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Amiloride Does not Alter NaCl Avoidance in Fischer-344 Rats

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Abstract

Fischer-344 (F-344) rats differ from other common rat strains in that they fail to show any prefererence for NaCl at any concentration in two-bottle preference tests. Because 100 μM amiloride partially blocks the NaCl-evoked chorda tympani (CT) response in electrophysiological studies, we tested NaCl preference (0.068–0.273 M) in F-344 rats with and without 100 μM amiloride solution as the solvent. A third group was tested with unadulterated NaCl solutions following CT transection. Amiloride had no significant effect on the NaCl preference–aversion function, whereas CT transection significantly reduced NaCl avoidance. These results suggest that the amiloride-sensitive component of the NaCl response is not necessary for F-344 rats to display avoidance of NaCl, but the entire CT input is.

Introduction

Sprague–Dawley, Wistar and some other rat strains prefer low- and mid-range concentrations of NaCl to water in two-bottle preference tests (e.g. Pfaffmann, 1952; Richter, 1956; Midkiff *et al.*, 1985; Fregly and Rowland, 1992). The Fisher 344 (F-344) strain differs in that these rats avoid all concen- trations of NaCl, even those maximally preferred by other rat strains (Midkiff *et al.*, 1985). This avoidance of NaCl was reversed to a preference following transection of the chorda tympani nerve (CT), which innervates taste buds on the anterior 2/3 of the tongue (Sollars *et al.*, 1991; Sollars and Bernstein, 1994). Transection of the glossopharyngeal nerve, which innervates about four times as many taste buds as the CT (Miller, 1977), did not alter NaCl avoidance in F-344 rats, indicating that the change in preference behavior following CT transection was not a general consequence of taste bud denervation in this strain (Sollars and Bernstein, 1994). Together, these findings prompted the hypothesis that the NaCl response of the CT nerve differs in F-344 rats compared with NaCl-preferring rat strains.

Electrophysiological studies have revealed that the CT response to NaCl in several species of rodents, including the rat, has at least two components: an amiloride-sensitive and an amiloride-insensitive component (Heck *et al.*, 1984; Brand *et al.*, 1985; DeSimone and Ferrell, 1985; Formaker and Hill, 1988; Ninomiya and Funakoshi, 1988; Hettinger and Frank, 1990). Amiloride is an epithelial sodium channel blocker (Benos, 1982) that when orally applied partially suppresses the CT response to NaCl (e.g. Heck *et al.*, 1984; Brand *et al.*, 1985; DeSimone and Ferrell, 1985; Formaker and Hill, 1988). The residual response to NaCl in amiloride-treated rats is presumably mediated by a transduction mechanism(s) distinct from the amiloride-sensitive sodium channel (Elliott and Simon, 1990; Ye *et al.*, 1993; Stewart *et al.*, 1997). Either or both transduction pathways could potentially be involved in the uncharacteristic avoidance of low and mid-range NaCl concentrations demonstrated by F-344 rats.

Bernstein *et al.* (1991) reported differences in the CT response of Wistar and F-344 rats. When the integrated response to NaCl (relative to a 0.5 M NH_4Cl standard) was compared between strains, the F-344 rats were found to have a significantly larger response to NaCl across a wide concentration range. In addition, application of a wide range of amiloride concentrations (1–500 μM) caused a greater percent reduction in the NaCl response in F-344 rats than in Wistar rats. Amiloride does not affect sodium responses in the glossopharyngeal nerve (Formaker and Hill, 1991). Hence, Bernstein *et al.* speculated that the greater amiloride-sensitive NaCl component of the CT response might underlie the greater NaCl avoidance behavior of the F-344 rats compared with other rat strains.

This hypothesis was recently buttressed by the observation that the postnatal development of amiloride sensitivity in the CT of the F-344 rat temporally corresponds with the appearance of the NaCl aversion in this strain (Schafe and Bernstein, 1997). We wanted to test this hypothesis more directly by comparing the NaCl preference behavior of intact and CT-transected F-344 rats with rats tested with the NaCl solutions mixed with a high concentration (100 μM) of amiloride. If the amiloride-sensitive component of the CT response were critical in maintaining NaCl avoidance, then sufficient adulteration of the NaCl solution with amiloride should cause the reversal

to a preference seen with CT transection, or at least cause a reduction in avoidance.

