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Amiloride Inhibits Taste Nerve Responses to NaCl and KCl in Sprague–Dawley and Fischer 344 Rats

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MINEAR, M. M., S. E. HAMMACK, R. F. LUNDY, JR. AND R. J. CONTRERAS. *Amiloride inhibits taste nerve responses to NaCl and KCl in Sprague–Dawley and Fischer 344 rats.* PHYSIOL BEHAV **60**(2) 507–516, 1996.—In a two-bottle test, Sprague–Dawley rats preferentially consume a greater amount of hypotonic and isotonic NaCl solutions relative to water, whereas inbred Fischer 344 (F344) rats fail to prefer NaCl solutions at any concentration relative to water. To determine whether taste contributes to this strain difference, we measured the integrated neural responses of the chorda tympani nerve to a concentration range of NaCl and KCl solutions. The amiloride-sensitive component of the taste nerve response was assessed by adding amiloride during salt stimulation in Experiment 1, and by pretreating the taste receptors with amiloride prior to salt stimulation in Experiment 2. Adding amiloride to NaCl during sustained neural activity suppressed chorda tympani nerve responses more than pretreating the tongue with amiloride. Adding amiloride during salt stimulation also partially suppressed chorda tympani neuron responses to KCl, a presumed control stimulus. The neural responses of the chorda tympani nerve to NaCl and KCl were similar for salt-avoiding F344 and salt-preferring Sprague–Dawley rats. However, amiloride pretreatment suppressed the taste nerve responses to NaCl significantly less in F344 rats than in Sprague–Dawley rats. The strain difference in the amiloride-sensitive component of the taste response may contribute to the difference in NaCl preference.

Salt preference Strain difference Taste electrophysiology Chorda tympani Nerve
Receptor mechanisms

ALTHOUGH sodium is an essential nutrient that must be consumed, there are large differences in NaCl intake among mammals. Some humans, rats, and mice presumably like the taste of NaCl and will consume it in large quantities, whereas others do not (15). For example, when given a two-bottle intake test between water and NaCl solution, many rat strains, as the outbred Sprague–Dawley, will preferentially consume a greater amount of hypotonic and isotonic NaCl solutions relative to water (9,13). In contrast to the Sprague–Dawley strain, the inbred Fischer 344 (F344) strain fails to prefer NaCl solutions at any concentration relative to water (34). This strain difference in intake seems to be unique to NaCl, as F344 rats' preference or aversion for sweet, sour, and bitter tasting solutions were similar to that of other strains. Additionally, F344 rats' response to sodium depletion by increasing hypertonic saline intake is modest at best compared to the robust salt appetite exhibited by other strains (35). The difference in NaCl intake between F344 and Wistar rats appears to be unrelated to differences in Na^+ excretion.

Taste is the primary sensory system that functions in the detection, recognition, and ingestion of NaCl. The chorda tympani nerve, which innervates the taste receptors on the anterior two-thirds of the tongue, is highly responsive to the monochloride salts, especially NaCl (3,22). After bilateral transection of chorda tympani nerves, rats trained to avoid the taste of NaCl exhibited a 50-fold increase in detection thresholds (46) and impaired ability to distinguish NaCl from KCl (45). Transection of the glossopharyngeal nerve, which innervates the taste receptors on the posterior one-third of the tongue, did not alter NaCl and KCl discrimination.

Several studies have shown that sodium salts stimulate taste receptors by Na^+ influx through ion channels on the apical plasma membrane of taste receptor cells (1,2,7,17,26,36). Amiloride hydrochloride, an epithelial Na^+ channel blocker, reduced the inward current produced by sodium ions (26,36), and partially suppressed the integrated responses of the salt-sensitive chorda tympani nerve to NaCl in rats (7,16,26), mice (23,39), hamsters (28), dogs (36,37), and rhesus monkeys (27). Insofar as amiloride treatment never completely suppressed taste nerve responses to NaCl, it appears that sodium taste processing involves both amiloride-sensitive and amiloride-insensitive transduction pathways (16,21).

The results from behavioral studies are generally consistent

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with findings from physiological studies. In human behavioral studies, amiloride suppressed NaCl taste intensity (40,42) and the saltiness of NaCl in some cases (20,33,47). In rat behavioral studies, amiloride treatment modified intake and licking rate to NaCl solution, suggesting perhaps that amiloride altered the rats' taste sensation of NaCl intensity and/or quality (5,11,14,30,32).

Taste appears to underlie the strain difference in NaCl intake between F344 and salt-preferring Wistar rats. As demonstrated with taste reactivity test in rats, microinfusion of taste solutions directly into the mouth elicit stereotypical patterns of mouth and body movements. These taste-elicited reflexes are divisible into positive ingestive and negative aversive responses that coincide with the intensity and quality of the taste stimulus (25). Using taste reactivity, F344 rats made taste-elicited aversive responses to all NaCl concentrations, whereas salt-preferring Wistar rats made no aversive responses and many ingestive responses (24). Bilateral transection of chorda tympani nerves in F344 rats reversed their NaCl aversion evident in a two-bottle intake test so that they preferred hypotonic and isotonic NaCl solutions like other rat strains (44). The mean relative responses of the chorda tympani nerve to a broad range of NaCl concentrations was greater in F344 than in Wistar rats; a larger amiloride-sensitive component appears to be responsible for the greater NaCl response in F344 rats (6).

The Sprague–Dawley rat has been the dominant experimental model for investigations dealing with salt intake behavior, salt appetite, and peripheral encoding mechanisms of salt taste. With few noteworthy exceptions (6,34,35), the Wistar rat is infrequently the subject of choice in studies of salt taste and salt appetite. The F344 rat is the predominant experimental model for cancer and aging research. There may be only one electrophysiological study of salt taste responses in F344 rats (6). The uniqueness of F344 rats' behavioral response to NaCl makes an electrophysiological comparison to the well-known Sprague–Dawley strain of special interest. We therefore compared the integrated responses of the chorda tympani nerve in F344 rats with the more widely studied Sprague–Dawley rats. The present study included a broad range of NaCl and KCl concentrations with and without amiloride. There are no prior studies of chorda tympani nerve responses to KCl in F344 rats.

