1 BSA) were given to eight rats to determine the magnitude of the sodium appetite 1 h after injection, i.e., during the period when recording normally took place. The procedure was the same as described above except that the rat was never anesthetized and, after the 1-h water-only access, both 3% NaCl and water were available (at 2:00 PM). Consumption of water and 3% NaCl was recorded at 0.25, 0.5, 1, 3, and 24 h. As in the recording sessions, urine was collected for 24 h before each injection and during the test period. Urine samples from both the recording and the behavioral tests were frozen for later analyses of Na⁺ and K⁺ content by flame photometry (Instrumentation Laboratories model 943). The amount of Na⁺ and K⁺ excreted was calculated by multiplying the concentration of each ion in the sample by the volume of urine collected for that time period.

Recording

The rat was lightly anesthetized (Nembutal, 35 mg/kg ip), mounted in the stereotaxic frame using the cranioskeletal cap, and the plastic plate and bone wax were removed from the skull aperture. To maintain a constant level of anesthesia, small amounts of Nembutal (2–3 mg/kg ip) were added approximately every 10 min. Rectal temperature was monitored and maintained between 36.5 and 37.5°C with a heating pad.

Glass-coated tungsten microelectrodes with a ball-shaped tip (2.5–5 Mohm at 1 kHz) were positioned using coordinates derived during the localization procedure. The electrode was advanced into the vicinity of the NST with a micromanipulator (SM-20, Narishige). Extracellular single-unit activity was recognized by consistent waveform and amplitude. Electrical activity was amplified conventionally and stored on magnetic tape for offline analysis. At the same time, unit activity was counted with a window discriminator. Spike frequencies were computed over 60 s using a microcomputer, displayed as peristimulus time histograms, and stored with physiological saline followed by 10% formalin. The brains were removed, blocked, and cut coronally in 50-μm sections on a freezing microtome. Alternate series of sections were stained with cresyl lecht violet and the Weil procedures. The 10th rat died in its cage without perfusion, and thus the histology from this brain was lost.

Stimulation

The taste stimuli consisted of sodium salts, non-sodium salts, sugars, Polycose, acid, and quinine hydrochloride (QHCl) presented at room temperature. The protocol called for testing four standard taste stimuli first (0.1 M NaCl, 0.3 M sucrose, 0.01 M citric acid, and 0.01 M QHCl), then a concentration series of NaCl and sucrose (0.01–1.0 M in 0.5-log steps), and finally the remaining stimuli. Only data from the standard stimuli and the concentration series are summarized here. The fluids (50 μl in 1.0 s by pressure) were delivered in a water-stimulus-rinse configuration from a 1.0-ml syringe through a polyethylene tube (PE-10) positioned over the ipsilateral anterior tongue with a stainless steel guide tube. During a trial, the minimal interval between the first water and the stimulus was 30 s and between the stimulus and the water rinse was 10 s. After each taste stimulation, the tongue was rinsed with 3–4 ml of distilled water at least three times. A minimum of 2 min elapsed between taste stimulations. The moment of stimulus contact with the tongue was marked by a TTL logic sensor (49).

Data Analysis

All analyses were based on neuronal activity in 5.0-s samples expressed as spikes per second. For water and stimulus trials, these samples began at the fluid-onset mark; for spontaneous activity, the samples 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, this was the raw water response minus the mean spontaneous rate; for taste, it was the raw taste response minus the mean raw water response. In both cases, the response was considered significant if it exceeded 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 ANOVA tests were performed on each data set, and statistically significant effects were evaluated further with Fisher’s least-significant different test (the GLM Procedure, SAS Users Group International). Pearson product-moment correlation coefficients for all possible pairs of responses were calculated (the CORR Procedure, SAS Users Group International) and used to conduct cluster analyses (average linkage method; the Cluster Procedure, SAS Users Group International) and multidimensional scaling (metric ratio and Euclidean model; the MDS Procedure, SAS Users Group International).

RESULTS

Fluid Intake

The intracerebroventricular renin elicited robust and reliable water intake in the 1 h after the infusion (16.3 ± 0.15 ml, mean ± SE, n = 20), but a similar injection of BSA did not (0.88 ± 0.33 ml). Nineteen of 20 renin injections elicited water intake that exceeded 10 ml. One renin injection (the 2nd for a rat) elicited only 3.5 ml of water intake.

