C(S)

Amiloride Effects on Taste Quality: Comparison of Single and Multiple Response Category Procedures

Corinne A. Ossebaard[1], Ilse A. Polet[2] and David V. Smith[1]

[1]Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD 21201, USA and [2]Psychological Laboratory, Utrecht University, 3584 CS Utrecht, The Netherlands

Correspondence to be sent to: C.A. Ossebaard, Chemosensory Perception Laboratory, Department of Surgery, University of California, San Diego, Mailcode 0957, La Jolla, CA 92093-0957, USA

Abstract

Although there is compelling evidence that amiloride reduces the intensity of Na^+ and Li^+ salts in humans, its effects on saltiness are conflicting. Many salts elicit not only a salty taste but also one or more side tastes (sweetness, sourness or bitterness). Some studies have shown a suppression of saltiness by amiloride; others show no effect on saltiness but a significant reduction in sourness. In the experiments demonstrating a reduction of *saltiness*, subjects estimated *only* saltiness; in those showing an amiloride effect on *sourness* and not saltiness, subjects estimated all qualities on each trial. The present study examines the role of the psychophysical method in these conflicting results. We have investigated the effects of amiloride on taste quality by modifying only the instructions to the subjects, keeping all other variables constant. One group of subjects (intensity-only) gave magnitude estimates of the overall intensity of a LiCl concentration series. A second group (salty-only) was instructed to estimate the saltiness of the stimuli, and a third group (sour-only) estimated their sourness. Finally, a fourth group (profile) rated all of the taste qualities on each stimulus presentation, using a modified taste profile method. The ratings of all groups were made comparable by the use of 0.1 mM quinine-HCl as a modulus. When subjects used only one response category, amiloride reduced their estimates (of intensity, saltiness or sourness), but if subjects attended to all four qualities, amiloride specifically reduced the sourness of LiCl and had no significant effect on its saltiness. Comparison of the saltiness estimates of the salty-only group to the sum of the salty and sour estimates of the profile group demonstrated that subjects combined these sensations when presented with only one response alternative. To reveal the effect of amiloride on a specific quality of a salt, the psychophysical method must allow subjects to attend to all qualities on each trial. These data and previous results suggest that apical Na^+ channels on the taste receptor cell membrane mediate the sourness but not the saltiness of Na^+ and Li^+ salts. **Chem. Senses 22: 267–275, 1997.**

Introduction

The transduction of Na^+ and Li^+ salts in many species has been shown to be partly mediated by ion channels on the apical membrane of taste receptor cells (Heck *et al.*, 1984; Brand *et al.*, 1985; Hill and Bour, 1985; Avenet and Lindemann, 1988; Formaker and Hill, 1988; Hellekant *et al.*, 1988; Ninomiya and Funakoshi, 1988; Hettinger and Frank, 1990; Nakamura and Kurihara, 1990). Amiloride-HCl blocks these epithelial ion channels and reduces the

NaCl-evoked response in the chorda tympani (CT) nerve (Heck *et al.*, 1984; Brand *et al.*, 1985; Hettinger and Frank, 1990; Ye *et al.*, 1993) and in single neurons of the nucleus of the solitary tract (NST; Scott and Giza, 1990; Giza and Scott, 1991; Smith *et al.*, 1996).

Psychophysical studies in humans have shown that the taste intensity of Na^+ and Li^+ salts is also suppressed by amiloride (Schiffman *et al.*, 1983; McCutcheon, 1992; Tennissen, 1992; Ossebaard and Smith, 1995, 1996; Smith and Ossebaard, 1995; Tennissen and McCutcheon, 1996). However, the effect of amiloride on the taste *quality* of these salts is subject to controversy; some studies show a reduction in their *saltiness* (McCutcheon, 1992; Tennissen, 1992; Tennissen and McCutcheon, 1996), whereas others demonstrate a specific effect on their *sourness* (Ossebaard and Smith, 1995, 1996). As a chemical group, salts are not only salty but evoke other taste qualities as well. Even NaCl, which is often used as a prototype of the salty taste quality, has several side tastes; it is salty and sweet at low concentrations, and salty and sour at mid-range and higher concentrations (Smith and McBurney, 1969; McBurney and Bartoshuk, 1973; Cardello, 1979; Schiffman *et al.*, 1980; Smith and van der Klaauw, 1995; van der Klaauw and Smith, 1995). The sour component of organic Na^+ salts is generally greater than the sourness of NaCl. LiCl is also both salty and sour, and its sourness is proportionally larger than that of NaCl (Smith and van der Klaauw, 1995; van der Klaauw and Smith, 1995; Ossebaard and Smith, 1996). At a concentration of 0.1 M, LiCl is almost equally salty and sour, whereas the sourness of NaCl at the same concentration is only about 25% of its overall intensity (Ossebaard and Smith, 1996).