In the present study, amiloride suppression of chorda tympani nerve responses to salt stimulation involved two procedures. First, we measured the magnitude of the response decrement that occurred from adding amiloride to ongoing NaCl stimulation. Second, we measured NaCl response amplitudes after pretreating the tongue with amiloride and compared them to those after water pretreatment. The former is a relatively new procedure perhaps more sensitive in detecting the amiloride-sensitive component of the taste nerve response (28). The latter is the more common procedure in studies of NaCl taste transduction. We used both procedures to maximize the possibility of uncovering a strain difference in salt taste sensitivity. As far as we know, this is the first time both amiloride suppression procedures have been compared in the same study.

EXPERIMENT 1

METHOD

Subjects

Data for 0.3, 1.0, and 10 μM amiloride were obtained from nine adult male Sprague–Dawley and nine F344 rats, with mean weights of 454 and 276 g, respectively. Data for 100 μM amiloride were obtained from three adult Sprague–Dawley rats (two female and one male) with a mean weight of 436 g and four adult F344 rats (three female and one male) with a mean weight of 285 g. The F344 rats were typically 100 g smaller than Sprague–Dawley rats of similar age. All animals came from the Wilmington & Raleigh divisions of Charles River Laboratories. Animals lived individually in standard wire hanging cages in a room on a 12:12 light dark cycle with lights on at 0500 h. The animals had free access to Purina Rat Chow 5001 and distilled water.

Neural Recording Procedure

Surgeries began approximately 4 h into the animals' light cycle. Anesthesia consisted of urethane (1.5 g/kg body weight) given in two IP injections spaced 15 min apart. Supplements of 0.1 ml were given as needed to maintain a deep level of anesthesia. The animals received a tracheotomy. A heating pad maintained body temperature between 36 and 38°C throughout recording. A nontraumatic head holder secured the animal's head. The chorda tympani branch of the seventh cranial nerve was exposed using a mandibular approach. The overlying auriculotemporal, mylohyoid, and inferior alveolar nerves were transected and removed. The chorda tympani was then isolated from where it joins the lingual nerve to the bulla where it was cut. The peripheral end was desheathed and placed upon a nichrome wire recording electrode. A similar indifferent electrode was placed into nearby tissue. The nerve was covered and bathed with mineral oil to prevent drying. The tongue was secured and positioned with a length of suture and remained covered with a cotton wad soaked in deionized water throughout surgery.

Chorda tympani nerve responses were differentially amplified 1000 ×, full wave rectified, and integrated with a Grass 7P3b R-C integrator (time constant = 0.2 s). Responses were recorded on a Grass polygraph for later quantification and stored on a Vetter video cassette recorder. All responses were simultaneously monitored via a Tektronix oscilloscope and a Grass audio monitor.

Solutions

Test solutions of NaCl, KCl, NH_4Cl, and amiloride hydrochloride were made from doubly distilled water and chemicals obtained from Sigma Chemical Company. They were presented at room temperature (22–24°C).

Stimulus Protocol

Test solutions were delivered to the tongue by gravity flow from 60-ml syringes at an approximate rate of 3 ml/min. A concentration series was always bracketed by a 10–15-s delivery of 0.1 M NH_4Cl to ensure the stability of the response over time. At all other times the tongue was continually bathed by a constant flow of distilled water.

NaCl and KCl

The first stimulus series determined the normal peak and tonic responses of the chorda tympani nerve to an ascending series of NaCl and KCl concentrations (0.01, 0.03, 0.1, 0.3, and 1.0 M). Each stimulus was presented for 10 s. A minimum 1-min distilled water rinse preceded each test stimulus (this was longer for strong concentrations). To assess the specificity of amiloride hydrochloride inhibition, KCl was used as stimulus. In rats, amiloride is significantly less effective in inhibiting chorda tympani nerve responses to KCl as compared to NaCl (7,16,29).

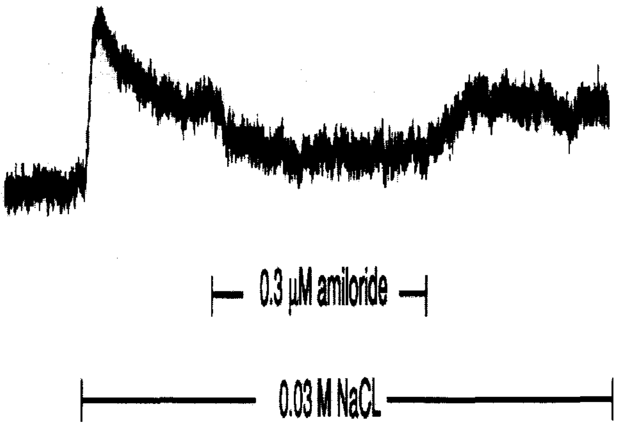

FIG. 1. Representative integrated response of the whole chorda tympani nerve to NaCl stimulation with and without amiloride. The bottom horizon line represents the entire 30-s period of stimulation. The upper horizontal line represents the middle 10-s segment when amiloride was added.

Amiloride

The second stimulus series examined the suppressive effects of 0.3, 1.0, 10, and 100 μ*M* amiloride on chorda tympani nerve responses to an ascending series of NaCl and KCl concentrations (0.01, 0.03, 0.1, 0.3, 1.0 *M*). Test stimuli were presented for 30 s followed by a 1-min distilled water rinse (longer for strong concentrations). The 30-s stimulation period was divided into three continuous 10-s segments with the stimulus presented alone, mixed with the amiloride, and again alone, respectively (see Fig. 1 for an example). A given animal was tested with only one amiloride concentration.

Response Quantification and Analysis

The chorda tympani responses to each salt solution were standardized to the mean peak response to 0.1 *M* NH_4Cl before and after a concentration series. The peak response was measured as the maximum response amplitude occurring within 2 s after stimulus onset, and the tonic response as the response amplitudes occurring 5 and 10 s after stimulus onset. Amiloride suppression was scored as the ratio in response magnitudes during amiloride (second 10-s segment) relative to the average control responses before and after amiloride (first and third 10-s segments). The control responses were measured 2 s before and 4 s after amiloride and averaged. Measurement at these time points allowed for response stabilization and controlled for possible progressive changes in the chorda tympani nerve response over time. Amiloride responses were measured 5 and 10 s after amiloride onset. Amiloride suppression was computed using the equation: 100{amiloride response/[(Tonic1 + Tonic2)/2]}. Suppression scores could not be computed for 0.01 *M* concentrations of NaCl and KCl because the tonic activity was either too small or lacking for these salt concentrations to observe an amiloride effect.

