Amiloride-sensitive signals and NaCl preference and appetite: a lick-rate analysis

MICHELLE D. BROT,1 CHAE H. WATSON,2 AND ILENE L. BERNSTEIN2

Departments of 1Orthodontics and 2Psychology, University of Washington, Seattle, Washington 98195

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Brot, Michelle D., Chae H. Watson, and Ilene L. Bernstein. Amiloride-sensitive signals and NaCl preference and appetite: a lick-rate analysis. Am J Physiol Regulatory Integrative Comp Physiol 279: R1403–R1411, 2000.—Rats prefer hypotonic and isotonic NaCl solutions to water in long-access drinking paradigms. To focus on the role of taste signals in NaCl preference, licking patterns of rats with 30-s exposure to NaCl solutions (0–0.5 M) were examined when they were either water deprived, sodium depleted, or not deprived (NaCl mixed in dilute sucrose). In all three conditions, rats displayed a preference for NaCl. The addition of 100 μM amiloride, a sodium channel blocker, to NaCl did not change rats' licking when they were sodium replete but dramatically reduced licking when they were deplete. Transection of the chorda tympani (CT) nerve, an afferent pathway for amiloride-sensitive Na⁺ signals, had no effect on NaCl preference in nondeprived rats and only a modest effect on those that were Na⁺ deplete. Amiloride was found to exert significant suppression of NaCl intake in Na⁺-depleted rats with transection of the CT, supporting the existence of other afferent pathways for transmission of amiloride-sensitive Na⁺ signaling. Together, these studies argue for the involvement of different neural signalling mechanisms in NaCl preference in the presence and absence of explicit Na⁺ need.

SODIUM IS CRITICAL for normal physiological function, and its levels within the body are tightly regulated. Sodium depletion or deprivation creates a need state to which animals respond by avidly ingesting sodium salts, a behavior known as sodium appetite. In addition, both humans and rats will ingest NaCl in the absence of any physiological need, with most rat strains preferring hypotonic and isotonic NaCl solutions to water (8, 17).

Behavioral and metabolic responses to sodium depletion have been well studied, and the hormonal mechanisms that promote these responses are becoming well understood (e.g., 23). Understanding NaCl preference in the absence of need has proven to be a greater challenge, and it remains unclear why animals consume NaCl when sodium replete. A frequent explanation is that they find NaCl palatable at low concentrations, but this is essentially a restatement of the finding. One approach to further evaluating this “palatability” hypothesis would be to significantly modify the gustatory effectiveness of NaCl stimulation through nerve transection or lingual application of a sodium channel blocker (amiloride) and observe effects on NaCl intake and preference. To the extent that specific types of gustatory signals contribute to the palatability of NaCl solutions, selective alteration of those signals would be expected to alter drinking patterns. Furthermore, if the contribution of these signals to palatability differs depending on the animal’s need state, then different patterns of effect on licking in response to such treatments would be expected under different motivating conditions.

Available methods for assessment of NaCl preference (as opposed to NaCl appetite) in rats has certain drawbacks. Long-term intake tests, such as the commonly used 24-h two-bottle choice between water and NaCl solutions, are simple to administer and provide clear evidence that NaCl solutions are “preferred” relative to water at hypotonic and isotonic concentrations. However, the shape of the preference/aversion function generated with the use of 24-h tests can be influenced by the postigestive effects of NaCl solutions as well as by their taste (19, 21). In contrast, short-term (less than several minutes) evaluation of ingestion offers the benefit of minimizing postigestive consequences, thereby focusing interpretation on the animal’s response to taste. Short-term consumption can be tested discretely with the use of a lickometer to measure lick frequency. One of the goals of the present research was to develop a method for using lick-frequency testing in brief access tests to evaluate NaCl preference and appetite. Lick frequency can be a good index of palatability of NaCl solutions when evaluated within the first minute or two of access, that is, before significant postigestive influences. A similar approach was adopted by Breslin and colleagues (6) testing sodium-deplete and -replete rats with a range of NaCl concentrations in brief access tests. The main emphasis of that work was the nature of the response-concentration functions generated under the two conditions as a test of hypotheses regarding the effects of sodium de-
pletion on taste perception. Our focus is somewhat different in that we were interested in examining the role of amiloride-sensitive sodium signals and the chorda tympani (CT) nerve in NaCl preference and appetite.