Materials and methods

Subjects

Twenty-four male F-344 rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN), ~60 days old, weighing 248–272 g at the start of the experiment, were used. Rats were individually housed in standard stainless steel cages and were given *ad libitum* access to tap water and Purina 5001 laboratory chow (Ralston-Purina, St. Louis, MO), except where otherwise noted. Food was given in pellet form initially, but was then given in powder form starting 4 days before the onset of preference testing. Light, temperature and humidity were automatically controlled in the colony room. All experimental manipulations were performed during the light phase.

[Due to a malfunction in the automatic timer, the animal room was in constant light for part of the testing phase. The problem was discovered and immediately fixed prior to the first test with 0.273 M NaCl. Although we cannot rule out that a change in the light cycle might have affected solution preference, because control rats were subject to these same environmental conditions, it is unlikely that our ability to detect differences among groups was compromised.]

Surgery

The rats were anesthetized with an i.m. injection of Ketaset (125 mg/kg) and Xylazine (5 mg/kg). Eight rats had the CT sectioned bilaterally (CTX group). The external auditory meatus was retracted with five blunted and curved hypodermic needles, and the tympanic membrane and CT were cauterized with a hand-held cautery unit (Roboz, Rockville, MD). We have found that cauterizing both the rim of the tympanic membrane and the CT substantially reduces the likelihood of nerve regeneration through the middle ear (unpublished observations). One rat in the CTX group exhibited a precipitous loss of body weight and was euthanized, leaving a sample size of $n = 7$. Rats from the other two groups (CON, control and AMIL, amiloride; $n = 8$ for both groups) had the tympanic membrane punctured but the CT left undisturbed. Following surgery the rats were placed back in their original cages where they remained for 4 days, and were then transferred to clean metabolism cages so urine output could be measured. The rats were given 16–17 days to recover, prior to the preference testing regimen.

Testing

Urine was collected over a 21 h period once the rats were transferred to the metabolism cages and continuing throughout the majority of the experiment. Urine volume, food intake and urinary sodium excretion were measured. Despite our precautionary measures, however, we found food spillage and the consequential contamination of urine samples to be a persistent problem in some rats, particularly in the CTX group. Therefore, the data regarding food intake and urinary sodium levels are not included.

Table 1 Preference test schedule

Days	Stimuli
1–2	Water versus Water
3–4	Water versus 0.034 M NaCl[a]
5–6	Water versus Water
7–8	Water versus 0.068 M NaCl[a]
9–10	Water versus Water
11–12	Water versus 0.137 M NaCl[a]
13–14	Water versus Water
15–16	Water versus 0.205 M NaCl[a]
17–18	Water versus Water
19–20	Water versus 0.273 M NaCl[a]
21–22	Water versus Water
23–24	Water versus 0.068 M NaCl[a]
25–26	Water versus 0.06 M Sucrose
27–28	Water versus 0.205 M NaCl[a]

[a]Both stimuli were made up in 100 μM amiloride for the rats in the AMIL group.

Preference testing occurred over 24 days. The rats received an ascending series of NaCl concentrations, with selected concentrations repeated at the end of the series to test for order effects (see Table 1). Rats in the AMIL group had 100 μM amiloride hydrochloride in the 'water' bottle and also as the solvent for the NaCl solution. Concentrations (0.034, 0.068, 0.137, 0.205 and 0.271 M) were chosen to match those used by Sollars and Bernstein (1994). A preference test for 0.06 M sucrose was also conducted to determine if CT transection had nonspecific effects on gustatory preferences (see Table 1). Solutions were mixed fresh daily, using reagent grade chemicals (taste stimuli: Fisher Scientific, Orlando, FL; amiloride hydrochloride: Sigma Chemical, St. Louis, MO). In addition, all rats were always given a 2 day water-only test prior to a 2 day NaCl test, in which both bottles contained pure distilled water. The primary purpose of these water-only days was to minimize any potential cumulative effects of repeated amiloride exposure.

Stimuli were presented in two Pyrex graduated cylinders (~100 ml) which were wrapped in aluminum foil to prevent the potential degradation of amiloride by light. Solutions were available for 21 h, and were removed for 3 h while the bottles were weighed, cleaned and refilled, and other measures (previously described) were taken. The bottles were then returned with their side positions reversed to control for position preferences. Preference (amount of NaCl solution consumed divided by total fluid consumed and multiplied by 100) and intake (amount of NaCl solution consumed) for each day of the 2 day test was averaged to

obtain overall preference or intake for the 2 day test (i.e. results from each day were weighted equally).