Sodium appetite was checked just before anesthesia for each rat. In the renin trials that elicited robust water intake, the rats also drank 3% NaCl as soon as it was offered. In the one trial in which renin produced only a modest effect on water intake, the rat failed to ingest 3% NaCl within the test period. In 15 of the 20 BSA injections, the same rats did not ingest 3% NaCl during the test period. After one BSA injection, one rat licked the salt spout a few times with a long latency (~25 s) and then ceased. In four other BSA trials, the rats immediately began licking the 3% NaCl and continued until the bottle was removed. The tendency to ingest NaCl after an intracerebroventricular injection of BSA was related to the rat’s prior history with renin infusions. In the five cases in which BSA was injected...
before any experience with intracerebroventricular renin, none of the rats touched the 3% NaCl bottle during the test period. In 2 of the 10 BSA trials that followed a first renin injection, one rat ingested NaCl immediately and one after a longer latency. After two experiences with renin, in three of the five BSA trials, the rats licked the NaCl spout immediately.

The day after a renin recording session, the rats ingested significant amounts of 3% NaCl compared with intake after the BSA sessions (Fig. 1A) \(F(1,37) = 11.3, P < 0.01\). Although NaCl intake did not differ between the first and second BSA trials, they each did differ from intake after both the first and second renin trials, and the renin trials differed from one another \(F(3,35) = 4.54, P < 0.01\) followed by post hoc least significant difference test (LSD), \(P < 0.05\). Thus intracerebroventricular renin increased 3% NaCl intake, and repeated renin injections enhanced the intake. Water intake also increased the day after renin infusions compared with BSA (Fig. 1B) \(F(1,37) = 6.43, P < 0.02\), but the trial effect was marginal, at best \(F(3,35) = 2.42, P = 0.08\).

There was a modest but significant correlation between water intake during the 1-h test period that immediately followed an intracerebroventricular injection and 3% NaCl intake the next day \(r = 0.55, P < 0.01\). The most striking difference between the rats infused with renin and those that received BSA was in their water intake during the 1-h test. With one exception, the distributions were nonoverlapping; after BSA, water intake was \(<6.0\) ml; after renin, it was \(>10.0\) ml. The day after the infusions, in all of the BSA trials that resulted in \(>5.0\) ml of NaCl intake, the rats had had prior experience with renin.

As described in METHODS, intracerebroventricular BSA and renin injections were given again to eight rats 2 wk after completing the neural recording sessions. Compared with BSA, renin significantly increased intake of water during the 1-h period immediately after the intracerebroventricular injection \((3.06 \pm 0.77\) ml for BSA; \(9.25 \pm 2.15\) ml for renin; Fig. 2B, inset) \(F(1,14) = 7.32, P < 0.05\). Renin also increased the intake of 3% NaCl in the 24-h sodium appetite test that began immediately after the water test \(F(1,78) = 13.8, P < 0.02\), but the trial effect was marginal, at best \(F(3,35) = 2.42, P = 0.08\). The most striking difference between the rats infused with renin and those that received BSA was in their water intake during the 1-h test. With one exception, the distributions were nonoverlapping; after BSA, water intake was \(<6.0\) ml; after renin, it was \(>10.0\) ml. The day after the infusions, in all of the BSA trials that resulted in \(>5.0\) ml of NaCl intake, the rats had had prior experience with renin.

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During this longer, sodium appetite test, however, water intake did not differ as a function of the intracerebroventricular infusion \([F(1,58) = 1.16, P = 0.29; \text{Fig. } 2B]\). The intake pattern was similar to that seen after the second intracerebroventricular injection during the recording trials (see Fig. 1). A strong positive correlation existed between water intake for the 1-h test after renin injections during the recording session and the amount of 3% NaCl ingested during this subsequent, behavioral test (H₂O and 15 min NaCl, \(r = 0.89, P < 0.01\); H₂O and 24-h NaCl intake, \(r = 0.84, P < 0.01\)).

