The Journal of Neuroscience, December 15, 1996, *16*(24):8115–8122

Amiloride Disrupts NaCl versus KCl Discrimination Performance: Implications for Salt Taste Coding in Rats

Alan C. Spector, Nick A. Guagliardo, and Steven J. St. John

Department of Psychology, University of Florida, Gainesville, Florida 32611

Amiloride, an epithelial sodium channel blocker, suppresses the responsiveness of narrowly tuned sodium-responsive taste afferents when orally applied in the rat. Broadly tuned salt-responsive taste afferents, which respond to sodium and nonsodium salts and acids, are relatively unaffected by the drug. We used amiloride treatment to examine the consequences of the specific removal of input from narrowly tuned sodium-responsive afferents on taste discrimination. Five water-restricted rats were trained in a gustometer to press one lever after licking NaCl and another lever after licking KCl across a range of concentrations (0.05, 0.1, and 0.2 M). Correct responses were rewarded with brief water access, and incorrect responses were punished with a time-out. After training, animals averaged about 90% correct responses and maintained competent performance during subsequent control sessions. Amiloride was then placed in all solutions at a given concentration (1–100 μM) for single test sessions. Control sessions were interposed between amiloride sessions. At high amiloride concentrations, overall responding was reduced to 50% correct and progressively improved as the drug concentration was lowered. The sigmoidal dose–response functions corresponded quantitatively with electrophysiological findings. Performance deficits occurred primarily with NaCl and were concentration dependent; performance during KCl trials was relatively undisturbed by amiloride adulteration. At high amiloride concentrations, rats treated NaCl as if it were KCl. Given that amiloride is tasteless to the rat, these results provide convincing evidence of the importance of narrowly tuned afferents in the discrimination between sodium and nonsodium salts and suggest that this is a general coding principle in the gustatory system.

Key words: amiloride; salt; psychophysics; taste transduction; gustatory system; sensory coding; NaCl; KCl; discrimination learning

Amiloride is an epithelial sodium channel blocker that has been shown to affect NaCl taste transduction in several species, including rats (e.g., Heck et al., 1984; Brand et al., 1985; Hellekant et al., 1988; Hettinger and Frank, 1990; Hill et al., 1990; Avenet and Lindemann, 1991; Gilbertson et al., 1992; Smith and Ossebaard, 1995). Oral application of amiloride suppresses the sodium responsiveness of the chorda tympani nerve (e.g., Brand et al., 1985; DeSimone and Ferrell, 1985; Hellekant et al., 1988; Hettinger and Frank, 1990; Nakamura and Kurihara, 1990), which innervates the anterior tongue. The inhibition of responses to NaCl in the rodent chorda tympani nerve is not absolute; even at high doses of the drug a portion of the salt response remains (Brand et al., 1985; DeSimone and Ferrell, 1985; Hettinger and Frank, 1990). Thus, with respect to NaCl, there is an amiloride-sensitive and an amiloride-insensitive taste transduction pathway. The amiloride-sensitive component of the whole-nerve chorda tympani response to NaCl seems to involve primarily the so-called N-units (Ninomiya and Funakoshi, 1988; Hettinger and Frank, 1990), which are narrowly tuned to respond to sodium and lithium salts (Boudreau et al., 1983; Frank et al., 1983). Amiloride does not affect the more broadly tuned sodium responsive H-units as effectively; these units respond to sodium and nonsodium salts, acids, and quinine. Moreover, based on whole-nerve electrophysiology, the sodium responsiveness of the glossopharyngeal nerve, which innervates taste buds in the posterior tongue, is entirely unaffected by amiloride (Formaker and Hill, 1991). The chorda tympani and glossopharyngeal nerves collectively innervate close to 80% of the taste bud population in the rat (Miller, 1977).

The fact that amiloride selectively suppresses activity in N-units makes the drug uniquely suited for experiments aimed at determining whether narrowly tuned units provide critical information that underlies taste discrimination. Although such a hypothesis is intuitively appealing, it remains to be explicitly tested. As might be expected from the electrophysiology, amiloride disrupts taste-guided behavioral responsiveness to NaCl. For example, amiloride abolishes the expression of a depletion-induced sodium appetite in rats (Bernstein and Hennessy, 1987; McCutcheon, 1991). In hamsters, the typical aversion to NaCl in a long-term two-bottle test is changed to indifference by amiloride adulteration of the stimuli (Hettinger and Frank, 1990). These particular experiments, however, were not designed to determine whether the effects were a result of alterations in the intensity of the stimulus or to a change in its perceptual taste quality. When NaCl was used as a conditioned stimulus in a taste aversion paradigm, rats treated with amiloride during conditioning uncharacteristically generalized the learned aversion to nonsodium salts (Hill et al., 1990). This finding suggests that amiloride actually changes the qualitative perceptual characteristics of NaCl, making this stimulus taste more like nonsodium salts.

With few exceptions, in all of the behavioral work conducted so far, a single 100 μM concentration of amiloride was used. The

Received May 8, 1996; revised Sept. 23, 1996; accepted Sept. 26, 1996.

Supported by National Institute on Deafness and Other Communication Disorders Grant R01-DC01628. A.C.S. is the recipient of Research Career Development Award K04-DC00104 from the National Institute on Deafness and Communication Disorders, and S.J.S. is the recipient of a Graduate Research Fellowship from the National Science Foundation. We thank Stacy Markison and Camille Tessitore King for providing comments on this manuscript.

Correspondence should be addressed to Dr. Alan C. Spector, Department of Psychology, University of Florida, Gainesville, FL 32611-2250.

amiloride concentration, however, that produces ½ maximal inhibition, as measured electrophysiologically, is ≤6 μM for midrange concentrations of NaCl (Brand et al., 1985; DeSimone and Ferrell, 1985; Hettinger and Frank, 1990; Gilbertson et al., 1992). To our knowledge, a behaviorally assessed dose–response function for the effect of amiloride on salt taste perception has not been determined for any species. The absence of a dose–response function derived from behavioral measures precludes attempts to quantitatively relate peripheral receptor function to gustatory perceptual processes.