Data analysis

The preference, intake and urine volume data were analyzed using analysis of variance (ANOVA) procedures. Two-way ANOVAs were used to test for main effects of NaCl concentration, group treatment and their interaction. Simple effects were tested using one-way ANOVAs at specified levels and further group differences within this analysis were tested with Tukey's honestly significant difference (HSD) test. In certain cases, supplemental paired comparisons were conducted with the nonparametric Mann–Whitney *U*-test. This was done to buttress the findings of the parametric analyses by providing an additional assessment of group differences using a procedure that has less assumptions concerning the variance of the distributions in question. The conventional $P \leq 0.05$ level of statistical confidence was applied.

Histology

Immediately following the final preference test, the rats were deeply anesthetized with an i.p. injection of sodium pentobarbital and perfused with isotonic saline followed by 10% buffered formalin. The tongues were extracted and stored in 10% buffered formalin for at least 24 h at room temperature prior to histological analysis. The anterior tongue was soaked in distilled water for 30 min, dipped briefly in 0.5% methylene blue and rinsed with water. The lingual epithelium was then removed, placed between two glass slides and analyzed by an observer unaware of the surgical condition of the subject. The numbers of taste pores and fungiform papillae were quantified.

Results

Histology

Rats in the CTX group were found to have a significantly lower percentage of fungiform papillae containing a taste pore (4.37 ± 1.5%) than rats in the CON (95.16 ± 1.2%) or AMIL (86.41 ± 4.1%) groups [$F(2,20) = 35.5$, $P < 0.00005$], and no individual subject in the CTX group had >11.3% of the fungiform papillae containing a taste pore. The presence of a few taste pores following CTX is consistent with other studies (Whitehead *et al.*, 1987; St. John *et al.*, 1995). Thus, regeneration of the CT did not appear to occur.

Sodium preference

On the second day of testing for 0.034 M NaCl, the left/right bottle positions were mistakenly not reversed. As a result, the data from this concentration were not included in the analysis. Preference for NaCl decreased as a function of concentration (Figure 1). A two-way ANOVA indicated a significant effect of Group [$F(2,20) = 9.7$, $P < 0.001$] and Concentration [$F(3,60) = 31.6$, $P < 0.0005$]. Tests for simple

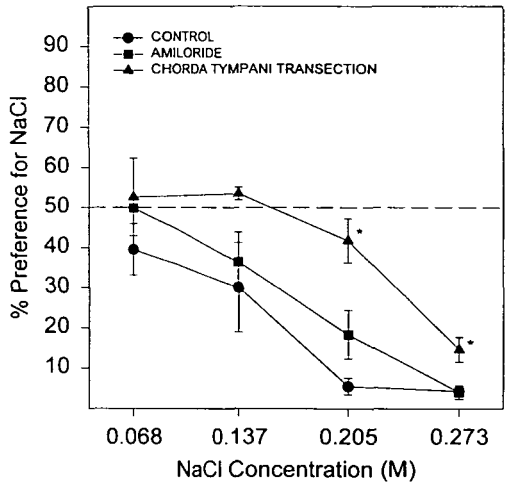

Figure 1 Mean (± SE) NaCl preference for each group as a function of concentration. An asterisk indicates a significant difference (Tukey's HSD test, $P < 0.05$) from controls.

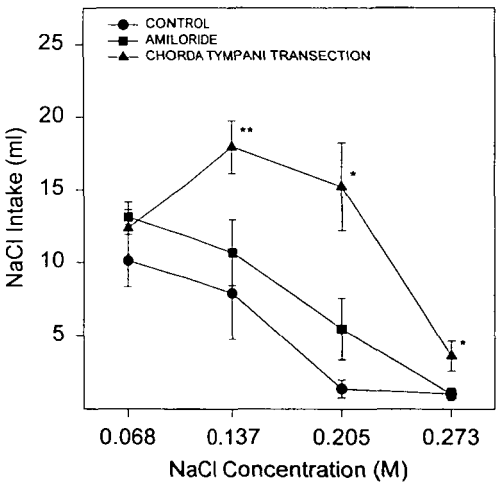

Figure 2 Mean (± SE) NaCl intake for each group as a function of concentration. An asterisk indicates a significant difference (Tukey's HSD test, $P < 0.05$) from controls and amiloride groups; a double asterisk indicates a significant difference from controls only.

effects (one-way ANOVAs) at each concentration revealed a significant difference between groups only at the 0.205 M [$F(2,20) = 14.06$, $P < 0.001$] and the 0.273 M [$F(2,20) = 8.24$, $P = 0.002$] concentrations. At both concentrations the CTX group had significantly higher preference scores compared with the other two groups (Tukey's HSD test, all *P*-values <0.01), which in turn did not differ from one another. Similar paired comparisons conducted with the non-parametric Mann–Whitney *U*-test had identical outcomes.