The studies that have demonstrated a specific effect of amiloride on the sour side taste of NaCl, Na-gluconate or LiCl (Ossebaard and Smith, 1995, 1996) have employed a psychophysical procedure in which subjects give a numerical estimate of the total intensity of a solution and then divide this estimate among the appropriate taste qualities (Smith and McBurney, 1969). This procedure yields taste profiles depicting simultaneously obtained estimates (i.e. within one trial) of the total intensity and of the component taste qualities of a stimulus. Taste profiles obtained for NaCl, Na-gluconate, and LiCl showed that their sourness was significantly reduced by amiloride treatment, but that their saltiness was unaffected (Ossebaard and Smith, 1995, 1996). This result was independent of the ratio between saltiness and sourness before amiloride treatment; the sourness was always virtually eliminated by amiloride. The sourness of 3.2 mM citric acid, which was also used as a stimulus in each of these studies, was unaffected by amiloride (see also Tennissen and McCutcheon, 1996), suggesting that different transduction mechanisms mediate the sourness of Na^+/Li^+ salts and the sourness of citric acid (Ossebaard and Smith, 1996).

Other studies have reported an effect of amiloride on *saltiness* (McCutcheon, 1992; Tennissen, 1992; Tennissen and McCutcheon, 1996). These experiments, however, assessed only saltiness and did not evaluate any other taste qualities. We have previously suggested that the instructions in these studies, which restricted the subjects' responses to a single category, could have prevented subjects from differentiating between a salty main taste and a sour side taste, resulting in their combining ('dumping') sourness and saltiness into a single estimate (Ossebaard and Smith, 1995, 1996). Consequently, an effect of amiloride on sourness would be reflected in the estimate for saltiness. Psychophysical studies with taste–odor mixtures and mixtures of two tastants have shown that such 'dumping' occurs when subjects do not have the opportunity to rate all of the appropriate attributes, but that providing this opportunity eliminates the 'dumping' effect (Frank and Byram, 1988; Frank *et al.*, 1993; Clark and Lawless, 1994).

However, the number of available response categories was not the only difference between studies showing an amiloride effect on sourness and those finding an effect on saltiness. Additional differences occurred in the concentration of amiloride, the stimulation method, the stimulated region of the tongue, the flow rate of the solution, and the bitterness of amiloride. The present study was designed to investigate the effects of amiloride on taste quality by modifying only the instructions to the subjects, keeping all other variables constant. LiCl was used as a stimulus because of its proportionately large sour side taste. One group of subjects (intensity-only) gave magnitude estimates of the overall intensity (strength) of the stimuli. A second group (salty-only) was instructed to estimate the saltiness of the stimuli, and a third group (sour-only) estimated their sourness. Finally, a fourth group (profile) rated all of the taste qualities on each stimulus presentation, using a slightly modified taste profile method. The ratings of all groups were made comparable by the use of 0.1 mM quinine-HCl (QHCl) as a modulus. We expected to find an effect of amiloride on the saltiness of LiCl for the group that estimated only its saltiness and to find a specific effect on the

sourness of LiCl combined with a lack of an effect on its saltiness for the group that estimated all taste qualities. We also expected amiloride to suppress the magnitude estimates of the groups that rated only sourness or intensity.

Materials and methods

Subjects

Seventy students or employees of the University of Maryland served as paid subjects. They were not users of tobacco in any form. All subjects in the profile group were able to perceive the dominant taste of 0.1 M NaCl, 0.1 M sucrose, 3.2 mM citric acid and 0.1 mM QHCl as salty, sweet, sour and bitter respectively, as determined by a screening task prior to the experiment. Subjects in the salty-only and sour-only groups were able to identify the saltiness and sourness of 0.1 M NaCl and 3.2 mM citric acid respectively.

Stimuli

Two sets of stimuli were formed; one dissolved in purified water [Hydro Ultrapure (Research Triangle Park, NC) water system, resistance >18 MΩ] and the other in 10 μM amiloride-HCl (Sigma Chemical Co., St Louis, MO). Each stimulus set consisted of five concentrations of LiCl (0.02, 0.05, 0.10, 0.21 and 0.45 M), 0.1 M sucrose, 3.2 mM citric acid, 0.1 mM QHCl, 10 μM amiloride and purified water. All tastants were reagent grade, except for QHCl. Stimuli were presented after each of two adapting solutions, which were the same as the solvents: 10 μM amiloride for the stimuli dissolved in amiloride, and purified water for the set dissolved in water. Adapting the tongue to amiloride eliminates its bitter taste during stimulus presentation (Ossebaard and Smith, 1995, 1996; Smith and Ossebaard, 1995).

Apparatus and stimulus application

Solutions were heated in a thermostatically controlled water bath and delivered to the tongue at 34°C. Stimuli were applied by a gravity flow system similar to that described by McBurney (1966). Subjects sat with their tongues extended between the lips so that the solutions stimulated the dorsal anterior tongue surface. Stopcocks controlled a continuous flow of either water or 10 μM amiloride and the application of the test solutions. The flow rate of the adapting and test solutions was 3.5 ml/s and stimulus duration was 10 s.