Accordingly, the purpose of our experiment was to establish the dose–response relationship of the effect of amiloride on the ability of the rat to discriminate NaCl from KCl. Furthermore, the operant task used was designed to assess the degree to which responding to each salt (i.e., NaCl and KCl) was influenced by amiloride adulteration, allowing for an evaluation of the stimulus specificity of the effect of the drug. In addition, these results could be compared to previous studies that tested the NaCl versus KCl discrimination performance of rats after bilateral transection of the chorda tympani nerve (Spector and Grill, 1992; St. John et al., in press). In these studies, transection of the chorda tympani nerve severely disrupted salt discrimination performance. The effect could be a result of the removal of the amiloride-sensitive or the amiloride-insensitive salt transduction pathway, or both. Finally, because amiloride selectively suppresses sodium responsiveness in N-units, the use of amiloride in this behavioral paradigm offered an opportunity to explicitly test the hypothesis that narrowly tuned afferents are critical in taste discrimination.

MATERIALS AND METHODS

Subjects

Five male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) served as subjects. These rats had served as control animals in a previous experiment (St. John et al., in press). At the start of this experiment the rats weighed 463–543 g. They were housed individually in stainless steel wire hanging cages. They had free access to laboratory chow (Purina, 5001) and distilled water, except where noted below. The lighting (12 hr/12 hr light/dark), temperature, and humidity in the colony room were controlled automatically.

Apparatus

The taste-testing apparatus used was a modified version of the gustometer described in detail elsewhere (Spector et al., 1990). It was altered to include two levers that were positioned on either side of the stimulus access slit in the side wall of the chamber. In addition, a vertically oriented 3.3 cm stainless steel drinking spout, electrically insulated except for the tip, was attached to the shaft of the stepping motor. It was mounted at a 160° angle to the taste stimulus delivery spout and served as the source of water reinforcement. Finally, one clear incandescent cue light was mounted 4.2 cm above each lever.

Procedure

Training. The rats had been trained previously in the gustometer to differentially press one of two levers depending on whether the stimulus was NaCl or KCl (St. John et al., in press). Briefly, rats were maintained on 23.3 hr restricted water access schedule for 5 d a week; *ad libitum* distilled water was available on the home cage for the remaining 2 d. Three of the rats were trained to lick a drinking spout for a small taste sample (10 licks at 5 μl/lick) and to press the left lever if the stimulus was NaCl and the right lever if the stimulus was KCl; the other two rats had the lever contingencies reversed. If the rat responded correctly it received immediate access to water reinforcement (40 licks at 5 μl/lick, 10 sec reinforcement period); an incorrect response resulted in a 30 sec time-out, during which the house lights and cue lights were turned off. The time-out served as a punishment because it further delayed the opportunity of the water-restricted rat to obtain fluid reward. After reinforcement or punishment there was a 10 sec intertrial interval, during which the chamber was dark. A white masking noise remained on during the entire session. The beginning of a trial was signaled by the house lights. Once the rat made spout contact it had 3 sec to sample the stimulus and then 5 sec to make a response (this limited hold was signaled by the lighting of the two cue lamps above the levers); if the latter time period expired with no response, the spout was rotated out of the reach of the rat and the rat received the 30 sec time-out. In each session, three concentrations of each salt (0.05, 0.1, and 0.2 M) were presented in randomized blocks of six. Concentration was varied to render intensity an irrelevant cue. The animal could initiate as many trials as possible in the 40 min session. On average, sessions consisted of about 75 trials. All salt solutions were mixed daily in distilled water with reagent grade chemicals (Fisher Scientific, Orlando, FL).

Testing. Testing began with three consecutive control sessions without amiloride mixed with the salt solutions. Then, the salt solutions were made with the use of 100 μM amiloride hydrochloride (Sigma, St. Louis, MO) as the solvent. The same concentration of amiloride served as the water reinforcement. After this session, two to three control sessions without amiloride were interposed between the following amiloride test sessions. This was done to maintain and measure stimulus control of behavior. The order of amiloride concentrations tested across sessions was 100, 30, 3, 1, 10, and 100 μM. The replication of the 100 μM dose was conducted to test for order effects. In the final session, three fluid reservoirs were filled with distilled water, and the other three were filled with the respective KCl concentrations. The purpose of this manipulation was to test the hypothesis that responding to NaCl adulterated with amiloride emulated what would be seen if water were pitted against KCl. Simply put, it tested whether rats treated amiloride-adulterated NaCl as if it were water.

Data Analysis

The percent correct was quantified for: (1) the session as a whole, (2) each salt collapsed across concentration, and (3) each stimulus concentration. This was done for each animal and these values served as scores. Trials for which the animal did not respond were not included in deriving the percent correct. The data were analyzed in standard parametric ANOVAs. The conventional $p = 0.05$ level of statistical confidence was used.

A logistic function was fit to the amiloride concentration-response data:

$$f(x) = \left(\frac{a - d}{1 + (x/c)^b} \right) + d, \quad (1)$$

where a is a constant representing asymptotic maximum performance as determined by the mean of the control sessions that preceded each of the amiloride sessions, b is the slope, c is the midpoint concentration between the asymptotic maximum and minimum, d is the asymptotic minimum, and x is the amiloride concentration. The relationship between chorda tympani nerve responsiveness to NaCl and amiloride concentration is sigmoidal. As will be shown, a sigmoidal logistic function also accounts for the behavioral data quite well. To compare the effective range of amiloride as assessed by electrophysiological and behavioral measures, we chose to focus on the c parameter, which defines the midpoint concentration between the performance asymptotes on the amiloride dose–response curve. Thus, this parameter can be compared with the inhibition constant, which, in the context of electrophysiological measures, defines the amiloride concentration that produces ½ maximal inhibition of chorda tympani nerve responsiveness. In cases in which the amiloride dose–response function is derived for a single NaCl concentration, a direct comparison between the c parameter and the inhibition constant is meaningful. If nothing else, the logistic function provides a quantitatively comprehensive and accurate description of the nature of the effect of amiloride on discrimination performance.