Spontaneous 24-h fluid intake was averaged over 3 days for three different periods during the experiment (Table 1): before any intracerebroventricular injections, 1 wk after the final recording session, and after the final salt appetite tests, i.e., 2 wk after recording. Salt, but not water, intake changed across the three periods \([F(2,75) = 19.8, P < 0.0001]\). Post hoc LSD tests revealed that the daily 3% NaCl intake was smallest in the first period and largest in the third. The five rats that increased NaCl intake after BSA injections also increased daily 3% NaCl intake in period 2.

Urine Output

Urine volume, sodium excretion, and potassium content are summarized in Table 2. In the 24 h before a trial (Table 2), neither urine volume, sodium, nor potassium differed between conditions (Fisher’s LSD, \(P = 0.41\) for volume, \(P = 0.99\) for sodium, and \(P = 0.44\) for potassium). Compared with BSA, on the day of a renin trial, there was a small but significant \((P < 0.05)\) increase in urine output, but no changes in total sodium or potassium excretion (Table 2; \(P < 0.05\) for volume, \(P = 0.73\) for sodium, and \(P = 0.93\) for potassium). This may reflect the fact that intracerebroventricular renin often elicited >15 ml of water intake during the 1 h before anesthetization, but after BSA, the water intake was minimal and was only brought up to 15 ml by gavage after the urine was collected. Furthermore, there was a clear increase in urine volume the day after renin injections compared with the same period after BSA (Table 2; \(P < 0.001\)). The day after a renin trial, potassium efflux did not differ from a similar period after BSA (\(P = 0.92\)), but sodium was greater (\(P < 0.001\)).

Table 1. Effects of experience with intracerebroventricular injections on spontaneous daily intake of water and 3% NaCl

<table>
<thead>
<tr>
<th></th>
<th>Baseline (Before ICV Injections)</th>
<th>After ICV Injections</th>
<th>After Behavioral Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water, ml</strong></td>
<td>47.8 ± 1.69</td>
<td>49.8 ± 1.76</td>
<td>48.2 ± 8.91</td>
</tr>
<tr>
<td><strong>3% NaCl, ml</strong></td>
<td>1.8 ± 0.28</td>
<td>4.88 ± 0.93*</td>
<td>8.91 ± 1.52*</td>
</tr>
</tbody>
</table>

Values are mean ± SE. ICV, intracerebroventricular. *Statistically significant differences in post hoc tests such that the second intake is greater than the first and the third greater than the second.

Table 2. Effects of ICV injections on urine volume and sodium and potassium excretion

<table>
<thead>
<tr>
<th>Collection Period/ICV Injection</th>
<th>Urine Volume, ml</th>
<th>Na⁺, mmol</th>
<th>K⁺, mmol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day before injection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA</td>
<td>27.3 ± 1.02</td>
<td>3.04 ± 0.09</td>
<td>5.77 ± 0.19</td>
</tr>
<tr>
<td>Renin</td>
<td>25.3 ± 1.73</td>
<td>3.03 ± 0.13</td>
<td>5.52 ± 0.14</td>
</tr>
<tr>
<td><strong>Day of injection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA</td>
<td>18.5 ± 1.09*</td>
<td>1.57 ± 0.13</td>
<td>2.60 ± 0.21</td>
</tr>
<tr>
<td>Renin</td>
<td>21.7 ± 1.09*</td>
<td>1.81 ± 0.12</td>
<td>2.57 ± 0.24</td>
</tr>
<tr>
<td><strong>Day after injection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSA</td>
<td>24.9 ± 2.31</td>
<td>5.34 ± 0.80</td>
<td>5.34 ± 0.30</td>
</tr>
<tr>
<td>Renin</td>
<td>34.4 ± 3.95*</td>
<td>8.76 ± 1.25*</td>
<td>5.31 ± 0.32</td>
</tr>
</tbody>
</table>

Day of injection includes the 1-h period after the ICV injection and the period when the rat was in its home cage after the recording session, which typically lasted ~6 h. *Renin values significantly different from BSA.