An analogous analysis of NaCl intake (Figure 2) revealed generally the same pattern of results. There was a significant effect of Group [$F(2,20) = 10.8$, $P < 0.001$] and Concentration [$F(3,60) = 25.1$, $P < 0.001$] as well as a significant

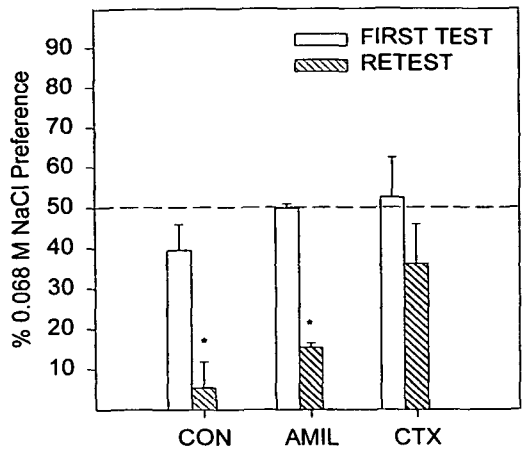

Figure 3 Mean (± SE) preference for 0.068 M NaCl during the initial concentration series (open bars) and a retest after the initial concentration series (hatched bars). An asterisk indicates a significant difference relative to the initial test (paired *t*-test, $P < 0.05$). Group abbreviations: CON = controls, AMIL = amiloride, CTX = chorda tympani transection.

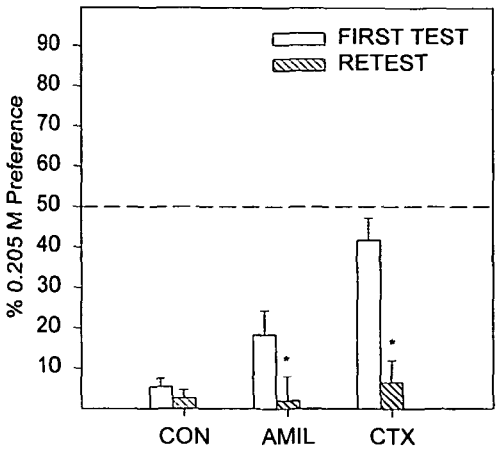

Figure 4 Mean (± SE) preference for 0.205 M NaCl during the initial concentration series (open bars) and a retest after the initial concentration series (hatched bars). An asterisk indicates a significant difference relative to the initial test (paired *t*-test, $P < 0.05$). Group abbreviations: CON = controls, AMIL = amiloride, CTX = chorda tympani transection.

interaction [$F(6,60) = 3.3$, $P < 0.01$]. Tests for simple effects (one-way ANOVAs) conducted for each NaCl concentration indicated a significant difference between groups at 0.137 M [$F(2,20) = 4.1$, $P < 0.04$], 0.205 M [$F(2,20) = 11.6$, $P < 0.001$] and 0.273 M [$F(2,20) = 5.5$, $P < 0.02$]. At the 0.137 M concentration, the CTX group drank significantly more NaCl compared with the CON group (Tukey's HSD, $P < 0.03$), but not the AMIL group ($P > 0.13$). At 0.205 and 0.273 M, the higher NaCl intake of the CTX group reached statistical significance compared with both the CON and AMIL groups (all *P*-values <0.03). In no case did the CON group differ from the AMIL group on this measure. A nonparametric version of these paired comparisons indicated that the higher NaCl intake of the CTX significantly differed from the other two groups at the three highest NaCl concentrations. The NaCl intake of the CON and AMIL groups did not significantly differ as assessed by the Mann–Whitney *U*-test.