RESULTS

The rats used in this experiment were well trained in the salt discrimination task as a result of their extensive experience in a previous experiment (St. John et al., in press). This is evident by their performance during control sessions (Fig. 1, *open circles*). There were no statistically significant differences in performance across the control sessions immediately preceding the amiloride sessions on any measure. Therefore, the performance on these control sessions was collapsed into a single mean for each animal,

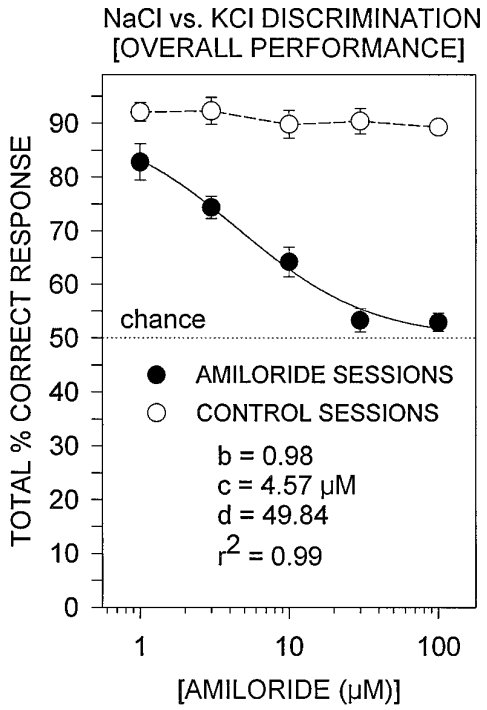

Figure 1. Mean ± SE total percent correct responses as a function of amiloride concentration. Data are collapsed across salts and concentration. *Open circles*, Control session without amiloride immediately preceding the amiloride sessions. *Closed circles*, Amiloride session at the indicated concentration. The curve representing performance during the amiloride sessions was based on a least squares regression using the equation shown in Materials and Methods. *b*, Slope; *c*, midpoint amiloride concentration between maximum and minimum asymptotes; *d*, asymptotic minimum performance; r^2, percentage of the variance accounted for by the fit.

and this value was used in all of the subsequent ANOVAs and served as the asymptotic maximum constant (*a*) in the logistic curve fits.

Analysis of overall performance

Amiloride adulteration disrupted overall performance in a dose-dependent manner [$F_{(5,20)} = 44.2$, $p < 0.0001$; Fig. 1]. At the 30 and 100 μM concentrations, amiloride reduced the total percent correct to essentially 50%. As the concentration of amiloride decreased, performance progressively improved. A logistic function (see equation) for which *a* was set to 90.8% (mean of control sessions) was fit to the mean data. The value of *c* (midpoint concentration between asymptotes) was 4.57 μM. The slope (*b*) was 0.98 and the minimum asymptotic performance (*d*) was 49.8%. The mean curve corresponded remarkably well to the curves fit for individual animals (Fig. 2). The averages of the parameters for individual rats were: $c = 5.40$ μM, $b = 1.00$, and $d = 48.9\%$.

Because the amiloride concentrations were presented in roughly descending order (except for 10 μM), a second session was conducted with the 100 μM amiloride concentration at the end of the series to rule out the possibility of carry-over effects accounting for the concentration–response relationship. There were no significant differences (matched *t* tests) observed in the overall performance observed between the two sessions (Table 1). Therefore, discrimination performance seemed to be related to amiloride concentration and not some other factor associated with repeated testing.

Table 1. Percent correct, 100 μM amiloride replication[a]

	Test 1	Test 2	Mean	*t* test[b]
Overall[c]	52.9 (±1.7)	50.2 (±4.0)	51.6 (±2.4)	$p = 0.52$
NaCl[d]	24.6 (±7.5)	32.8 (±11.0)	28.7 (±6.6)	$p = 0.58$
KCl[d]	81.8 (±6.3)	67.4 (±5.6)	74.6 (±4.2)	$p = 0.16$

[a]Mean (± SE).
[b]Matched *t* test results for test 1 vs test 2 comparison.
[c]Based on all trials collapsed across salt and concentration.
[d]Collapsed across concentration.

Analysis by salt

Amiloride clearly had its greatest effect on responses to NaCl and exerted only a minor influence on KCl performance (Fig. 3). In fact, separate ANOVAs indicated that there was a significant effect of amiloride concentration on the percentage of correct responses to NaCl [$F_{(5,20)} = 34.3$, $p < 0.0001$] but not to KCl [$F_{(5,20)} = 1.26$, $p = 0.32$]. The percentages of correct responses to NaCl at the high amiloride concentrations were significantly lower than 50% [30 μM: $t_{(4)} = 3.52$, $p < 0.05$; 100 μM: $t_{(4)} = 3.39$, $p < 0.05$]. A logistic function (see equation), for which *a* was set to 92.4% (mean of control sessions), was fit to the mean data for NaCl. The value of *c* (midpoint amiloride concentration between asymptotes) was 7.41 μM. The slope (*b*) was 0.75 and the asymptotic minimum performance (*d*) was 15.02%. The fact that the overall performance collapsed across all trials (i.e., Figs. 1, 2) at the 100 μM amiloride concentration approximated 50% demonstrates that each animal pressed the KCl-associated lever on NaCl trials with the same probability that it did on KCl trials. In other words, if an animal had a very high (well above 50%) "hit rate" (i.e., percentage correct) to KCl, then it had a proportionally low (well below 50%) hit rate on NaCl trials during the 100 μM amiloride session. Likewise, under the same conditions, if an animal had only a moderately high hit rate to KCl then it had only a moderately low hit rate to NaCl. Collectively, the results of the analyses of trials collapsed across concentration suggest that the rats treated NaCl mixed with high concentrations of amiloride as if it were essentially identical to KCl.

A final session was conducted in which the 3 KCl concentrations were pitted against water. The correct response for the water stimulus was the lever previously associated with NaCl. The intent of this manipulation was to confirm that the high concentrations of amiloride were doing more than simply rendering the NaCl stimuli tasteless. The mean percentage of correct responses to NaCl adulterated with 100 μM (mean across both sessions) was significantly lower (matched *t* test, $p < 0.05$) than the percentage observed for water on the final session, which, in turn, was essentially at chance (Fig. 4). This finding provides evidence that amiloride-adulterated NaCl was not treated by the rat as if it were water.