Neuronal Activity

Based on the behavioral data described above, the rats exhibited a sodium appetite in 19 of 20 renin trials, but they also showed an appetite in several BSA trials. Neuronal activity was recorded during the 19 effective renin trials and 20 BSA trials. Because the apparent sodium appetite in four BSA trials might confound the control condition, the data were analyzed with and without the results from neurons recorded during these sessions. In fact, there were no differences between the two analyses, so only the complete data set is summarized here.

Histology. Fifteen marking lesions were evident in the histology of the four brains subjected to this procedure. All of these were either in or on the dorsal edge of the NST at an anteroposterior level ±0.5 mm from the posterior border of the dorsal cochlear nucleus. This corresponds closely with the terminal zone of CT afferent axons that innervate the taste buds on the anterior tongue, the area stimulated in this study. Of the brains without lesions, three had electrode tracks in the vestibular nuclei overlying the rostral NST and two had no obvious damage in the medulla.

Spontaneous activity and evoked responses to standard taste stimuli. A total of 85 single gustatory neurons was isolated in the rostral NST and tested at least once with the four standard stimuli and water (average = 8.5/rat, range = 4–12/rat). Of these cells, 39 were recorded after BSA infusions and 46 after renin. The cells were classified on the basis of the largest response to the four standard taste stimuli, i.e., NaCl-best, sucrose-best, acid-best, and QHCl-best neurons (Table 3). In Fig. 3, the spontaneous activity and the response profiles of the neurons recorded after BSA (Fig. 3A) and renin (Fig. 3B) infusions are arranged in descending order of response magnitude, beginning with the NaCl-best neurons, followed by sucrose-best cells, then the citric acid-best, and finally the QHCl-best neurons. Based on these best-stimulus categories, the sampling ratios were similar in the two treatment conditions. There were somewhat more sodium-specific...
Table 3. Classification of 85 NST taste neurons

<table>
<thead>
<tr>
<th></th>
<th>BSA Injections (n = 39)</th>
<th>Renin Injections (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Best stimulus</td>
<td>Second-best stimulus</td>
</tr>
<tr>
<td>NaCl = 27(69.2%)</td>
<td>Specific* = 9(23.1%)</td>
<td>Sodium = 3(7.7%)</td>
</tr>
<tr>
<td></td>
<td>Citric acid = 10(25.6%)</td>
<td>QHCl = 5(12.8%)</td>
</tr>
<tr>
<td>Sucrose = 6(15.4%)</td>
<td>Sodium = 6(15.4%)</td>
<td>Sucrose = 5(10.9%)</td>
</tr>
<tr>
<td>Citric acid = 4(10.3%)</td>
<td>Specific* = 1(2.6%)</td>
<td>Citric acid = 5(10.9%)</td>
</tr>
<tr>
<td>QHCl = 2(5.1%)</td>
<td>Sodium = 2(5.1%)</td>
<td>QHCl = 3(6.5%)</td>
</tr>
</tbody>
</table>

Values are nos. of neurons classified on the basis of the most effective of the 4 standard stimuli, 0.1 M NaCl, 0.3 M sucrose, 0.01 M citric acid, and 0.01 M quinine HCl (QHCl). Nos. in parentheses represent percentage of sample of neurons for each infusion condition. NST, nucleus of the solitary tract. *None of the other 3 standard stimuli elicited a significant response, i.e., ± 2.5 SD different from water.

Fig. 3. Spontaneous firing rates and response profiles of nucleus of the solitary tract (NST) taste neurons using 4 standard taste stimuli and water recorded after intracerebroventricular BSA injections (A; n = 39) and intracerebroventricular renin (B; n = 46). Taste neurons were grouped into best-stimulus categories and then arranged within those categories in descending order of response magnitude to the best stimulus. Taste responses were adjusted for average water response; the response to water was adjusted for spontaneous rates. Spontaneous rates are shown at bottom with unit numbers. Filled bars indicate significant responses. Excitatory responses are up; inhibitory responses, down. None of the inhibitory taste responses reached the 2.5-SD criterion for significance.
sapid stimuli as a function of intracerebroventricular treatment.