Statistical analysis was done using analysis of variance (ANOVA) tests with repeated measures on Strain, Tastant (NaCl

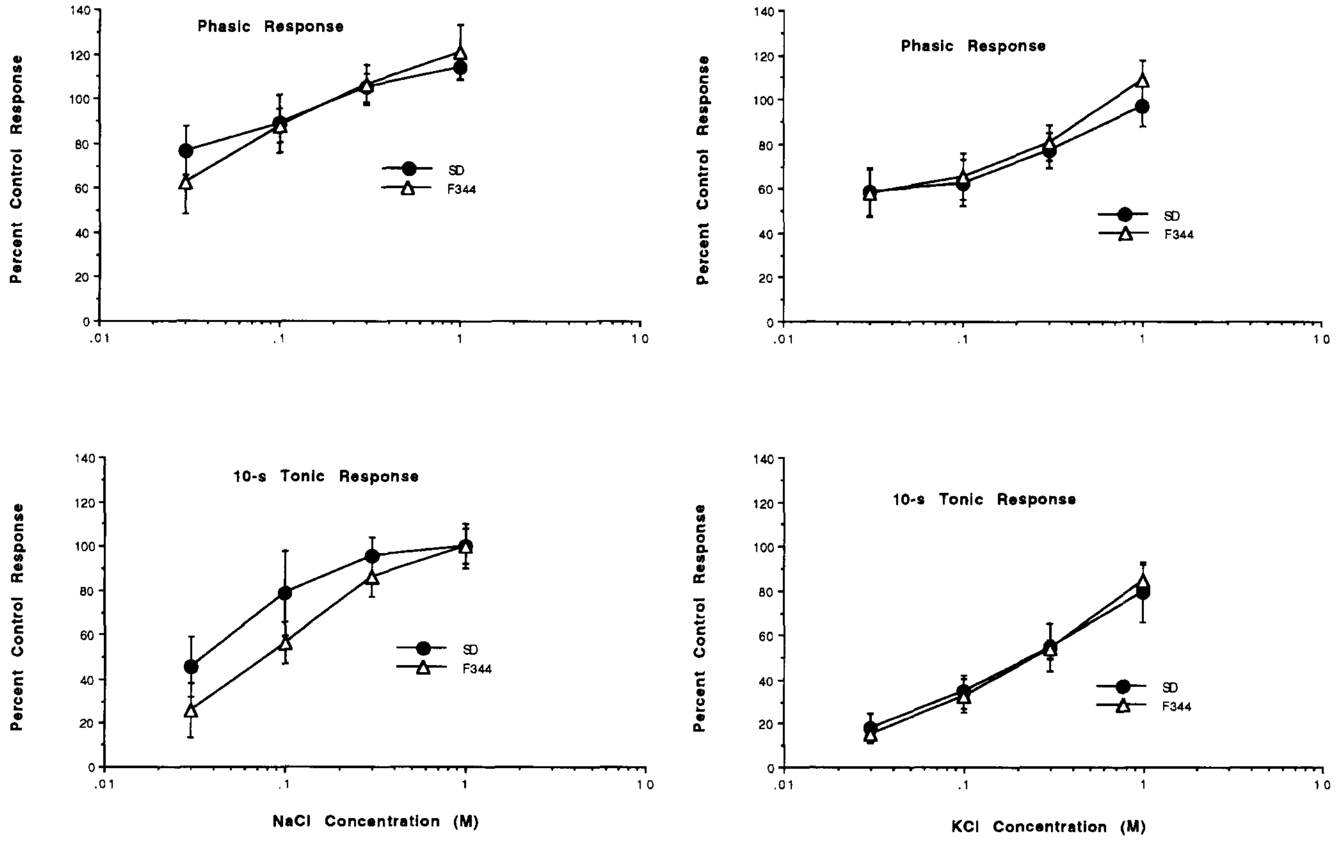

FIG. 2. The mean phasic and tonic responses to NaCl and KCl relative to 0.1 *M* NH_4Cl in Fischer 344 and Sprague–Dawley rats.

or KCl), Amiloride Concentration, and Time of Measure variables. Post hoc analyses were performed using univariate F and Wilk's lambda multivariate F-tests. Significant differences were reported as $p < 0.05$. In addition, paired samples t-tests were conducted on salt concentration (NaCl vs. KCl) across amiloride concentration.

RESULTS

NaCl and KCl Responses

Neural responses were expressed as a percentage of the responses to 0.1 M NH_4Cl, which were similar (F344 = 8.9 ± 0.5; SD = 8.8 ± 0.4) in magnitude between the two strains. As can be seen in Fig. 2, the peak and 10-s tonic responses of the chorda tympani nerve increased with NaCl and KCl concentration (all $p < 0.0001$). There were no differences between F344 and Sprague–Dawley rats in either mean peak responses to NaCl, $F(1, 16) = 1.406$, $p = 0.253$, or to KCl, $F(1, 16) = 0.157$, $p = 0.697$. There were also no strain differences in the 10-s tonic responses to NaCl, $F(1, 15) = 2.153$, $p = 0.163$, or to KCl, $F(1, 16) = 0.957$, $p = 0.342$. Similar results were obtained for the 5-s tonic measure.

NaCl Suppression

As can be seen in Fig. 3, the suppression functions for 0.3, 1.0, 10, and 100 μM amiloride were similar for F344 and Sprague–Dawley rats, $F(3, 16) = 0.193$, $p = 0.9$. There was a significant main effect for Amiloride Concentration, $F(3, 16) = 24.763$, $p < 0.0001$, as suppression increased with concentration with 100 $\mu M >$ 10 $\mu M >$ 1.0 $\mu M =$ 0.3 μM (all $p < 0.01$).

Figure 3 also shows that amiloride's effectiveness varied across NaCl concentration. Both 0.3 μM, $F(1, 4) = 48.636$, $p = 0.001$, and 1 μM amiloride, $F(1, 4) = 48.269$, $p = 0.001$, were effective only for the two weakest NaCl concentrations. Amiloride at concentrations of 10 and 100 μM suppressed chorda tympani responses to all four NaCl concentrations. For all four amiloride concentrations, the amount of suppression tended to decline with increasing NaCl concentration, particularly for 0.3 and 1.0 μM amiloride.

KCl Suppression

Contrary to our expectation, amiloride significantly suppressed KCl responses. There was a significant main effect for Amiloride Concentration, $F(3, 16) = 7.648$, $p = 0.002$, as suppression increased with concentration (100 $\mu M =$ 10 μM and 100 $\mu M >$ 1.0 $\mu M =$ 0.3 μM; all $p < 0.05$). As can be seen in Fig. 4, the suppression functions for 0.3, 1.0, 10, and 100 μM amiloride were similar for F344 and Sprague–Dawley rats, $F(3, 16) = 1.657$, $p = 0.216$. Figure 4 shows that suppression of KCl responses, although less than suppression of NaCl responses, also increased as amiloride concentration increased.