Analysis by salt concentration

A two-way ANOVA (NaCl concentration × amiloride concentration) revealed significant main effects for NaCl concentration [$F(2,8) = 12.83$, $p = 0.0032$] and amiloride concentration [$F_{(5,20)} = 33.9$, $p < 0.0001$] and a significant interaction [$F_{(10,40)} = 2.86$, $p = 0.0089$; Fig. 5]. A test for simple effects (with unpooled error terms) indicated that performance did not vary significantly across NaCl concentration under control conditions [$F_{(2,8)} = 2.24$, $p = 0.17$], but did so at each amiloride concentration (all *p* values < 0.05). At each NaCl concentration amiloride affected the percent

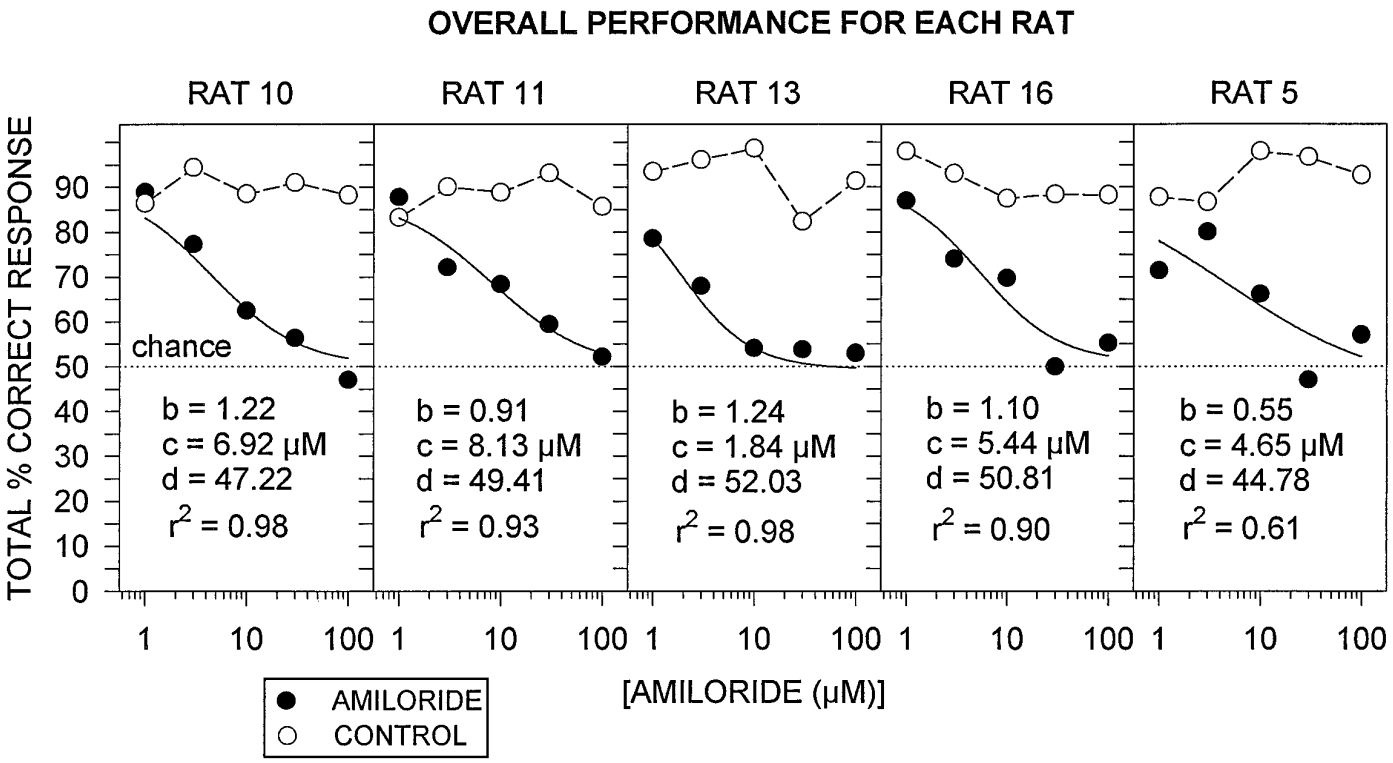

Figure 2. Total percentage of correct responses as a function of amiloride concentration shown for each rat. See legend to Figure 1 for more details.

correct in a dose-dependent manner (all p values < 0.0001). Logistic functions were fit to the data for the 0.05 M and the 0.2 M NaCl concentrations; a curve could not be reliably fit to the 0.1 M NaCl data presumably because of the outlying data point at the 10 μM amiloride concentration. Nevertheless, the c parameter seemed to vary with concentration (0.05 M NaCl: $c = 2.32$ μM; 0.2 M NaCl: $c = 8.55$ μM).

A two-way ANOVA (KCl concentration × amiloride concentration) revealed a significant main effect only for KCl concentration [$F_{(2,8)} = 5.14, p = 0.037$; Fig. 6]. The main effect for amiloride concentration and the interaction were both not significant ($p > 0.16$). Judging from the data, we were somewhat surprised by the lack of a significant interaction. Amiloride seemed to have some effect on responses to 0.2 M KCl. We suspected that statistical