**Breadth of responsiveness.** This measure of entropy (45) provides a criterion-free assessment of the number of stimuli a category of neurons responds to. The values were calculated for both treatment conditions using the excitatory components of the responses to the four standard stimuli (Table 4). The NaCl-best neurons tested after intracerebroventricular renin were slightly but significantly more broadly tuned than those tested after BSA (P < 0.05). This is consistent with the fact that, based on our criterion, NaCl-best neurons responded significantly to more of the four standard stimuli after intracerebroventricular renin (2.8/4) than after BSA (2.1/4). There were no significant differences between the breadth of tuning indexes for the other best-stimulus categories as a function of intracerebroventricular treatment.

**NaCl and sucrose concentration series.** A number of cells were stimulated with the NaCl (n = 55) and sucrose (n = 52) concentration series; the numbers tested in each best-stimulus category are summarized in Table 5. With NaCl there was a concentration effect \[F(4,212) = 135.6, P < 0.0001\] as well as an interaction between intracerebroventricular infusion and concentration \[F(4,212) = 5.49, P < 0.001\] both for the total

Table 4. Breadth of responsiveness (entropy) for each best-stimulus category of taste neurons

<table>
<thead>
<tr>
<th></th>
<th>BSA Injections</th>
<th>Renin Injections</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl-best</td>
<td>0.52 ± 0.04</td>
<td>0.64 ± 0.03*</td>
</tr>
<tr>
<td>Sucrose-best</td>
<td>0.71 ± 0.06</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>Citric acid-best</td>
<td>0.85 ± 0.04</td>
<td>0.88 ± 0.03</td>
</tr>
<tr>
<td>QHCl-best</td>
<td>0.63 ± 0.11</td>
<td>0.68 ± 0.10</td>
</tr>
<tr>
<td>Total</td>
<td>0.59 ± 0.03</td>
<td>0.68 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Statistically significant difference between the renin and BSA conditions.

Table 5. Numbers of neurons tested with a concentration series of NaCl and sucrose in the 2 experimental conditions

<table>
<thead>
<tr>
<th></th>
<th>NaCl Injections</th>
<th>Sucrose Injections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA Renin</td>
<td>BSA Renin</td>
</tr>
<tr>
<td>NaCl-best</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Sucrose-best</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Citric acid-best</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>QHCl-best</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>26</td>
</tr>
</tbody>
</table>

between the breadth of tuning indexes for the other best-stimulus categories as a function of intracerebroventricular treatment.

Fig. 4. Comparison of averaged spontaneous firing rates and response profiles to the 4 standard taste stimuli and water recorded after intracerebroventricular BSA and intracerebroventricular renin injections by best-stimulus category. Total, Nb, Sb, Cb, and Qb correspond to total, NaCl-best, sucrose-best, citric acid-best and QHCl-best neurons.

Fig. 5. Comparison of the average response profiles of all (A) and NaCl-best neurons (B) to the NaCl concentration series during the BSA and the renin conditions. A concentration series for sucrose produced no differences between conditions and thus is not shown. *Significant post hoc comparisons.

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sample (Fig. 5A) and for the NaCl-best cells (Fig. 5B). Post hoc tests revealed that, for the total sample, the cells tested after intracerebroventricular renin responded less than those after BSA when stimulated with 0.3 and 1.0 M NaCl ($P < 0.05$), but their responsiveness was equivalent at the lower concentrations. For the NaCl-best sample, only the highest concentration produced a significant difference between the two intracerebroventricular infusion conditions ($P < 0.05$). When the data were compared across trials, repeated renin injections did not significantly change the reduction in responsiveness to NaCl at higher concentrations ($P = 0.34$).

When the entire sample or just the NaCl-best neurons were tested with sucrose, concentration effects were evident [entire sample, $F(4,192) = 19.9, P < 0.0001$; NaCl-best, $F(4,120) = 9.51, P < 0.0001$], but the type of intracerebroventricular infusion did not matter, i.e., neither the main effect of condition [entire sample, $F(1,48) = 0.42, P = 0.52$; NaCl-best, $F(1,30) = 0.82, P = 0.37$] nor the interaction term was significant [entire sample, $F(1,48) = 0.42, P = 0.52$; NaCl-best, $F(1,30) = 0.82, P = 0.37$]. Similarly, when the sucrose-best cells were tested with either NaCl or sucrose, there was a concentration effect, but no effect of infusion and no interaction [NaCl: concentration, $F(4,20) = 33.3, P < 0.0001$; intracerebroventricular, $F(1,5) = 0.085, P = 0.78$; interaction, $F(4,20) = 1.33, P = 0.29$; sucrose: concentration, $F(4,20) = 25.8, P < 0.0001$; intracerebroventricular, $F(1,5) = 0.048, P = 0.84$; interaction, $F(4,20) = 0.038, P = 0.997$].