Figure 4 also shows that amiloride's effectiveness varied across KCl concentration. Both 0.3 μM, $F(1, 4) = 9.342$, $p = 0.002$, and 1.0 μM, $F(1, 4) = 2.797$, $p = 0.086$, amiloride were

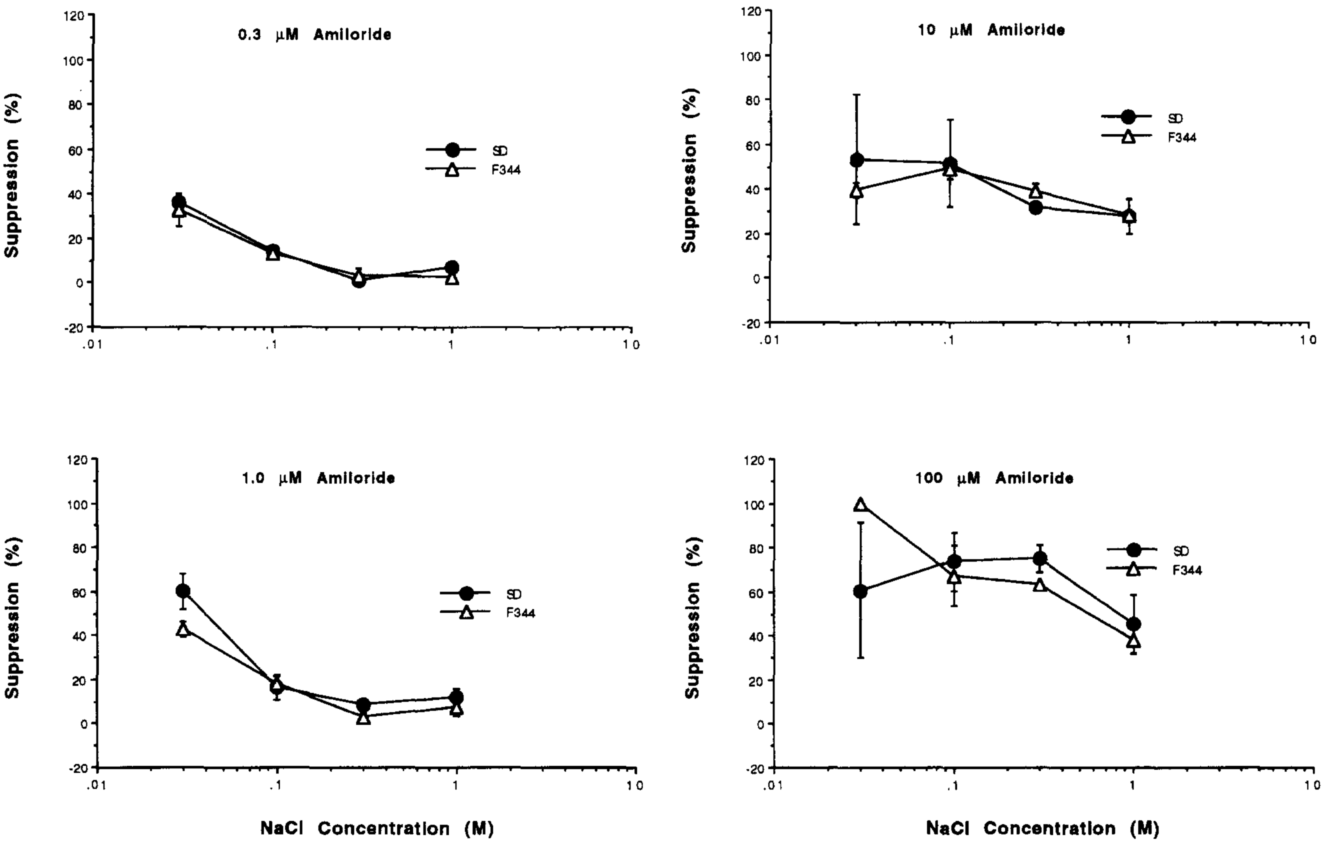

FIG. 3. The average percent suppression of chorda tympani nerve responses to four concentrations of NaCl with 0.3, 1.0, 10, and 100 μM amiloride in Fischer 344 (F344) and Sprague–Dawley (SD) rats.

effective in suppressing chorda tympani nerve responses to only 0.03 *M* KCl. This was not the case for 10 μM, $F(1, 4) = 0.938$, $p = 0.453$, or 100 μM, $F(1, 4) = 1.717$, $p = 0.217$, for which suppression was very similar across KCl concentrations. Amiloride concentrations of 10 and 100 μM suppressed chorda tympani responses to all four KCl concentrations. For all four amiloride concentrations, the amount of suppression tended to decline with increasing KCl concentration.

NaCl and KCl Comparisons

Because there were no significant strain differences in amiloride suppression, the data from both strains were combined to compare NaCl and KCl suppression. Amiloride suppression differed significantly by salt, $F(7, 40) = 18.007$, $p < 0.001$. Post hoc analysis (see Fig. 5) indicates that NaCl and KCl responses begin to diverge significantly only in response to 10 and 100 μM amiloride. As shown in Fig. 5, 10 μM amiloride suppressed 0.1 *M*, $F(1, 40) = 4.306$, $p = 0.044$, and 0.3 *M*, $F(1, 40) = 8.248$, $p = 0.006$, NaCl to a greater extent than the same concentrations of KCl. With 100 μM amiloride, there was even a greater divergence in suppression among 0.1 *M*, $F(1, 40) = 13.828$, $p = 0.001$, and 0.3 *M*, $F(1, 40) = 73.95$, $p < 0.001$, salt concentrations. There were no differences in suppression between 0.03 *M* NaCl and KCl and between 1.0 *M* NaCl and KCl with 10 μM or 100 μM amiloride (all $p > 0.05$).

Response Recovery From Amiloride

Suppression responses were scored at four times, 5 s apart. The first and second time samples occurred during amiloride application. The last two were taken during recovery of the response to the salt stimulus. This was done to reveal any possible differences in sensitivity across time between the two strains. A Strain × Time effect was not found for either NaCl, $F(3, 48) = 1.809$, $p < 0.158$, or KCl, $F(3, 48) = 0.828$, $p < 0.485$.