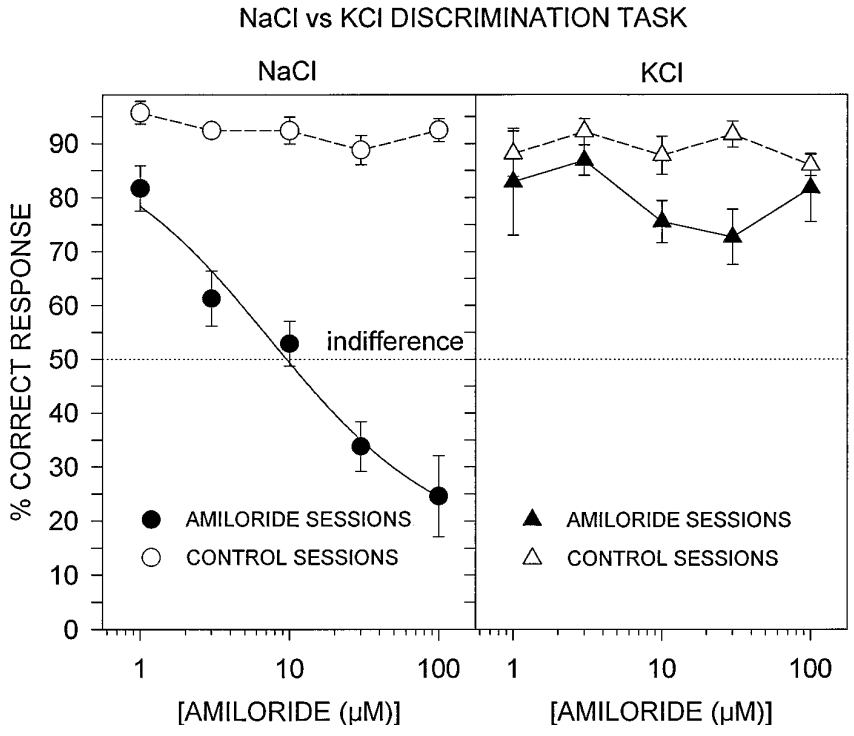

Figure 3. Mean ± SE percent correct responses on trials with NaCl (*left*) and KCl (*right*) collapsed across concentration. Notice that responses on NaCl trials when the amiloride concentration was ≥30 μM was below 50% correct. This means that the rats were pressing the KCl-associated lever more than the NaCl-associated lever.

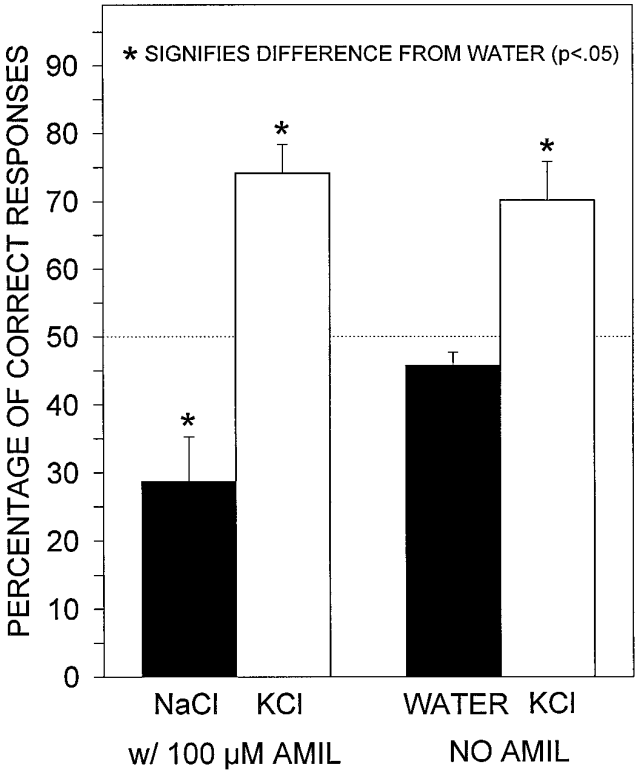

Figure 4. Mean ± SE percent correct responses on trials with NaCl and KCl when adulterated with 100 μM amiloride (*w/ 100 μM AMIL*). This is compared to performance when water was pitted against KCl on the final session (*NO AMIL*). Matched *t* tests were used in statistical comparisons.

confirmation of this difference may have been obscured by the overall variablity in the two-way ANOVA. Accordingly, we conducted a separate one-way ANOVA testing the effect of amiloride concentration on responses to 0.2 M KCl. This test did reveal a significant effect of amiloride [$F_{(5,20)} = 2.84$, $p = 0.043$] on responses to this concentration. Similar tests on the other KCl concentrations were not significant ($p > 0.31$ for both). Oddly, paired comparisons between the control condition and each amiloride concentration indicated that responses to 0.2 M KCl were significantly disrupted at the 10 μM ($p = 0.0037$) and 30 μM ($p = 0.0196$) concentrations, but not at the 100 μM concentration ($p = 0.29$), suggesting that the drug had a nonmonotonic effect with respect to this taste stimulus.

DISCUSSION

Amiloride exerted a potent influence on salt discrimination performance in a monotonic dose-dependent manner. Overall, performance was reduced to 50% at the high amiloride doses and then progressively improved as the concentration of the drug was lowered. A first-order logistic function accounted for the variance almost perfectly. The amiloride concentration producing one-half asymptotic performance corresponded well with the inhibition constant (dose of the drug that produces half-maximal inhibition) in electrophysiological examinations of peripheral taste receptor cells or fibers in rats and hamsters (see Brand et al., 1985; DeSimone and Ferrell, 1985; Hettinger and Frank, 1990; Avenet and Lindemann, 1991; Gilbertson et al., 1992). At midrange NaCl concentrations, the amiloride inhibition constant is electrophysiologically estimated to be ≤6 μM (e.g., DeSimone and Ferrell, 1985; Avenet and Lindemann, 1991). As a result of the varied concentrations of salt used, a direct comparison between the electrophysiological results and the overall performance curve is not entirely appropriate. Nevertheless, the *c* values from the curve fits of performance during single concentrations of NaCl support the correspondence. The effect of amiloride was clearly inversely related to the concentration of NaCl as has been shown in electrophysiological examinations of the degree of amiloride-induced suppression of chorda tympani nerve responses in both rats and hamsters, suggesting competitive inhibition. It should be noted that there is strong evidence that, contrary to the case in humans (Smith and Ossebaard, 1995), amiloride is essentially tasteless to the rat (Bernstein and Hennessy, 1987; Hill et al., 1990; Markison and Spector, 1996). On the whole, it seems that salt discrimination behavior in this task can be explained simply and quantitatively by peripheral receptor processes.