In several cases, preferences displayed during a retest of 0.068 and 0.205 M were reduced (Figures 3 and 4). Specifically, the CON and AMIL rats showed a reduced preference for 0.068 M NaCl on the second exposure relative to the first [matched *t*-tests, CON: $T(7) = 6.2$, $P < 0.001$; AMIL: $T(7) = 4.7$, $P < 0.003$]. The CTX group, however, did not reduce their preference for this stimulus [$T(6) = 1.2$, $P > 0.29$]. Preference for 0.205 M was reduced in the AMIL and CTX groups during the retest [matched *t*-tests, AMIL: $T(7) = 2.7$, $P < 0.03$; CTX: $T(6) = 6.3$, $P < 0.001$]. A reduction in preference in the CON group may have been obscured by floor effects (Figure 4).

A one-way ANOVA indicated that the groups did not differ in preference [$F(2,20) = 1.1$, $P > 0.36$] or intake [$F(2,20) = 0.73$, $P > 0.49$] for 0.06 M sucrose (Table 2).

Table 2 Results of the Water versus 0.06 M Sucrose preference test

Group	Sucrose preference	Sucrose intake (ml)
Control	88.1 (± 6.88)	31.1 (± 3.03)
Amiloride	89.2 (± 5.67)	35.6 (± 3.65)
Chorda tympani transected	76.5 (± 7.58)	30.4 (± 3.29)

Urine volume

To determine whether amiloride had a diuretic effect, we compared the volume of urine excreted on the second day of four NaCl preference tests between the CON and AMIL groups. An ANOVA revealed no significant effect of Group [$F(1,14) = 2.28$, $P > 0.15$] or a Group by Days interaction [$F(3,42) = 0.21$, $P > 0.88$]. There was, however, a slight difference in total fluid intake between the groups [$F(1,14) = 6.45$, $P < 0.03$] that was not >3.8 ml greater in the AMIL group than the CON group on any day. Because there was a small difference in total intake, an ANOVA was also conducted on the difference between total fluid intake and urine output; it indicated no effect of Group [$F(1,14) = 0.84$, $P > 0.37$] nor a significant Group by Days interaction [$F(3,42) = 0.02$, $P > 0.99$]. In conclusion, the amiloride concentration used did not have obvious diuretic effects in the present paradigm.

Discussion

Despite the prominent amiloride sensitivity of the CT in F-344 rats, at least compared with Wistars (Bernstein *et al.*, 1991), our results do not lend strong support to the hypothesis that the amiloride-sensitive NaCl transduction pathway is necessary for F-344 rats to avoid NaCl concentrations that are preferred by Wistar and Sprague–Dawley

rats. In our study, 100 μM amiloride was mixed with both the NaCl and water stimuli for rats in the AMIL group. These rats showed no change in the preference–aversion function for NaCl (Figure 1), whereas rats that received CT transection showed a striking reduction in the avoidance of NaCl. Thus, it does not appear that the amiloride-sensitive component of the CT (and perhaps other nerves) is necessary in maintaining this avoidance, but the CT nerve as a whole is clearly necessary. In Wistar rats and in the golden hamster, oral application of amiloride selectively suppresses responsiveness to sodium salts in the narrowly tuned N-units of the CT compared with that seen in the broadly tuned H-units (Ninomiya and Funakoshi, 1988; Hettinger and Frank, 1990). These H-units are broadly responsive to a variety of salts and acids, and quinine (Frank *et al.*, 1983). If the CT in the F-344 rat is organized similarly, then our results would additionally imply that N-units do not make a necessary contribution to the avoidance of NaCl by these rats.

Our interpretation could be challenged if 100 μM amiloride were shown to have an aversive taste quality. Indeed, concentrations as low as 10 μM appear to produce a bitter taste quality in humans (e.g. Smith and Ossebaard, 1995). If amiloride itself were unpalatable to F-344 rats, then this taste could counteract a hypothetical reduction in the aversiveness of NaCl. The possibility that an aversive taste of amiloride could have affected preference was controlled in this study, however, because amiloride was present in *both* the water and the NaCl solutions. In any event, it is interesting to note that amiloride may not have a potent taste in rats as is the case with humans. The fact that Sprague–Dawley rats were unable to form a taste aversion to 100 μM amiloride, despite three conditioning trials with a potent dose of LiCl, provides a strong argument against amiloride having a detectable taste to these animals (Markison and Spector, 1995; see also Hill *et al.*, 1990). Moreover, water-deprived Long–Evans rats do not decrease their licking responses to amiloride-adulterated water (Bernstein and Hennessy, 1987) and hamsters do not avoid amiloride solution relative to water in a long-term two-bottle preference test (Hettinger and Frank, 1990). Until a similar study is conducted with F-344 rats, however, it remains possible that amiloride has a detectable and aversive taste to this rat strain.