One impediment to the use of lickometer testing to study NaCl preference is that without fluid or sodium deprivation, rats do not consume significant amounts of NaCl solutions in short tests (9). Because reliable and prompt licking is required for brief-access lickometer assessment, we sought a modification that would generate high levels of licking without fluid or sodium deprivation. The approach used here was based on the view that taste is considered an analytic sense, meaning that the individual components of a taste mixture are discernible and retain their unique identities (7). We therefore produced taste mixtures that took advantage of the rat’s eagerness to consume sucrose solutions when nondeprived to examine the effect on lick rate of adding NaCl to sucrose solutions. We offered a constant 0.15 M sucrose background as the “vehicle” for a range of NaCl concentrations (0–0.5 M) to generate preference functions for NaCl in brief-access tests. We also generated preference functions, in the identical testing apparatus, for straight NaCl (0–0.5 M) solutions in animals motivated to drink by fluid deprivation and sodium depletion with furosemide. The effects of these three methods of motivating licking for NaCl solutions were compared with regard to overall lick rates and the shape of the preference curve. This made it possible to then examine the contribution of afferent signals from the CT nerve and amiloride-sensitive sodium channels to patterns of licking for NaCl under different motivating conditions.

The CT nerve is the primary source of information about sodium signals received from the anterior portion of the tongue (2, 12). However, behavioral studies indicate that bilateral transection of the CT (CTX) has little effect on normal NaCl intake and preference (1, 24, 31). When rats are sodium depleted, a role for the CT emerges more clearly. Breslin and colleagues (6) found that after sodium depletion, rats with CTX appeared less able to discriminate between salt solutions that contain sodium and those lacking sodium. Nonetheless, they still ingested more of the Na-containing solutions. Similarly, sodium-depleted Wistar rats with CTX show a reduced and delayed NaCl intake, while still drinking enough NaCl to restore sodium balance (27). Thus signals conveyed by the CT appear to be important in the detection of Na⁺-containing solutions and to the selective ingestion of those solutions. However, CTX does not completely eliminate differential responding to sodium solutions during expression of a sodium appetite.

In the rat, a significant mechanism for gustatory detection of NaCl involves the passage of sodium ions into taste buds through epithelial channels that are blocked by amiloride, the sodium channel blocker. Electrophysiological studies have demonstrated that the CT response to NaCl has components that are amiloride sensitive and amiloride insensitive (14, 15).

The lingual application of amiloride reversibly suppresses the CT whole nerve response to NaCl solutions, while having little or no effect on the response to nonsodium salts such as KCl or NH₄Cl (4, 11, 14). Behavioral analysis of the response to amiloride indicates that the perceptual characteristics of NaCl may be altered by lingual application of amiloride (16) and that the effects of amiloride treatment on preference may depend on the sodium balance of the animal (3).

The present studies used three approaches to motivate NaCl solution intake: 1) 15-h water deprivation, 2) overnight sodium depletion with the use of furosemide, and 3) providing a mixture of NaCl and sucrose with no deprivation. Rats consumed the solutions in a lick-detection apparatus, which counted the number of licks during a series of 30-s presentations. By measuring the short-term licking response of Wistar rats under sodium-replete and sodium-deplete conditions, we could also determine whether the physiological need for NaCl altered the shape of the response-concentration function. Test protocols were then used to explore the contribution of signals conveyed by the CT nerve and amiloride-sensitive sodium signals to the NaCl response-concentration function.

EXPERIMENT 1

Method

Subjects. Ten Wistar male rats (Simonsen Labs), weighing 300–350 g at the start of the experiment, were housed individually in a room with a 12:12-h light-dark cycle (lights on at 0700). Rats had ad libitum access to chow pellets (Teklad Rodent Diet 8640, containing 0.4% sodium) and water, unless noted otherwise.

Apparatus. The Davis MS160 lick-detection system (DiLog Instruments, Tallahassee, FL), or lickometer, was used to present a range of NaCl concentrations to the rat. The lickometer apparatus consists of a Plexiglas cage (29 cm long × 14.5 cm wide × 23 cm high) with a metal wire mesh floor, clear walls on the side, and a front metal wall containing a slot. In front of the cage, a sliding rack that can hold up to 16 tubes is positioned so that the spout on each tube lines up with the open slot in the front of the cage once it moves into place. A sliding metal door blocks this slot to prevent the rat’s access to the spout between solution presentations. The lickometer measures the number of licks made by the animal by passing a current of <60 nA through the rat each time its tongue contacts the drinking tube. Data are stored on a computer for later analysis.