With regard to stimulus specificity, amiloride seemed to exert only a weak effect, at best, on responses to KCl. In fact, the only statistical evidence of such an effect was seen with the 0.2 M concentration. The fact that performance during those stimulus presentations was nonmonotonically related to amiloride concentration calls into question whether the effect involved KCl transduction processes. As an alternative explanation, the performance decrement observed to KCl may have been a result of a generalized extinction effect. In other words, the failure to reinforce responses to amiloride-adulterated NaCl when the rats pressed the KCl-associated lever may have been responsible for a slight and relatively unsystematic decay in performance during trials with the high concentration of KCl.

Although amiloride did not have a robust effect on KCl responses in the present study, Contreras and Studley (1994) found that 100 μM amiloride significantly increased unconditioned licking of KCl at midrange concentrations (including 0.2 M) during brief access trials in mildly water-deprived rats. In the evaluation of this disparity, it is important to consider what was being measured. Our study focused on discrimination. Their study focused on taste-related hedonics. Perhaps amiloride affects the intensity and/or motivational properties of KCl without affecting its qualitative perceptual characteristics. In this regard, it would be interpretively useful to behaviorally assess the effects of amiloride on KCl detection thresholds. Although the responsiveness of N-units displays a remarkable degree of specificity for sodium (and lithium) salts, there is some electrophysiological evidence suggesting that amiloride blocks the relatively weak responses of these units to high concentrations of KCl in the rat chorda tympani nerve (Ninomiya and Funakoshi, 1988; see also Herness, 1987). It follows then that the perceived quality of amiloride-adulterated KCl would become less similar to and more discriminable from NaCl. Accordingly, amiloride would not be expected to compromise KCl responses in the present discrimination paradigm, but given that the peripheral signal representing KCl was affected, unconditioned licking behavior to this salt could perhaps be altered, as was seen in the Contreras and Studley (1994) experiment.

At high amiloride concentrations, NaCl seemed to taste more similar to KCl. This finding extends the results of Hill et al. (1990), who reported that when 0.5 M NaCl served as a conditioned stimulus in a learned taste aversion paradigm, rats treated with 100 μM amiloride subsequently generalized the aversion to non-sodium salts including KCl. Generalization paradigms provide a way of assessing the perceived similarity of stimuli, whereas discrimination tasks provide a way of assessing the perceived difference between stimuli. The distinction between these two behav-

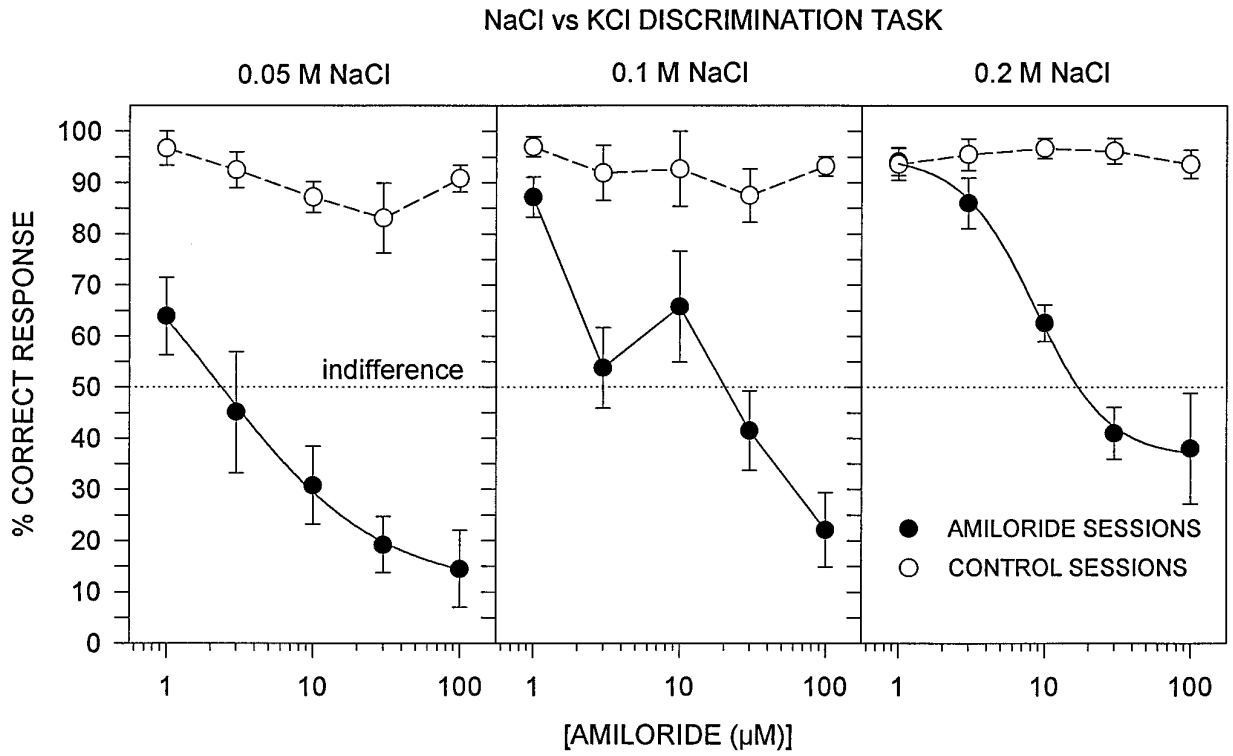

Figure 5. Mean ± SE percent correct responses on trials with 0.05 M (*left*), 0.1 M (*middle*), and 0.2 M (*right*) NaCl. *Open circles*, Control session without amiloride immediately preceding the amiloride sessions. *Closed circles*, Amiloride session at the indicated concentration. A logistic function (see equation in Materials and Methods) was fit (least squares) to the data from amiloride sessions for 0.05 and 0.2 M. The asymptote was set to the mean for the control sessions shown (0.05 M: $b = 0.80$, $c = 2.32$ μM, $d = 10.78\%$; 0.2 M: $b = 1.73$, $c = 8.55$ μM, $d = 36.35\%$). A curve could not be reliably fit to the data for the 0.1 M concentration.

ioral procedures is perhaps subtle, but real. It is possible that rats can treat two stimuli as being somewhat similar, yet be able to clearly discriminate between them. Such seems to be the case with the two sugars, sucrose and maltose (Nissenbaum and Sclafani, 1987; Spector and Grill, 1988; Spector et al., in press). Nevertheless, the results from the generalization and discrimination tasks involving the effects of amiloride on NaCl perception in rats apparently converge on a similar conclusion.