Another concern is whether 100 μM amiloride is a high enough concentration to effectively block NaCl channels in F-344 rats. Bernstein *et al.* (1991) found that a 100 μM pretreatment of the tongue caused a 60% suppression in the CT response to 0.5 M NaCl, a concentration of NaCl which is nearly double the highest concentration used in our study. Minear *et al.* (1996) reported a similar degree of suppression in F-344 rats when 0.1 and 0.3 M NaCl solutions were adulterated with 100 μM amiloride. Although it is not clear if larger amiloride doses would further attenuate the CT response to NaCl in F-344 rats, it is clear that a sizable portion of the neural response is eliminated by 100 μM amiloride.

In behavioral studies such as ours, amiloride can potentially affect taste receptors throughout the entire oral cavity. There is some evidence to suggest that the glossopharyngeal nerve does not contain an amiloride-sensitive NaCl response (Formaker and Hill, 1991), but the possible amiloride sensitivity of other nerves innervating rat taste buds (i.e. the greater superficial petrosal nerve or the superior laryngeal nerve) remains equivocal. Harada and colleagues (1997) reported that amiloride did not suppress the GSP response to NaCl, whereas Sollars and Hill (1997) found that it did. In Sprague–Dawley rats, 100 μM amiloride eliminates the behavioral discrimination between NaCl and KCl at low- and mid-range concentrations (Spector *et al.*, 1996), whereas CT transection only attenuates performance on this task (St. John *et al.*, 1997). Based on this latter finding, Spector *et al.* (1996) concluded that amiloride-sensitive receptors might exist in taste receptor cells innervated by other gustatory nerves in addition to the CT. If this is the case in the F-344 rat, then our interpretation is still valid provided that the amiloride-sensitive signals from the CT do not have opposite effects on preference behavior compared with those of other nerves.

We confirmed previous reports that CT transection causes notable effects on the avoidance of NaCl in F-344 rats (Sollars *et al.*, 1991; Sollars and Bernstein, 1994). In those experiments, however, CT transection reversed the avoidance to a preference, whereas in the present study CT transection merely attenuated the avoidance. Furthermore, when rats in the CTX group were retested with 0.205 M NaCl, they demonstrated a pronounced avoidance of the stimulus. Subtle differences in procedure and the fact that the rats were obtained from different breeders could perhaps explain the differences in outcome between this study and the Sollars and co-workers studies (Sollars *et al.*, 1991; Solars and Bernstein, 1994). A reduction in avoidance and reversal to a preference are fundamentally different results, however, and in order to understand how the various NaCl-responsive afferents are contributing to the taste-based behavior of the animal, it will be important to investigate precisely what effect CT transection is having. For example, a reduction in avoidance could be explained rather simply as a decrease in the perceived intensity of the stimulus, whereas a reversal to a preference implies that a change in the qualitative features of the NaCl stimulus may occur after CT transection. The F-344 strain does not normally prefer NaCl at *any* concentration, and that is why a simple decrease in stimulus intensity cannot easily explain a preference reversal.

Transection of the CT does not affect preference for 0.06 M sucrose. This concentration was chosen because it is normally preferred by other rat strains, but is low enough that any subtle effects of nerve transection could be seen.

This result indicates that the effects of CT transection on preference behavior in F-344 rats is somewhat stimulus-specific.

When the 0.068 and 0.205 M NaCl concentrations were retested after the initial ascending series of stimuli was completed, some differences were found, always in the direction of increased avoidance. At 0.068 M NaCl, CON and AMIL rats had a significantly reduced preference relative to the initial test. This effect was not unexpected; rats become sensitized to the presentation of high concentrations of a substance and will subsequently show a heightened avoidance to lower concentrations. Such an effect can produce markedly different preference–avoidance functions depending upon whether an ascending or descending series is used (Rowland and Fregly, 1990; Fregly and Rowland, 1992). It is notable that despite this effect, seen in the other two groups, the CT-transected rats did not significantly differ in the retest at this concentration. When 0.205 M NaCl was tested, the AMIL and CTX groups both showed a reduced preference in the second test, and it is likely that a floor effect prevented the same effect from surfacing in the CON group (Figure 4).