Procedure. Testing occurred between 0900 and 1300. Rats were habituated to the cage for an hour per day for 2 days after which they were allowed access to fluid in the cage after overnight water deprivation. Initially, 0.15 M sucrose was used as the tantast. When the rats licked readily in a 30-min period, brief-access training began. Rats were given 2-min exposure to water and two sucrose solutions (0.15 and 0.3 M), with each solution presented twice in a random order. The access
time was gradually reduced to 30 s. Once the rats licked promptly for sucrose solutions without water deprivation, one of three test conditions to motivate the rats to consume NaCl was employed. The NaCl test conditions were 1) 15-h water deprivation, 2) overnight sodium depletion with the use of furosemide, and 3) providing a mixture of NaCl and sucrose with no deprivation. The concentrations of NaCl tested were 0, 0.05, 0.089, 0.158, 0.281, and 0.5 M, on the basis of work by Breslin and colleagues (5). These molar concentrations of NaCl correspond to the following percentages: 0.3, 0.5, 0.9, 1.6, and 2.9%. In the case of the NaCl-sucrose condition, 0.15 M (5%) sucrose served as the solvent for all NaCl concentrations. Each concentration was presented in random order, three times during the test in blocked trials, totaling 18 presentations. The length of access to each solution was fixed at 30 s from the time of the first lick with 20 s between presentations. In addition, the rat had 40 s to begin licking after which time the door would close and the next solution would be presented 20 s later. Rats were tested in all three conditions, typically with 2 additional training days between test days.

The diuretic, furosemide (Astra Pharmaceuticals; 10 mg/kg), in two subcutaneous doses an hour apart was used to induce sodium depletion following a procedure developed by Wolf (34). Animals were weighed, and food and water were removed from the cages before the first injection. A minimum 20-g overnight weight loss was considered confirmation of diuresis, and no rats were excluded on this basis. Distilled water and sodium-deficient diet (ICN Biochemicals) were available in the cages overnight, and testing occurred the next day.

Analysis. The results were calculated separately for each condition by averaging the number of licks made across all three presentations for each concentration. A minimum criterion of five licks was set for inclusion of data, and these numbers for each rat were averaged for all the rats at each concentration. The percentage of times averaged data did not meet criterion was very low: 1) 0% for NaCl-sucrose, 2) 7% for water deprivation, and 3) 3% for Na depletion. The 30-s lick-rate data were analyzed in a two-factor (condition × NaCl concentration) repeated-measures ANOVA with statistical significance set at $P < 0.05$. A one-way ANOVA and post hoc Scheffé’s test were done to determine the source of the significant main effects.

Results and Discussion

Lick rates for NaCl over a range of concentrations can be viewed in Fig. 1. Repeated-measures ANOVA indicate a significant effect of condition, $F(2,10) = 3.859, P < 0.05$; concentration, $F(5,10) = 27.77, P < 0.0001$; and their interaction, $F(5,110) = 6.07, P < 0.0001$.

Figure 1A represents licking for the sucrose-NaCl mixture in the absence of any deprivation. The shape of the curve is strikingly similar to those that are typically generated in long-term two-bottle tests (e.g., 21, 27). Those curves represent preference as a ratio of NaCl intake to total fluid intake, whereas the present curve represents lick rate for sucrose solutions, with and without added NaCl, over 30 s. The rise in the curve toward isotonicity, although not statistically significant, has been seen by us repeatedly. It suggests that adding NaCl to an already palatable sucrose solution increases its palatability. The similarity in both the ascending and descending limb of the preference curve between long-term tests and the present brief-access protocol provides support for the notion that taste factors alone can generate an NaCl preference curve. Furthermore, the similarity between the curves supports the use of this mixture as a method for assessing a range of factors that may play key roles in NaCl preference in the rat.