EXPERIMENT 2

In Experiment 1 the integrated responses of the whole chorda tympani nerve to a broad range of NaCl and KCl concentrations were similar for F344 and Sprague–Dawley rats. Furthermore, the degree of NaCl taste suppression with a broad range of amiloride concentrations was similar for F344 and Sprague–Dawley rats. Unlike the results of some prior studies (7,26), amiloride also suppressed the neural responses to KCl solutions. This was the first study to use amiloride addition to examine a strain difference in salt taste sensitivity and assess the specificity of amiloride blockade. We conducted Experiment 2 to determine whether our observations were procedurally specific to the way of measuring amiloride suppression.

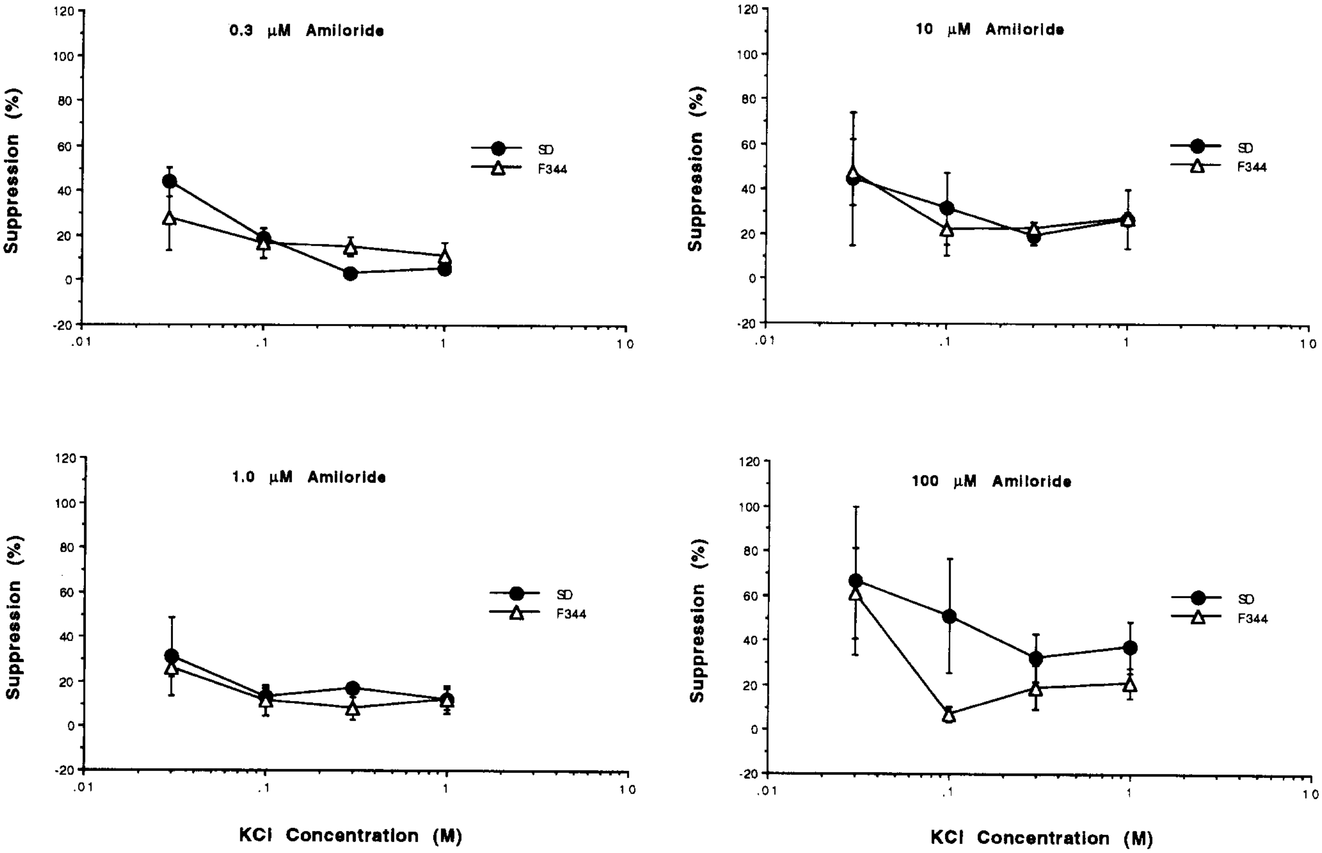

FIG. 4. The average percent suppression of chorda tympani nerve responses to four concentrations of KCl with 0.3, 1.0, 10, and 100 μM amiloride in Fischer 344 (F344) and Sprague–Dawley (SD) rats.

METHOD

Whole nerve recordings were obtained from six adult F344 (mean = 359 g) and six adult Sprague–Dawley (mean = 661 g) rats in response to stimulation with an ascending concentration series of NaCl (0.06, 0.1, 0.3, and 1.0 M NaCl) and to 0.1 M NH_4Cl. Responses to 0.1 M NH_4Cl before and after each concentration series were used to assess the stability of neural recording. The complete set of NaCl concentrations was tested using distilled water as the rinse solution, a 1-min rinse preceding each taste stimulus. The same NaCl solutions were then tested with 100 μM amiloride hydrochloride as the rinse solution (23). With a new stimulus delivery system (31), taste solutions and rinses were presented to the tongue at a precisely controlled flow rate of 25 μl/s and temperature of 25°C. The data were stored on a video cassette recorder and analyzed off-line on a Power PC computer equipped with a GW Instrument 15-μs data acquisition board and SuperScope II data analysis software. The neural responses were quantified by the area under the 20-s response curve using SuperScope II. All other conditions were identical to those in Experiment 1.

RESULTS

As was shown in Experiment 1, there were no differences in relative NaCl responses after water adaptation between F344 and Sprague–Dawley rats, $F(1, 40) = 0.308$, $p = 0.599$. There were also no strain differences in relative NaCl responses after amiloride adaptation, $F(1, 40) = 1.491$, $p = 0.229$. Although there were no strain differences for either rinse condition, the neural responses to 0.06 and 0.1 M NaCl tended to be smaller after the water rinse in F344 rats (0.3 ± 0.06; 0.52 ± 0.10) compared to Sprague–Dawley rats (0.48 ± 0.06; 0.78 ± 0.11). The opposite was seen after amiloride rinse as the responses to 0.06 and 0.1 M NaCl tended to be larger in F344 rats (0.39 ± 0.03; 0.53 ± 0.04) compared to the same responses in Sprague–Dawley rats (0.31 ± 0.04; 0.49 ± 0.06). These trends for the two weakest NaCl concentrations are evident by comparing the NaCl response functions for F344 and Sprague–Dawley rats (see Fig. 6).