It is clear from the experiments described here that CT transection substantially reduces the avoidance of low- and mid-range NaCl concentrations in F-344 rats. However, 100 μM amiloride did not mimic the effect of CT transection. This suggests that the amiloride-sensitive portion of the CT response to NaCl is not, in and of itself, necessary for F-344 rats to display NaCl avoidance, despite the fact that, electrophysiologically, these rats appear to have a more prominent amiloride-suppressible sodium response relative to the NaCl-preferring Wistar strain (Bernstein *et al.*, 1991). It should be noted that Minear *et al.* (1996) found that amiloride, when mixed in the solutions, was equally effective at suppressing CT responses to NaCl in F-344 rats compared with Sprague–Dawley rats. The fact that rats of the latter strain, unlike F-344 rats, prefer low to midrange concentrations of NaCl further suggests that the preference behavior is not directly related to the amiloride sensitivity of the CT. Furthermore, F-344 rats are unlike hamsters, another species that avoids NaCl at all concentrations, because amiloride has been shown to reduce NaCl avoidance in that species (Hettinger and Frank, 1990).

We close this discussion with a final caveat. The fact that amiloride did not significantly alter NaCl avoidance behavior in a long-term two-bottle test does not mean that it does not affect NaCl perception in this rat strain. The two-bottle preference test is not considered an optimal measure of taste function (see Grill *et al.*, 1987). More to the point, the taste-related aspects of the avoidance response in this kind of test depends on the animal's affective evaluation of the stimulus. It is possible that the discriminative features of the stimulus were altered by the amiloride, but it nonetheless remained 'unpalatable'. Given that stimulus adulteration by amiloride severely compromises performance in an operantly conditioned NaCl versus KCl discrimination task (Spector *et al.*, 1996) and impairs the expression of a sodium appetite (McCutcheon, 1991; Bernstein and Hennessy, 1987) in some other rat strains, there is reason to suggest that similar impairments might be observed in F-344 rats. Such a hypothesis remains to be tested.

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References

Benos, D.J. (1982) *Amiloride: a molecular probe of sodium transport in tissues and cells.* Am. J. Physiol., 242, C131–C145.

Bernstein, I.L. and **Hennessy, C.J.** (1987) *Amiloride-sensitive sodium channels and expression of sodium appetite in rats.* Am. J. Physiol., 253, R371–R374.

Bernstein, I.L., Longley, A. and **Taylor, E.M.** (1991) *Amiloride sensitivity of chorda tympani response to NaCl in Fischer 344 and Wistar rats.* Am. J. Physiol., 261, R329–R333.

Brand, J.G., Teeter, J.H. and **Silver, W.L.** (1985) *Inhibition by amiloride of chorda tympani responses evoked by monovalent salts.* Brain Res., 334, 207–214.

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

Elliott, E.J. and **Simon, S.A.** (1990) *The anion in salt taste: a possible role for paracellular pathways.* Brain Res., 535, 9–17.

Formaker, B.K. and **Hill, D.L.** (1988) *An analysis of residual NaCl taste response after amiloride.* Am. J. Physiol., 255, R1002–R1007.

Formaker, B.K. and **Hill, D.L.** (1991) *Lack of amiloride sensitivity in SHR and WKY glossopharyngeal taste responses to NaCl.* Physiol. Behav., 50, 765–769.

Frank, M.E., Contreras, R.J. and **Hettinger, T.P.** (1983) *Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat.* J. Neurophysiol., 50, 941–960.

Fregly, M.J. and **Rowland, N.E.** (1992) *Comparison of preference thresholds for NaCl solution in rats of the Sprague–Dawley and Long–Evans strains.* Physiol. Behav., 51, 915–918.

Grill, H.J., Spector, A.C., Schwartz, G.J., Kaplan, J.M. and **Flynn, F.W.** (1987) *Evaluating taste effects on ingestive behavior.* In Rowland, N. and Toates, F. (eds), Methods and Techniques to Study Feeding and Drinking Behavior. Elsevier, Amsterdam, pp. 151–188.

Harada, S., Yamamoto, T., Yamaguchi, K. and **Kasahara, Y.** (1997) *Different characteristics of gustatory responses between the greater superficial petrosal and chorda tympani nerves in the rat.* Chem. Senses, 22, 133–140.

Heck, G.I., Mierson, S. and **DeSimone, J.A.** (1984) *Salt taste transduction*

occurs through an amiloride-sensitive sodium transport pathway. Science, 223, 403–405.