There is strong evidence that amiloride selectively inhibits the response of N-units in both rats and hamsters (Ninomiya and Funakoshi, 1988; Hettinger and Frank, 1990). These units are narrowly tuned to respond to sodium (and lithium) salts. More broadly tuned afferents in the peripheral gustatory system are also stimulated by NaCl, but these units also respond to nonsodium salts and acids (and sometimes quinine) and are not affected by amiloride. The data presented here offer insight into the peripheral coding process for taste. It is now quite clear that narrowly tuned units play an important role in taste discrimination. Although this is expected based on parsimony, to our knowledge it has never been explicitly demonstrated. Spector and Grill (1992) hypothesized that the effectiveness of chorda tympani transection to severely disrupt a NaCl versus KCl discrimination was based on the removal of N-units from the signal, but such neurotomy removes more than just these narrowly tuned afferents from the total peripheral taste input. The behavioral consequences of the selective elimination of a very specific component of the taste signal through pharmacological blockade, however, provides convincing evidence of the importance of narrowly tuned afferents (N-units) in the discrimination between a sodium and a nonsodium salt and suggests that this may be a general coding principle in the gustatory system. Such a hypothesis awaits further experimental scrutiny.

Using the same task as presented here, St. John et al. (in press) demonstrated that transection of the chorda tympani only partially disrupted overall salt discrimination performance; that is, such rats responded significantly above chance levels, and responding to both NaCl and KCl was affected. In contrast, high concentrations of amiloride essentially reduced overall responding to chance levels in the present experiment, and responding to NaCl was much more severly affected than responding to KCl. Taken together, these findings imply that there are amiloride-sensitive receptors in taste fields other than the anterior tongue. Given that the glossopharyngeal nerve seems to be insensitive to the inhibitory effects of amiloride (Formaker and Hill, 1991), and transection of that nerve does not impair salt discrimination (Spector and Grill, 1992), we hypothesize that the greater superficial petrosal nerve may show amiloride-induced suppression of NaCl responsiveness. This nerve, which innervates taste buds on the palate, responds well to NaCl (Nejad, 1986). Whether there are N-units in this nerve remains unknown, because a single-fiber analysis has yet to be conducted. Likewise, the amiloride sensitivity of the greater superficial petrosal nerve remains untested. Another possibility is the superior laryngeal nerve, which innervates the gustatory receptors in and around the epiglottis accounting for about 5–10% of the total taste buds (Miller, 1977; Travers and Nicklas, 1990). On the basis of their anatomical location and electrophysiological response properties, however, these taste buds have been suspected of playing a large role in protection of

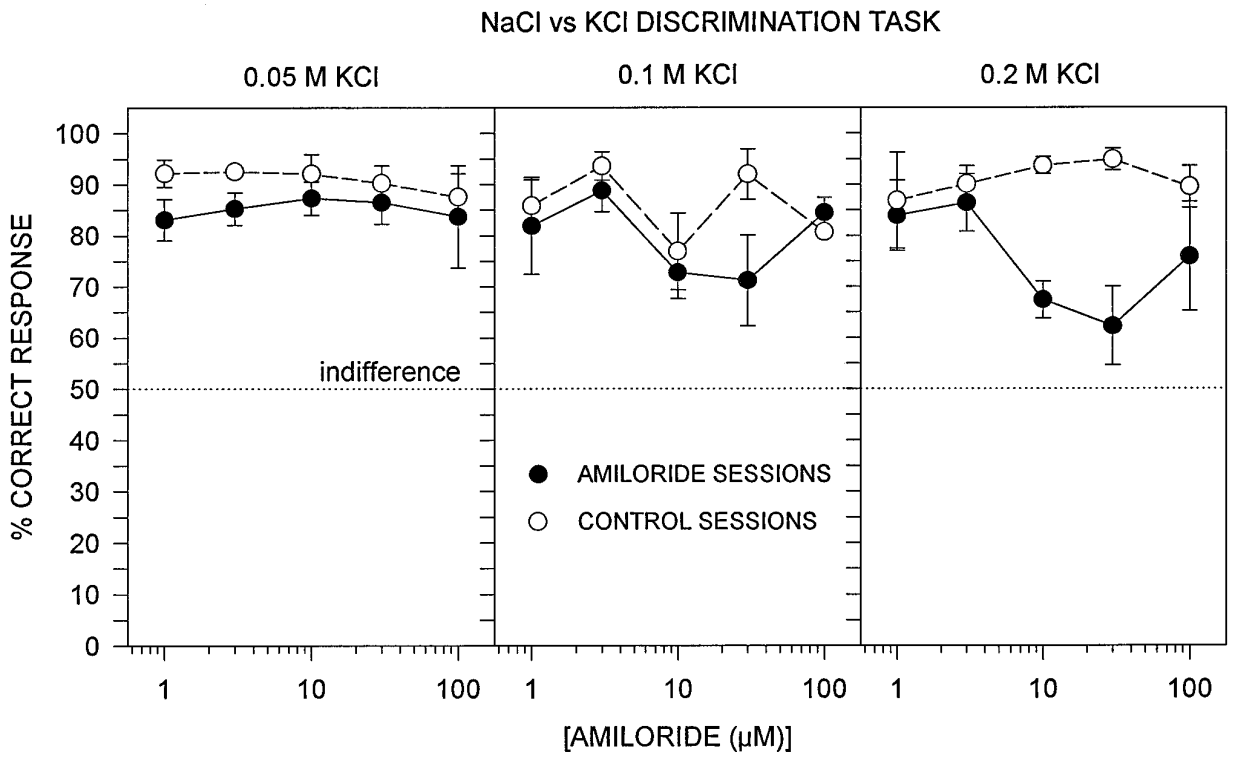

Figure 6. Mean ± SE percent correct responses on trials with 0.05 M (*left*), 0.1 M (*middle*), and 0.2 M (*right*) KCl. *Open circles*, Control session without amiloride immediately preceding the amiloride sessions. *Closed circles*, Amiloride session at the indicated concentration.

the airways rather than in perceptual processes per se (see Smith and Hanamori, 1991).