Fig. 1. Licking for NaCl solutions over a range of concentrations under conditions of NaCl mixed with 0.15 M sucrose (A), water deprivation (B), and sodium depletion (C). Each data point represents an average ± SE of 3 30-s presentations for each animal averaged across all animals ($n = 10$).
Sucrose was mixed with NaCl in this experiment to motivate the animals to drink in brief-access presentation regimens and thereby allow us to evaluate NaCl preference in brief-access tests. Given that animals are drinking a mixture and not NaCl alone, we need to address the question of whether changes in lick rate as a function of NaCl concentration are indeed attributable to changes in perception of the salt or due to some mixture interaction. Behavioral and electrophysiological studies have examined taste-mixture interactions in rats and other rodents. Behaviorally, rats appear to be able to identify individual components of most taste mixtures (20), including mixtures of NaCl and sucrose. In contrast, NaCl appears to interfere with the identification of bitter quinine. Electrophysiologically, there is evidence that some taste-mixture interactions are strong, such as the suppression of both bitter and sweet in sucrose-quinine interactions but that interactions between sucrose and NaCl are relatively weak. For example, recordings from the hamster CT nerve did not provide evidence for strong mixture interactions in sucrose-NaCl mixtures (10). Similarly, recordings from single neurons in the hamster parabrachial nucleus indicated that response frequencies evoked by sucrose-NaCl mixtures did not differ from those evoked by the most effective component alone (32). Thus, although mixture interactions cannot be completely ruled out, available evidence supports the idea that such interactions would be relatively weak under the present conditions. Furthermore, the similarity between the concentration-response functions generated using licking for mixtures and preference for NaCl in 2-bottle tests encourages us to view this method of preference testing as a useful addition to the behavioral tools available for assessing NaCl preference.

The other graphs in Fig. 1 represent licking for water and NaCl solutions under conditions of water deprivation (Fig. 1B) and sodium depletion (Fig. 1C). Both curves display an inverted U shape with an ascending and descending limb. The data are similar to those of Breslin et al. (6) with the highest lick rates occurring for concentrations around isotonicity. Those investigators point to a vertical shift in lick rates for NaCl in sodium-deplete vs. sodium-replete conditions but no “peak shift.” The present findings support this general trend, namely that manipulation of physiological state with either water deprivation or sodium depletion produces a similar response-concentration function to that of sodium deprivation.

The tendency to generate more licks for solutions containing NaCl, particularly around isotonic concentrations, is evident under all three motivating conditions. This is the case, despite the fact that increased NaCl intake serves an obvious “homeostatic” function only in animals that are sodium deplete. The shape of the curves does reflect sensitivity to need state. For example, the ascending limb of the curve for rats that are sodium deplete is extremely sharp, with very low levels of licking for water (P < 0.001; 1-factor ANOVA and Scheffe’s F test). Additionally, high lick rates are maintained out to 0.3 M NaCl, which is normally an unpalatable concentration (P < 0.0001; 1-factor ANOVA and Scheffe’s F test). In contrast, the descending limb of the curve for rats that are fluid deprived is very sharp, and once solution concentrations are higher than isotonicity, there is little licking for them. None of this is surprising, but it does reinforce the sensitivity of this technique to the detection of subtle variations in palatability.

**EXPERIMENT 2**

The concentration-response functions generated in experiment 1 support the use of these motivating paradigms for assessing NaCl preference in brief-access tests. The purpose of the next study was to evaluate the contribution of signals conveyed by amiloride-sensitive gustatory pathways to NaCl preference and appetite. The techniques used in experiment 1 are ideally suited to an examination of the effects of amiloride exposure because amiloride’s lingual blockade of sodium transport is relatively brief. Two possible predictions could be made regarding the effects of amiloride on licking for NaCl. One is that amiloride, by reducing the gustatory afferent response to NaCl, would reduce the perceived intensity of the NaCl solutions, leading to a rightward shift in the concentration-response curves. The other is that, because amiloride reduces that portion of the neural signal that is relatively specific to sodium, we might predict a change in perceived quality of the NaCl solutions and that would be likely to have its greatest effect on licking in the sodium-deplete condition. Presumably, amiloride could alter both the perceived intensity and quality of NaCl solutions, with somewhat more complex effects on licking.

**Method**

**Subjects.** The 10 Wistar rats from experiment 1 were used in the NaCl-sucrose segment of this study. For the other segments, a different set of 10 Wistar male rats (Simonsen Labs), weighing 285–335 g at the start of the experiment, were used.

**Procedure.** For this set of studies, amiloride hydrochloride (Sigma) was made up in a 100-μM solution in dH2O that was used to prepare NaCl solutions for all amiloride-exposure conditions. Amiloride was made fresh the morning of testing. It went into solution with constant stirring and heating for at least 1 h. Because amiloride is light sensitive, the beaker and individual testing tubes were wrapped in aluminum foil. The amiloride solution was cooled to room temperature before presentation.