As supported by a main effect for Rinse, amiloride significantly suppressed the chorda tympani nerve responses to NaCl in both F344, $F(1, 40) = 5.973$, $p = 0.02$, and Sprague–Dawley rats, $F(1, 40) = 30.201$, $p < 0.001$. As revealed in post hoc tests, the source of this suppression was due to the responses to 0.3 M ($p = 0.03$) and 1.0 M ($p = 0.004$) NaCl in F344 rats. In Sprague–Dawley rats, the source of the amiloride suppression was due to 0.1 M ($p = 0.048$, one-tailed), 0.3 M ($p < 0.0001$), and 1.0 M ($p = 0.0009$) NaCl. Given these ANOVA results and by comparing the functions shown in Fig. 6, amiloride suppression seemed to be more significant for Sprague–Dawley rats than

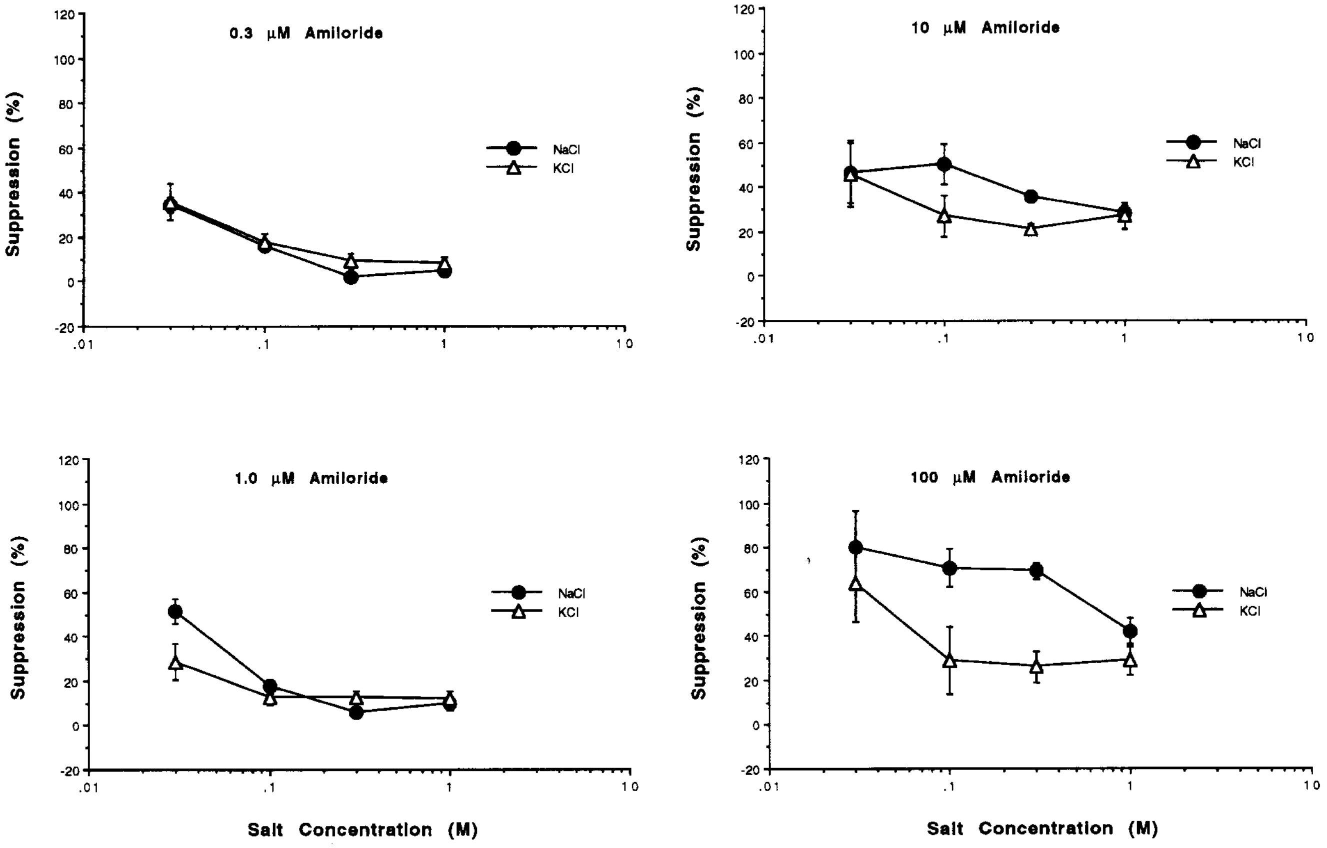

FIG. 5. A comparison of amiloride suppression between NaCl and KCl responses. Because there were no strain differences, the data from Fischer 344 and Sprague–Dawley rats were combined.

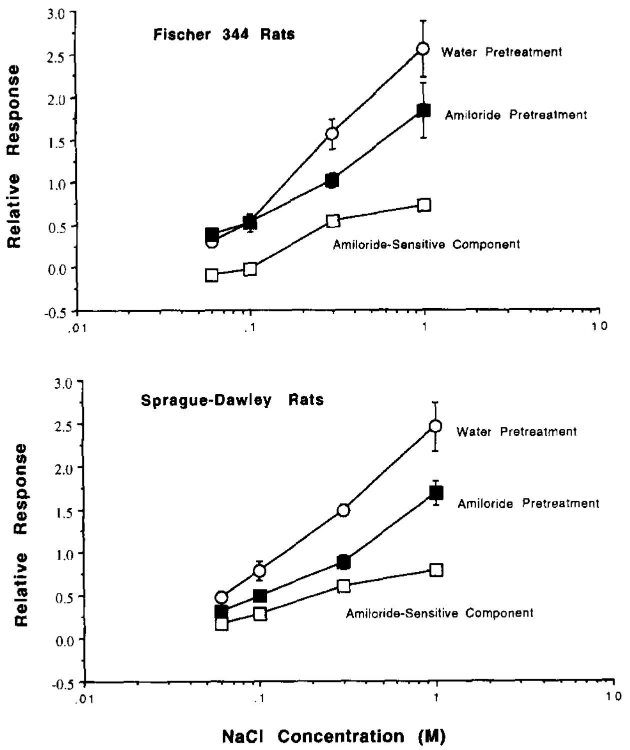

FIG. 6. The average chorda tympani responses to NaCl relative to 0.1 *M* NH_4Cl after pretreatment with distilled water or 100 μ*M* amiloride in Fischer 344 (top) and Sprague–Dawley (bottom) rats. The amiloride-sensitive component of the nerve response was determined by subtracting the responses after amiloride pretreatment from the responses after water pretreatment.

for F344 rats, particularly for the two weakest NaCl concentrations.