Hettinger, T.P. and **Frank, M.E.** (1990) *Specificity of amiloride inhibition of hamster taste responses*. Brain Res., 513, 24–34.

Hill, D.L., Formaker, B.K. and **White, K.S.** (1990) *Perceptual characteristics of the amiloride-suppressed sodium chloride taste response in the rat*. Behav. Neurosci., 104, 734–741.

Markison, S. and **Spector, A.C.** (1995) *Amiloride is an ineffective conditioned stimulus in taste aversion learning*. Chem. Senses, 20, 559–563.

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

Midkiff, E.E., Fitts, D.A., Simpson, J.B. and **Bernstein, I.L.** (1985) *Absence of sodium chloride preference in Fischer-344 rats*. Am. J. Physiol., 249, R438–R442.

Miller, I.J. (1977) *Gustatory receptors of the palate*. In Katsuki, Y., Sato, M., Takagi, S. and Oomura, Y. (eds), Food Intake and Chemical Senses. University of Tokyo Press, Tokyo, pp. 173–186.

Minear, M.M., Hammack, S.E., Lundy, R.F., Jr and **Contreras, R.J.** (1996) *Amiloride inhibits taste nerve responses to NaCl and KCl in Sprague–Dawley and Fischer 344 rats*. Physiol. Behav., 60, 507–516.

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

Pfaffmann, C. (1952) *Taste preference and aversion following lingual deafferentation*. J. Comp. Physiol. Psychol., 45, 393–400.

Richter, C.P. (1956) *Salt appetite of mammals: its dependence on instinct and metabolism*. In L'Instinct dans le comportement des animaux et de l'homme. Masson et cie editeurs, Paris, pp. 577–629.

Rowland, N.E. and **Fregly, M.J.** (1990) *Thirst and sodium appetite in Dahl rats*. Physiol. Behav., 47, 331–335.

Schafe, G.E. and **Bernstein, I.L.** (1997) *Development of the enhanced neural response to NaCl in Fischer 344 rats*. Physiol. Behav., 61, 775–778.

Smith, D.V. and **Ossebaard, C.A.** (1995) *Amiloride suppression of taste intensity of sodium chloride: evidence from direct magnitude scaling*. Physiol. Behav., 57, 773–777.

Sollars, S.I. and **Bernstein, I.L.** (1994) *Gustatory deafferentation and desalivation: effects on NaCl preference of Fischer 344 rats*. Am. J. Physiol., 266, R510–R517.

Sollars, S.I. and **Hill, D.L.** (1997) *Amiloride sensitivity of the greater superficial petrosal nerve in Sprague–Dawley and Fischer 344 rats*. Abstr. Soc. Neurosci., 23, 1037.

Sollars, S.I., Sollars, P.J. and **Bernstein, I.L.** (1991) *Reversal of the sodium chloride aversion of Fischer 344 rats by chorda tympani nerve transection*. Behav. Neurosci., 105, 603–605.

Spector, A.C., Guagliardo, N.A. and **St. John, S.J.** (1996) *Amiloride disrupts NaCl versus KCl discrimination performance: implications for salt taste coding in rats*. J. Neurosci., 16, 8115–8122.

St. John, S.J., Markison, S. and **Spector, A.C.** (1995) *Salt discriminability is related to number of regenerated taste buds after chorda tympani nerve transection in rats*. Am. J. Physiol., 269, R141–R153.

St. John, S.J., Markison, S., Guagliardo, N.A., Hackenberg, T.D. and **Spector, A.C.** (1997) *Chorda tympani nerve transection and selective desalivation differentially disrupt two-lever salt discrimination performance in rats*. Behav. Neurosci., 111, 450–459.

Stewart, R.E., DeSimone, J.A. and **Hill, D.L.** (1997) *New perspectives in gustatory physiology: transduction, development and plasticity*. Am. J. Physiol., 272, C1–C26.

Whitehead, M.C., Frank, M.E., Hettinger, T.P., Hou, L.-T. and **Nah, H.-D.** (1987) *Persistence of taste buds in denervated fungiform papillae*. Brain Res., 405, 192–195.

Ye, Q., Heck, G.L. and **DeSimone, J.A.** (1993) *Voltage dependence of the rat chorda tympani response to Na^+ salts: implications for the functional organization of taste receptor cells*. J. Neurophysiol., 70, 167–178.

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