Habituation and procedures for the water-deprivation and NaCl-sucrose testing were similar to those described in experiment 1. To assess effects of amiloride exposure, rats were tested twice, once with amiloride exposure and once without it, with order of testing counterbalanced.

For the sodium-depletion condition, procedures for depletion were the same as those described in experiment 1. To avoid repeated days of sodium deprivation,
only one concentration of NaCl was presented, and rats were tested either with or without amiloride in a single session. Rats were given a sequence of solution presentations consisting of two 30-s presentations each of 0.1 M NaCl and water, followed by three 30-s presentations of either 0.1 M NaCl mixed with 100 μM amiloride (amiloride group) or 0.1 M NaCl (control group), and then two 30-s water presentations and a single 0.1 M NaCl presentation. The water presentations were included to act as “rinses” for the amiloride. This same sequence was repeated a second time. As in the other studies, there was a 20-s interval between solution presentations and a 40-s latency for the rat to begin licking before the door closed.

Analysis. The results for the studies with the use of water deprivation and NaCl-sucrose were analyzed as described in experiment 1, except that the ANOVA tested amiloride × NaCl concentration as the two factors. In addition, a paired t-test was performed to determine whether the increase in licking when NaCl was added to sucrose was significant. For the sodium-deprivation study, each half of the session was calculated as a unit, and then the data from each were averaged together. For example, the two baseline NaCl presentations were averaged for each animal as were the three NaCl or NaCl-amiloride presentations, etc. The final mean for each animal for each solution was then averaged to determine a group mean and SE. As in experiment 1, a one-factor ANOVA with post hoc Scheffe’s test was performed to follow up any significant main effects.

Results and Discussion

Lick rates for a range of NaCl concentrations with and without amiloride can be viewed in Fig. 2. Figure 2A represents licking for the NaCl-sucrose mixture in the absence of any deprivation (data repeated from Fig. 1), and it is evident that the addition of amiloride to the solution had no effect on the shape of the curve or on absolute number of licks. Note that in the amiloride condition, the addition of NaCl to sucrose was associated with a statistically significant increase in licking at 0.09 M [t(9) = −5.37, P < 0.001], suggesting that the palatability enhancement associated with NaCl is not dependent on amiloride-sensitive sodium signals. Thus these data demonstrate that in nondeprived rats, amiloride does not alter licking for a NaCl-sucrose mixture over a range of NaCl concentrations.

Figure 2B represents licking for water and NaCl solutions under conditions of water deprivation. Overall, the effects of amiloride treatment are not statistically significant, but there is a significant condition by concentration interaction [F(1.5) = 4.47; P < 0.01]. Further analysis revealed a reduced effect of amiloride-NaCl at 0.281 M NaCl. This is consistent with results from Weisinger et al. (33) demonstrating that water depriving rats resulted in an increase in sodium excretion concurrent with a decrease in sodium intake to produce an overall sodium deficit. Moreover, water-deprived rats increase their intake of both water and NaCl solution. In light of these data and those of Weisinger et al. (33), it appears that a water-deprivation condition is ambiguous with regard to whether animals are in a state of sodium need.

Licking for NaCl under conditions of sodium depletion can be viewed in Fig. 3. A two-factor repeated-measures ANOVA did not show a significant main effect, however, the interaction was significant [F(2,8) = 20.02; P < 0.001]. It is evident that the addition of amiloride to 0.1 M NaCl dramatically reduces licking (post hoc Scheffe’s test, P < 0.05). The effects of amiloride were assessed with only a single NaCl concentration in sodium-deplete animals, but with that concentration it was clear that amiloride in the solution had a powerful effect on behavior toward the solution. In animals receiving amiloride, reductions in lick rate to ∼20–25% of baseline (preamiloride) levels were seen. Amiloride effects were promptly reversed after water was presented, with licking returning to baseline levels. Control animals show stable
significant reduction in licking for NaCl vs. baseline and postamiloride NaCl solutions. This suggests that the dilute NaCl lost much of its taste, and the more concentrated NaCl lost its distinctive “sodium” quality. If blockade of amiloride-sensitive sodium signalling leads to such changes in taste quality and intensity, one would expect this to be reflected in licking for NaCl in the absence of sodium need by differences in the shape of the response-concentration function. In our study of sodium-replete rats, we observed no change in licking over the concentration curve when amiloride was added to the NaCl solutions.