Figure 7 presents a direct comparison of amiloride suppression of the four NaCl concentrations between F344 and Sprague–Dawley rats. There was a significant main effect for strain indicating that amiloride suppression was greater for Sprague–Dawley rats, $F(1, 40) = 8.619$, $p = 0.006$. The source of this strain difference was due to 0.06 *M* ($p = 0.01$) and 0.1 *M* ($p = 0.01$) NaCl, but not for 0.3 *M* ($p = 0.511$) or 1.0 *M* ($p = 0.976$) NaCl.

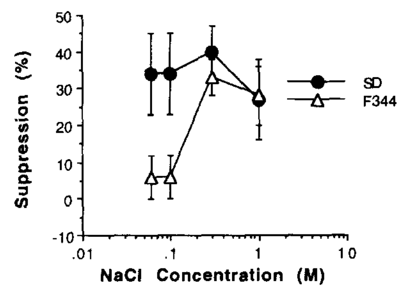

FIG. 7. The average percent suppression of chorda tympani nerve responses to four concentrations of NaCl with 100 μ*M* amiloride pretreatment in Fischer 344 (F344) and Sprague–Dawley (SD) rats.

DISCUSSION

In two separate experiments, we found that the integrated responses of the chorda tympani nerve to NaCl were similar for salt-avoiding F344 and salt-preferring Sprague–Dawley rats. We did, however, find a small strain difference in the amiloride-sensitive portion in the chorda tympani response to NaCl after amiloride pretreatment, but not after the addition of amiloride during NaCl stimulation. Amiloride partially suppressed chorda tympani neuron responses to NaCl and unexpectedly as well to KCl, a presumed control stimulus.

Strain Differences in NaCl Taste Preference

We expected our results to be similar to prior work (6) comparing F344 rats with salt-preferring Wistar rats and finding a larger relative NaCl response and greater amiloride sensitivity in F344 rats. If salt taste mechanisms play a role in salt preference, then one may expect salt-preferring Wistar and Sprague–Dawley rats to be similar in relative neural response and amiloride sensitivity and equally different from F344 rats. However, we classify Wistar and Sprague–Dawley as salt preferring only from their preference performance in two-bottle intake tests. In these long-term tests, taste as well as many postingestive factors, contributes to the preference behavior. As far as we know, there are no studies directly comparing Wistar and Sprague–Dawley either in long-term or in brief exposure tests that minimize postingestive factors. The possibility exists that Wistar rats may also differ from Sprague–Dawley rats in their salt taste behavior. If this were the case, then one may expect Wistar and Sprague–Dawley rats to differ as well in chorda tympani response and amiloride sensitivity.

In rat studies of taste and salt intake behavior, the Sprague–Dawley is the animal model most often used in the experimental literature. The salt-sensitive chorda tympani of Sprague–Dawley rats responds strongly to a broad range of concentrations. The relative NaCl responses found in the present study are similar to those reported by us (12) and others (7,21,26) in the experimental literature. There may be only one electrophysiological study of salt taste responses in F344 rats (6). The Wistar rat is also an uncommon model for electrophysiological taste research. Because of its long history of use in taste electrophysiology, the Sprague–Dawley may be a better model with which to compare chorda tympani responses with other strains like the F344.

Despite some differences in the anesthetic, stimulus protocol and flow rate, integrator time constant, and normalizing NH_4Cl concentration, the relative NaCl responses of F344 from the present and earlier study (6) compare favorably. The neural responses of F344 to strong NaCl concentrations are larger than the normalizing NH_4Cl stimulus in both the earlier and present study, particularly in Experiment 2. The NaCl responses of the chorda tympani in Wistar rats were smaller than the normalizing NH_4Cl stimulus in the previous study (6). Bernstein and her coworkers (6) found the threshold amiloride concentration for suppression to be at least as low as 1 μ*M* amiloride in F344 compared to the mimimum of 5 μ*M* in Wistar. In the present study, 0.3 μ*M* amiloride was equally effective in suppressing chorda tympani responses to NaCl in F344 and Sprague–Dawley. Thus, the amiloride threshold for F344 and Sprague–Dawley appears to be the same. Bernstein, et al. (6) found greater NaCl suppression using a higher 500 μ*M* amiloride concentration to block all amiloride-sensitive channels than the 100 μ*M* concentration used in the present study. However, a higher amiloride concentration should not change the pattern of results found in the present study.

Hill and his associates (30) conducted an important study in rats suggesting that the amiloride-sensitive portion of the functional taste response to NaCl mediates a taste associated with sodium cations, whereas the residual amiloride-insensitive portion mediates a taste associated with nonsodium cations. Control rats trained to avoid the taste of 0.5 *M* NaCl generalized their aversion only to sodium salts specifically to 0.1 *M* NaCl and to 0.1 and 0.5 *M* sodium acetate. Amiloride-treated rats, in contrast, generalized their conditioned aversion of 0.5 *M* NaCl to 0.1 and 0.5 *M* concentrations of KCl, NH_4Cl, and ammonium acetate, but not to sodium salts. Furthermore, rats prefer NaCl over KCl and NH_4Cl as revealed in short-term intake (10) and in lick rate (8) tests in water-deprived rats. From these findings, the amiloride-sensitive component seems necessary to distinguish between the preferred sodium salts from the nonsodium salts.

In Experiment 2, there was a small strain difference in the amiloride-sensitive portion of the chorda tympani nerve as assessed by the relative response amplitudes to NaCl after water and amiloride adaptation. In F344 rats, an amiloride-sensitive component of the chorda tympani nerve response was evident during stimulation with hypertonic 0.3 and 1.0 *M* NaCl, but not during stimulation with hypotonic 0.06 and 0.1 *M* NaCl. In Sprague–Dawley rats, an amiloride-sensitive component was evident for all of the NaCl concentrations. With taste reactivity tests, Grill and Bernstein (24) demonstrated that F344, but not Wistar, reacted aversively to hypotonic and isotonic NaCl concentrations. Sprague–Dawley rats reacted with positive ingestive responses up to 0.3 *M* NaCl, and aversively to higher NaCl concentrations (20). If the amiloride-sensitive portion of the chorda tympani nerve response underlies the sodium taste in rats, then from Hill's work (30) we can infer that all NaCl concentrations elicit the same sodium-driven taste but vary in intensity across the concentration spectrum in Sprague–Dawley rats. To F344 rats hypertonic saline may elicit the sodium taste, but hypotonic saline may elicit another taste because of the absence of amiloride sensitivity. The excessively high concentration of hypertonic saline may contribute to the aversion of normally preferred sodium solutions in salt-preferring and salt-avoiding rats. The unpalatable, perhaps nonsodium quality may contribute to F344 rats' aversion for hypotonic and isotonic NaCl solutions.