Hierarchical cluster analysis and multidimensional scaling. The relations apparent from the best-stimulus categories can be checked using less arbitrary analyses based on correlation coefficients. All possible Pearson product-moment correlation coefficients were calculated from the response profiles produced by the four standard stimuli from the 39 cells in BSA condition (741 pairs, $39 \times 38/2$) and the 46 neurons in the renin condition (1,035 pairs, $46 \times 45/2$). Using these matrices, hierarchical cluster analyses for neurons were generated for each intracerebroventricular infusion condition using the average linkage method. The dendrograms resulting from these two analyses were quite similar and, therefore, are not shown. In both cases, the first two clusters segregated neurons into the smaller sucrose-best population and all the other cells at about zero correlation ($-0.03$ for the BSA sample and $-0.01$ for renin). In the larger cluster, the next three groupings separated the cells into a large, purely NaCl-best cohort and two others that contained the citric acid-best and QHCl-best neurons, each with an admixture of a few NaCl-best units.

The same sets of correlation coefficients also permitted multidimensional scaling (MDS). As with the cluster analyses, the resulting two-dimensional solutions for the BSA and the renin samples had quite similar patterns. Based on the corrected fit correlations from the MDS solutions, for both groups, the first two dimensions accounted for $96\%$ of the variance. In either case, adding a third or fourth dimension covered less than $1\%$ more of the variance. Both the hierarchical cluster and the MDS analyses serve to confirm the raw data displayed in Fig. 3. The neurons appear to have been drawn from the same population of cells, and the independent variable did not noticeably alter their overall response profiles.

**DISCUSSION**

**Behavioral Changes**

In virtually every intracerebroventricular renin trial (19/20), water intake increased significantly during the 1-h interval between the infusion and anesthesia. In the same trials, the rats ingested 3% NaCl when it was presented just before anesthetization. This implied that a sodium appetite had been raised. The following morning, these rats also increased intake of both water and 3% NaCl. This next-day NaCl intake by itself is not an unequivocal index that a sodium appetite was active shortly after the infusions, because intracerebroventricular renin has delayed consequences, such as increased blood pressure and diuresis, that also can influence fluid intake. During the 24 h after intracerebroventricular infusions of renin, urine volume and sodium content rose in parallel (17.3 and 15.3%, respectively) compared with the BSA controls, but only the change in urine volume was significant (Table 2). The effects of intracerebroventricular renin observed after the recording trials and those conducted several weeks later in the same rats were consistent with one another and with observations in previous studies (2, 3, 5, 35). Taken together, we assumed that a robust sodium appetite was in place during the recording sessions in which intracerebroventricular renin had been infused.

**Electrophysiological Changes**

In the present study the average response magnitude of NST neurons to the standard taste stimuli did not differ between the renin and BSA conditions. At hypertonic concentrations of NaCl (0.3 and 1.0 M), however, the responses after renin infusions were significantly lower than those after BSA. A similar effect occurred in the NaCl-best subset of cells but reached significance only at 1.0 M. In the other best-stimulus categories, the two intracerebroventricular infusion conditions did not significantly influence the responses to any concentration of NaCl, but individually these samples were small. The average responses to the sucrose concentration series did not differ as a function of the intracerebroventricular infusion.