One possible explanation for this lack of effect with amiloride is that there was, indeed, a change in the perceptual characteristics of the NaCl, but it was not associated with any change in preference and lick rate. However, because NaCl preference is both concentration dependent and cation dependent, the lack of effect of amiloride on NaCl preference presents something of a paradox: if rats’ perception of NaCl is so muddled by amiloride that they cannot distinguish between NaCl and KCl (29), why is their licking for NaCl unaffected by amiloride treatment in our Na-replete data? The unique “salty” taste of NaCl has been attributed to the amiloride-sensitive component of the rat’s taste response, whereas a “sour-salty” taste is carried by the portion of the NaCl response that is unaffected by amiloride (16). Thus one might consider the unlikely scenario that this perceived sour-salty taste after amiloride resulted in an identical lick rate to that of NaCl over a range of concentrations. Another possibility is that the amiloride was not working effectively to block Na+ channels, but this seems unlikely, as well, because a repetition of each experiment yielded very similar results. Furthermore, the amiloride was prepared and presented the same way for depleted and replete rats, but it was clearly effective only in the former.

**EXPERIMENT 3**

CTX has been reported to be associated with little effect on NaCl preference of most rat strains in long-term two-bottle preference tests (but see Ref. 26), and this contrasts with clear effects of CTX on expression of sodium appetite in a variety of tests (6, 10, 22). The effect of transection of the CT nerve, which affects both amiloride-sensitive and amiloride-insensitive signals, on NaCl preference and appetite in lickometer tests was assessed in the next study. The water-deprivation condition was omitted from this experiment for two reasons. One is that CTX results in partial desalivation (7, 25), which might be expected to confound drinking patterns particularly under conditions of fluid deprivation. The other reason is that, as previously discussed, there is some ambiguity about whether the water-deprivation condition does constitute a state of sodium need and, hence, effects on licking for NaCl after CTX would be difficult to interpret.

**Method**

**Subjects.** Wistar male rats, weighing 335–470 g and maintained as in the previous studies, underwent CTX or sham surgery (described in Surgery).

**Surgery.** Rats were anesthetized with a mixture of ketamine and xylazine and randomly assigned to the CTX or sham condition. Bilateral CTX was accomplished by removing the ear bones after penetration of the tympanic membrane with the use of a pair of fine forceps. This method severs the nerve and has been previously documented to be effective (6, 22). In addition, we confirmed CTX in these rats by counting taste pores on the tongue after the completion of the exper-
iment. The sham surgery consisted of simply penetrating the tympanic membrane without disturbing the bone. The rats were given 11 days after surgery to recover and retrain on sucrose licking.

Histology. To confirm nerve transection, at the end of the behavioral testing, rats were deeply anesthetized (100 mg/kg pentobarbital sodium), and their tongues were removed and postfixed in 4% paraformaldehyde in 0.1 M phosphate buffer. After a minimum of 10 days postfixing, tongues were removed and rinsed in distilled water. The anterior 5 mm of each tongue was removed and stained in 0.5% methylene blue for a few seconds and rinsed again in distilled water. Under a light microscope, fungiform papillae were identified and counted by an observer blind to the condition. In the intact rat, each fungiform papilla contains a single taste bud with a pore. If no pore exists, then the taste bud is believed to have degenerated, indicating that the innervation of the taste bud has been eliminated. The percentage of papillae with a pore served as a measure of denervation. A criterion of <15% of papillae with pores was set as an indication of a successful transection, on the basis of previous work (10, 12). Every CTX rat met the criterion, and none had to be excluded from the study.

Procedure. Ten rats (CTX = 5, Sham = 5) were used to assess effects of CTX on NaCl preference by testing them as described for NaCl-sucrose mixtures in experiment 1. Another 10 rats (CTX = 5, Sham = 5) were used to assess effects of CTX on NaCl appetite with the use of the test procedures in experiment 2. Rats were sodium depleted with the use of furosemide and tested with the use of NaCl solutions with and without amiloride. The sequence of solution presentations was as follows: 1) 30-s presentations with 20-s interpresentation intervals with the use of 0.1 M NaCl and 200 M amiloride in alternating blocks, with order of presentation counterbalanced.