Although this may be an intriguing explanation, the strain difference in amiloride sensitivity is small. The difference does not appear to be large enough to account completely for the striking behavioral difference between the two strains. The difference in amiloride sensitivity may contribute to the NaCl aversion in F344, but a central change in the evaluation of salt taste must also occur.

Amiloride Suppression of Whole Nerve Responses

Adding amiloride to NaCl during sustained neural activity suppressed chorda tympani nerve responses more than pretreating the tongue with amiloride. For example, in Sprague–Dawley rats we found that adding 100 μM amiloride to the four NaCl concentrations suppressed the sustained tonic responses by an average of 65%, whereas pretreatment suppressed the responses by only 34%. In F344 rats, adding 100 μM amiloride to the stimulating NaCl solution suppressed NaCl responses by 68%, whereas pretreatment suppressed the responses by 18%. This is the first instance to compare the two amiloride protocols within the same study. The present results are consistent with previous findings suggesting that amiloride addition may be a more sensitive method of assessing suppression than pretreatment (28).

There are at least two reasons why adding amiloride to the stimulating NaCl solution may be more effective than pretreatment (28). The duration of amiloride application, which is always longer with pretreatment than with addition, may be one key factor. In studies using pretreatment, amiloride application may be as long as 5 min before the onset of an ascending concentration series (7,26) coupled with shorter rinses of 1-min duration before each stimulus. When added to the stimulating NaCl solution, amiloride was present for only 10 s. The reversal of amiloride suppression after prolonged pretreatment may take minutes (16). The reversal after amiloride addition may take only a few seconds (28), as was observed in the present investigation. Lengthy pretreatment may alter the taste receptor membrane in a way to reduce amiloride suppression. For example, amiloride pretreatment may reduce the spontaneous firing frequencies of chorda tympani neurons (7), reflecting an alteration in the resting membrane characteristics of the taste cells.

The differential effect of amiloride on so called "N" and "H" fibers may be a second reason why amiloride addition is more effective than pretreatment. N and H fibers constitute in equal numbers the major fiber groups within the chorda tympani nerve (22). Both fibers respond well to NaCl but with distinct temporal patterns. The brunt of H fiber activity is immediate and brief, whereas N fiber activity remains high throughout NaCl stimulation (22). Amiloride exerted a greater suppressive effect on the responses of N fibers than on H fibers in both rats (38) and hamsters (28). Adding amiloride to NaCl stimulation would therefore be more effective by suppressing the tonic responses of N fibers than amiloride pretreatment that fails to suppress the brief responses of H fibers (28). Additionally, it is also possible that F344 rats may have a greater proportion of H fibers or that the threshold for activating N fibers is higher.

Amiloride-Sensitive Channels on Taste Cells

Not only was adding amiloride to a stimulating NaCl solution more effective in suppressing chorda tympani responses, it also seemed to be less selective than amiloride pretreatment. Studies conducted more than 10 years ago in rats found that amiloride pretreatment suppressed the integrated responses of the whole chorda tympani nerve relatively selectively to NaCl and LiCl, but not to KCl, RbCl, and NH_4Cl (7,26). Unexpectedly in the present study, amiloride addition also suppressed chorda tympani neuron responses to a stimulating KCl solution but to a lesser degree than amiloride suppression of NaCl responses. Amiloride suppression of KCl responses mirrored the same pattern of amiloride suppression of NaCl responses. That is, amiloride suppression increased with increasing amiloride concentration, and decreased with increasing salt concentration. The present findings are consistent with recent studies showing: 1) amiloride blockade of sodium and potassium conductance in isolated taste receptor cells from the frog tongue (1); 2) amiloride suppression of single fiber responses to KCl in the rat chorda tympani nerve (38); and 3) amiloride suppression of whole nerve responses to KCl in the dog chorda tympani nerve (37).

Our data indicate that the sodium channel may not be specific to sodium ions as previously envisioned, or that a component of the KCl response of taste cells may be susceptible to amiloride blockade. The latter explanation seems more likely insofar as amiloride partially suppressed the chorda tympani nerve responses to KCl, like NaCl. One possibility is that amiloride may block KCl-stimulated entry of Na^+ into the receptor cells (41). If this was the case, then the passive sodium current stimulated by KCl may not be as large as the sodium current generated from direct NaCl stimulation. This may explain why amiloride suppression is greater on NaCl than on KCl responses. A second possibility is that amiloride suppresses just the responses of N fibers that respond robustly to NaCl but may also respond less to KCl (38). This may also explain why amiloride suppression is greater on NaCl than on KCl responses.

With pretreatment, amiloride bathes the receptors before salt stimulation. For NaCl to stimulate taste receptors after amiloride pretreatment, sodium ions must displace amiloride from their location in channels on the plasma membrane. The ease with which sodium can displace amiloride would depend upon how tightly amiloride binds to the channels. As indicated above, this may depend also on the duration of amiloride application typically for long periods with pretreatment. The response envelope of the chorda tympani nerve to stimulation depends on how quickly and efficiently Na^+ displaced amiloride from the plasma membrane. Apparently, the chorda tympani nerve response to KCl may not require potassium displacement of amiloride, because amiloride pretreatment typically fails to suppress KCl responses. It is more likely that depolarization due to KCl stimulation indirectly displaces amiloride.

When added to the stimulating solution, taste cells see amiloride when they are highly active during stimulation, not when the cells are relatively quiescent during baseline. With NaCl stimulation, amiloride and sodium are available to compete during sustained tonic activity for available sodium channel sites on the plasma membrane. When the taste cells are active due to KCl stimulation, amiloride may influence cellular processes beyond that involved in cation detection and ion-amiloride competition, because amiloride not only blocks sodium channels, but also blocks sodium–hydrogen exchange (4). And as indicated above, amiloride may also block passive sodium entry into receptor cells triggered by taste stimuli other than NaCl (41).

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