Intake of 3% NaCl was significantly enhanced after the second renin injection compared with the first. This effect mirrors serial sodium appetites raised with other methods, such as diet or furosemide (30, 35, 38, 41). Despite this increased intake, the changes in neural responsiveness to sapid sodium were similar after the first and second renin injections both for the full sample and the NaCl-best cells. This stands in contrast to the effects of a
sodium appetite raised with furosemide in which NST NaCl-best taste cells became more responsive to sapid sodium in the later trials (3 and 4) compared with the earlier ones (1 and 2; Ref. 46). Although the current experiment and the prior furosemide one involved nearly identical preparations, the protocols differed on several dimensions. After intracerebroventricular renin, neurons were tested within 1–4 h; with furosemide, the recording session began 24 h later. Intracerebroventricular renin induces a need-free sodium appetite, while furosemide acts by inducing a body sodium deficit. The current experiment used four iterations, two BSA and two renin; the furosemide study used eight, four diuretic and four controls. These differences make it difficult to rule out procedure as an important variable in accounting for the differing results in the two experiments.

The results also differed because, in the present case, the higher concentrations of sapid NaCl elicited smaller responses after treatment with renin, but with furosemide, the lower concentrations produced larger responses in the NaCl-best neurons. This latter difference may reflect the time course of the two experiments. Both treatments probably raise circulating aldosterone levels, and this hormone is known to increase the number of amiloride-sensitive sodium channels on the apical membranes of taste buds (17). This could raise the overall responsiveness to sapid sodium. The difference is that with furosemide treatment the recording began the next day, but with renin, neurons were isolated and tested within a few hours of treatment. In the former case, the taste buds could be influenced, but in the latter, it is less likely. Dietary sodium restriction decreases the number of amiloride-sensitive sodium channels (18) and leads to decreased sodium responses in CT fibers and NST taste neurons (8, 9, 19, 30).

The present results from the NST paralleled those from the CT nerve in another study that used furosemide-induced sodium depletion (4). These investigators found a marginal reduction in the whole nerve response magnitude but also only at the highest concentration of NaCl tested (0.5 M). The changes observed in the current data were in the same direction as those in other CT studies that raised an appetite from sucrose-best neurons in rats fed a sodium-deprived diet (8, 9, 19, 30).

In addition to our furosemide experiment (46), three comparable studies have been done while recording in the NST; two used dietary sodium restriction and the third, intracranial renin (19, 23, 30). A fourth, brief report summarized responses from parabrachial taste neurons with and without furosemide treatment (44). Despite differences in the anesthetics and treatments, each of these studies found that sodium appetite reduced the magnitude of the neural responses to sapid NaCl. In some cases, the effective concentrations and the classes of affected cells differed, but the effect was similar throughout.

Of these studies, only one was done in awake, behaving animals that had had their sodium appetite raised by dietary deprivation and thus is most applicable to the natural situation (30). The present results are in the same direction as those in the chronic recording experiment, but the effects are smaller and restricted to only the highest two of five NaCl concentrations. What the current results do match very closely are those reported by McCaughey and Scott (23). In both studies, renin treatment reduced responses to NaCl at the two highest concentrations (0.3 and 0.5 M for them; 0.3 and 1.0 M for us) across the entire sample of taste cells. Similarly, in the NaCl-best groups in both studies, the effects were significant only at the highest concentration of NaCl.

Given that both studies raised a sodium appetite using intracranial infusions of renin, this correspondence might not seem surprising, but the experiments actually differed on a number of other dimensions. The anesthetics differed, as did the tongue area stimulated, the recording paradigm (within- vs. between-neuron comparisons), and the hormonal background of the rats. McCaughey and Scott (23) primed their rats with 6–7 days of DOCA specifically so that the rats would ingest NaCl before water. We did not pretreat with steroid but used a higher dose of renin (100 vs. 25 ng) and then allowed the rats 1.0-h access to water before beginning the recording session. Despite these considerable differences, the changes in responsiveness to NaCl were essentially identical.

Of the procedural differences, perhaps the most interesting is the aldosterone priming. Because McCaughey and Scott (23) did not have a steroid-only control group, it remained possible that some of their renin effects resulted from the aldosterone, which at higher concentrations does elicit a sodium appetite by itself. At first glance, our results appear to preclude any direct influence from the steroid priming. Our renin dose, however, was four times greater than theirs, and it is well established that, when combined, subthreshold doses of aldosterone and ANG II act synergistically to produce a robust sodium appetite (36). Thus it remains possible that the steroid priming contributed to the effect in the prior study (23), but regardless, the overall change in responsiveness to NaCl did not differ between the two experiments.