Analysis. Data for effects of CTX on licking for sucrose-NaCl mixtures were analyzed with a repeated-measures ANOVA. This was followed by one-factor ANOVA analysis and a post hoc Scheffe’s test to determine the source of the main effect. For the sodium-depletion series, data were averaged across NaCl presentations and across NaCl with amiloride presentations for each animal and then analyzed by repeated-measures ANOVA. The criterion for the minimum number of licks to NaCl for the data to be considered sufficient for a sodium appetite was set at 50. This differed from the minimum criterion of five licks in the previous experiments because it was expected that rats in a need state would respond by avidly licking a sodium-containing solution.

Results and Discussion

There was no significant effect of CTX when sodium- and fluid-replete Wistar rats were drinking the sucrose-NaCl mixtures (Fig. 4). The lack of effect mirrors the negative findings in experiment 2 using amiloride and agrees with previous work indicating that signals from the CT do not contribute significantly to need-free NaCl preference (27).

Figure 5 presents mean lick rates for 0.1 M NaCl with and without amiloride in CTX and intact rats depleted of sodium. As in experiment 2, amiloride significantly reduces licking for NaCl [F(1, 5) = 41.23; P < 0.001]. Although suppression was seen in transected animals as well, a significant interaction (chorda status by amiloride condition) [F(1, 8) = 8.18; P < 0.05] suggests that amiloride had a greater effect on intact animals. This was confirmed by a one-factor ANOVA and Scheffe’s post hoc test comparing the magnitude of the reduction between licks for NaCl and NaCl/amiloride in the two CT groups [F(1, 8) = 6.5; P < 0.05]. The reduction in NaCl licking in the CTX group is consistent with reports of others testing Na-deplete rats drinking NaCl in longer-access tests (13, 27). Because
amiloride still yields significant suppression of licking for NaCl in the CTx group, this indicates that although an intact CT nerve may be important for carrying amiloride-sensitive signals about NaCl taste, it is not the only pathway conveying such signals.

GENERAL DISCUSSION

The present set of studies provided a methodology for assessing the palatability of NaCl solutions in short-term access tests under a variety of motivating conditions and then utilized that methodology for a comprehensive examination of the effect of amiloride treatment and CTX on NaCl licking and appetite. Perhaps the most striking finding was the demonstration that the effect of these treatments differs significantly as a function of the sodium balance of the animal. Transection of the CT, and particularly elimination of amiloride-sensitive signals, dramatically affects the palatability of NaCl solutions when animals are motivated to lick NaCl as a result of sodium depletion. In this case, identification of the solution as containing sodium ions is key to the generation of robust, enthusiastic licking, and amiloride-sensitive neural signals are essential. The situation is entirely different when rats are motivated to drink by thirst or the presence of a sweet taste. In these cases, although the presence of NaCl in the solution appears to enhance its palatability, blocking sodium-specific signals by amiloride treatment does not interfere with its palatability. Thus amiloride-sensitive pathways do not appear to be critical for an animal to respond to NaCl in a sodium-replete state, whereas amiloride treatment clearly interferes with short-term licking for NaCl in a need state.

Sodium-deplete rats, with CTX, showed a significant reduction in licking for NaCl when it was mixed with amiloride. Suppression of licking by amiloride, in rats without a CT nerve, indicates that amiloride-sensitive gustatory signals must be carried by some other neural pathways and that these signals contribute to Na+-appetite. This is consistent both with a recent electrophysiological recording study of the greater superficial petrosal (GSP) nerve and with two behavioral studies. Sollars and Hill (28) have demonstrated that the ap- petrosal (GSP) nerve and with two behavioral studies. Physiological recording study of the greater superficial pathways and that these signals contribute to Na+-appetite. This is consistent both with a recent electrophysiological recording study of the greater superficial petrosal (GSP) nerve and with two behavioral studies. Sollars and Hill (28) have demonstrated that the application of amiloride markedly reduces whole nerve responses to Na+ stimulation in the GSP. St. John et al. (30) tested rats for ability to discriminate NaCl from KCl. Transection of the CT nerve impaired discrimination performance, but amiloride treatment impaired it even further, reducing discrimination to chance levels. Roitman and Bernstein (22) examined sham drinking for NaCl solutions in intact and CTX rats under sodium-depleted conditions. They observed a reduction in sham drinking in rats given amiloride mixed with NaCl. These studies offer evidence consistent with data presented here suggesting that additional amiloride-sensitive pathways must be functioning in CTX rats. The GSP nerve appears to be a likely candidate at this time. Additional work needs to be done to elucidate the neural basis for which the motivational state of the animal modifies the role of amiloride-sensitive signals in brief-access NaCl licking tests.

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REFERENCES