Finally, this behavioral assay shows promise in the evaluation of the functional consequences of manipulations suspected of influencing the number or properties of amiloride-sensitive taste receptors. For example, if a given manipulation markedly lowers the total number of amiloride-sensitive receptors, the asymptotic performance at weak amiloride doses may be lowered without a shift in the *c* parameter. Alternatively, it is possible that only a portion of the total number of amiloride-sensitive receptors is required for competent performance in this discrimination task. If true, changes in the total number of receptors may be reflected in shifts in the value of the *c* parameter. Given that there is indirect evidence supporting the existence of palatal amiloride-sensitive sodium receptors as discussed above, it would be instructive to test rats with bilateral chorda tympani transections using this behavioral assay to determine whether the amiloride dose–response curve shifts.

REFERENCES

Avenet P, Lindemann B (1991) Noninvasive recording of receptor cell action potentials and sustained currents from single taste buds maintained in the tongue: the response to mucosal NaCl and amiloride. J Membr Biol 124:33–41.

Bernstein IL, Hennessy CJ (1987) Amiloride-sensitive sodium channels and expression of sodium appetite in rats. Am J Physiol 253:R371–R374.

Boudreau JC, Hoang NK, Oravec J, Do LT (1983) Rat neurophysiological taste responses to salt solutions. Chem Senses 8:131–150.

Brand JG, Teeter JH, Silver WL (1985) Inhibition by amiloride of chorda tympani responses evoked by monovalent salts. Brain Res 334:207–214.

Contreras RJ, Studley JL (1994) Amiloride alters lick rate responses to NaCl and KCl in rats. Chem Senses 19:219–229.

DeSimone JA, Ferrell F (1985) Analysis of amiloride inhibition of chorda tymapni taste response of rat to NaCl. Am J Physiol 249:R52–R61.

Formaker BK, Hill DL (1991) Lack of amiloride sensitivity in SHR and WKY glossopharyngeal taste responses to NaCl. Physiol Behav 50:765–769.

Frank ME, Contreras RJ, Hettinger TP (1983) Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat. J Neurophysiol 50:941–960.

Gilbertson TA, Avenet P, Kinnamon SC, Roper SD (1992) Proton currents through amiloride-sensitive Na channels in hamster taste cells: role in acid transduction. J Gen Physiol 100:803–824.

Heck GI, Mierson S, DeSimone JA (1984) Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. Science 223:403–405.

Hellekant G, Dubois GE, Roberts TW, van der Wel H (1988) On the gustatory effect of amiloride in the monkey (*Macaca mulatta*). Chem Senses 13:89–93.

Herness MS (1987) Effect of amiloride on bulk flow and iontophoretic taste stimuli. J Comp Physiol [A] 160:281–288.

Hettinger TP, Frank ME (1990) Specificity of amiloride inhibition of hamster taste responses. Brain Res 513:24–34.

Hill DL, Formaker BK, White KS (1990) Perceptual characteristics of the amiloride-suppressed sodium chloride taste response in the rat. Behav Neurosci 104:734–741.

Markison S, Spector AC (1996) Amiloride is an ineffective conditioned stimulus in taste aversion learning. Chem Senses 20:559–563.

McCutcheon NB (1991) Sodium deficient rats are unmotivated by sodium chloride solutions mixed with the sodium channel blocker amiloride. Behav Neurosci 105:764–766.

Miller IJ (1977) Gustatory receptors of the palate. In: Food intake and chemical senses (Katsuki Y, Sato M, Takagi S, Oomura T, eds), pp 173–186. Tokyo: University of Tokyo.

Nakamura M, Kurihara K (1990) Non-specific inhibition by amiloride of canine chorda tympani nerve responses to various salts: do Na^+-specific channels exist in canine taste receptor membranes? Brain Res 524:42–48.

Nejad MS (1986) The neural activities of the greater superficial petrosal nerve of the rat in response to chemical stimulation of the palate. Chem Senses 11:283–293.

Ninomiya Y, Funakoshi M (1988) Amiloride inhibition of responses of rat single chorda tympani fibers to chemical and electrical tongue stimulations. Brain Res 451:319–325.

Nissenbaum JW, Sclafani A (1987) Qualitative differences in polysaccharide and sugar tastes in the rat: a two-carbohydrate taste model. Neurosci Biobehav Rev 11:187–196.

Smith DV, Hanamori T (1991) Organization of gustatory sensitivities in hamster superior laryngeal nerve fibers. J Neurophysiol 65:1098–1113.

Smith DV, Ossebaard CA (1995) Amiloride suppression of the taste intensity of sodium chloride: evidence from direct magnitude scaling. Physiol Behav 57:773–777.

Spector AC, Grill HJ (1988) Differences in the taste quality of maltose and sucrose in rats: issues involving the generalization of conditioned taste aversions. Chem Senses 13:95–113.

Spector AC, Grill HJ (1992) Salt taste discrimination after bilateral section of the chorda tympani or glossopharyngeal nerves. Am J Physiol 263:R169–R176.

Spector AC, Andrews-Labenski J, Letterio FC (1990) A new gustometer for psychophysical taste testing in the rat. Physiol Behav 47:795–803.

Spector AC, Markison S, St. John SJ, Garcea M (1996) Behavioral discrimination between sucrose and maltose by rats depends on the gustatory input of the seventh cranial nerve. Am J Physiol, in press.

St. John SJ, Markison S, Guagliardo NA, Hackenberg TD, Spector AC (1996) Chorda tympani nerve transection and selective desalivation differentially disrupt two-lever salt descrimination performance in rats. Behav Neurosci, in press.

Travers SP, Nicklas K (1990) Taste bud distribution in the rat pharynx and larynx. Anat Rec 227:373–379.