The results did differ in one respect, the responsiveness of sucrose-best neurons to sapid NaCl. In both studies, over the full 5.0-s response period, the effectiveness of NaCl in driving sucrose-best cells did not differ as a function of treatment. When McCaughey and Scott (23) examined the initial second of the responses, however, they found that NaCl elicited more neural activity from sucrose-best neurons in rats...
treated with renin. When we reexamined this period in the sucrose-best neurons in the present sample, we found a clear concentration effect for both NaCl and for sucrose but no difference between the renin and the BSA conditions [NaCl, $F(4,20) = 1.40, P = 0.27$; sucrose, $F(4,20) = 0.90, P = 0.48$].

It is possible that this difference reflects the influence of exogenous aldosterone. This seems unlikely, however, because similar parallels appeared in two earlier studies of NST gustatory neurons in rats with (or without) an appetite raised by dietary sodium restriction (19, 30). In these experiments, steroids were not manipulated, and it seems unlikely that endogenous levels would have differed over the time course needed to match McCaughey and Scott (23). In both these experiments, a sodium appetite reduced responsiveness more broadly, but the biggest decrease was in sodium responses from NaCl-best neurons. In the one done in acute, anesthetized animals (19), NaCl drove sucrose-best neurons more in deprived than in replete animals. The other set of data was collected in awake, behaving rats and found no such effect (30).

With the exception of furosemide’s action on NST taste neurons, gustatory responses to sapid NaCl are reduced during sodium appetite regardless of the method used to induce this motivational state. This observation has been made repeatedly since Contreras (8) first published data 25 years ago. The primary functional interpretation of this effect was that the decreased afferent barrage resulted in a lower perceived intensity of the NaCl, thus fostering greater intake particularly at higher stimulus concentrations, which are normally aversive (11).

A reduction in perceived intensity, however, cannot account for the apparent change in hedonic value of NaCl, the avid seeking for and ingestion of strong salt, that is the cardinal feature of sodium appetite. One proposed mechanism for this change is that, during sodium appetite, NaCl activates not only neurons that respond best to sodium but also those that respond best to sugars or other molecules that taste sweet to humans (19). Evidence for such a switch in the effectiveness of sapid sodium has been observed in two experiments, one that used dietary induction (19) and one that used intracerebroventricular renin (23). Unlike the reduction in the responsiveness of NaCl-best cells to salt, however, the increased effectiveness of sapid NaCl in driving sucrose-best cells has not been observed in the only other two studies in which it was specifically looked for, one of which is the present experiment. As mentioned above, the other experiment used dietary sodium deprivation to induce the appetite and recorded neural responses in awake, behaving animals (30). As these circumstances are the closest to the natural condition and other important procedural differences exist between the two dietary studies (19 and 30), the most that can be said of this proposed mechanism for changing the hedonic value of sapid sodium is that “the jury is still out.”

**Perspectives**

The current experiment matches the study by McCaughey and Scott (23) in its primary independent variable (intracerebroventricular injections of renin), in its primary dependent variable (the responses on NST neurons to sapid stimuli), and in its primary observation (reduced responsiveness to hypertonic NaCl). This might seem predictable were it not for the factors that did differ between the two investigations: hormonal background, renin dose, anesthetics, within- or between-neuron design, and stimulus application. The overall effect, reduced responsiveness of taste neurons to sapid sodium, has been observed now at three different levels of the nervous system using four different methods for raising an appetite (4, 8, 9, 11, 19, 20, 23, 30, 42–44, 47, 48). Although the effect is also observed on the periphery, both in the CT and the geniculate ganglion (8, 9, 42, 43), the application of renin intracerebroventricularly and, in the case of McCaughey and Scott (23), the time course of the change in responsiveness bespeak a central origin for the NST changes. A first test of this inference would use a similar regimen of intracerebroventricular renin injections but record taste responses from the CT nerve, while measuring circulating ANG II and steroid levels. If this failed to reveal differences on the periphery, then the original NST recording experiment could be repeated using serial administration of intracerebroventricular renin and an ANG II receptor blocker such as lorsartan.

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