Procedure

Each of the 70 subjects was randomly assigned to one of four different groups; these groups differed only in the instruction they received prior to the experiment. Twenty subjects were instructed to estimate the overall intensity (strength) of each stimulus ('intensity-only' group); 15 subjects estimated only the saltiness ('salty-only' group), 15 subjects rated only the sourness ('sour-only' group) and 20 subjects gave estimates of the saltiness, sourness, sweetness and bitterness of each solution on a single trial ('profile' group). Subjects in the profile group could also use the response category 'other' if their perception was not described by the given categories.

Prior to the experiment, each subject practiced with magnitude estimation by rating the length of lines. In order to make the estimates of each group comparable, we employed a form of cross-modality matching (Stevens and Marks, 1980; Marks *et al.*, 1988). Before each session all subjects were presented twice with a standard stimulus, or modulus, of 0.1 mM QHCl, dissolved in water and presented after a water rinse. A magnitude estimate of 10 was assigned to either the intensity (for the intensity-only group) or the bitterness (for the other groups) of this modulus. QHCl has no appreciable side tastes; its bitterness and intensity are equal (van der Klaauw and Smith, 1995). Subjects in the intensity-only group were instructed to compare the intensity of the stimuli to the intensity of the modulus. Subjects in the salty-only group gave magnitude estimates of the saltiness of the stimuli, in proportion to the bitterness of the modulus. Subjects in the sour-only group gave magnitude estimates of stimulus sourness, in proportion to the bitterness of the modulus. Subjects in the profile group were instructed to give magnitude estimates of all the taste qualities (salty, sour, sweet, bitter and other) on a single trial, each in proportion to the bitterness of the modulus. Subjects in this group were further instructed to assign the number zero to any taste quality they did not perceive. All groups assigned the number zero when they perceived no taste at all.

In the first half of each session either water or 10 μM amiloride was the adapting solution; in the second half the other adapting solution was used. The adapting rinse flowed for 60 s before each stimulus. The stimulus set dissolved in water was presented when water was the adapting solution and the set dissolved in amiloride when amiloride was the adapting solution. Stimuli in a set were presented twice in each condition. Subjects recorded their estimates on a sheet

of paper while their tongues remained under the flow; a new response sheet was provided after each trial. On the response sheets of the profile group the words *salty*, *sweet*, *sour*, *bitter* and *other* were printed; the sheets of the other groups were blank. After all the stimuli in a set were presented twice under one adapting condition, subjects were given a 10 min break, followed by the other adapting condition. The order of the stimuli in each condition was randomized and random orders were counterbalanced over adapting conditions and subjects. Each subject participated in two sessions, resulting in four replications for each stimulus under each adapting condition. The order of adapting conditions was counterbalanced over sessions and subjects. In one of the two sessions for each subject, water was the first adapting solution and in the other amiloride was the first.

Data analysis

We performed an adjustment procedure to remove any residual scale differences between subjects within a group. For each subject in the profile group the estimates for each of the taste qualities on a single trial were added, resulting in a derived estimate for 'total intensity'. The arithmetic mean of these derived estimates after water adaptation was calculated for each subject in this group; for the subjects in the other groups a similar procedure was performed using the magnitude estimates (of intensity, saltiness or sourness respectively) after water adaptation. Next, these individual means were averaged for each group. Dividing this group average by the mean for a subject in that group provided an adjustment factor for that subject. All magnitude estimates were multiplied by the appropriate factor; for the profile group the estimates for each of the taste qualities were adjusted in this way. Finally, the adjusted estimates of each stimulus replication were averaged for each subject and these means were used for all further analyses. This adjustment procedure resulted in equally weighted estimates for each subject *within* a group. However, it did not change the differences *between* the groups.

To determine whether the use of the QHCl modulus was successful in producing matching magnitude estimates, the intensity estimates of 0.1 mM QHCl (after water) for the intensity-only group were compared with the bitterness estimates of this solution for the profile group. Since QHCl has no side tastes, these estimates should be equal. The mean intensity estimate for the intensity-only group was 14.91 ± 1.51 (SEM); the mean bitterness estimate for the profile group was 15.84 ± 1.48. These means did not differ significantly (t = 0.44; df = 38; P = 0.33). A similar comparison was made between the mean intensity estimate of 0.1 M sucrose (after water) for the intensity-only group and the mean sweetness estimate of this stimulus for the profile group. Sucrose is almost purely sweet, so this analysis served as an additional test to confirm that the estimates of the groups were comparable. The mean intensity estimate of 0.1 M sucrose was 11.54 ± 1.11 for the intensity-only group; the mean sweetness estimate for the profile group was 11.51 ± 1.00. These means did not differ significantly (t = 0.03; df = 38; P = 0.49). Finally, the mean intensity estimate of 10 μM amiloride after water adaptation (6.00 ± 1.02) was compared with the mean bitterness estimate of this solution for the profile group (6.05 ± 0.81). Amiloride has no side tastes so the two mean estimates should be equal: they did not differ significantly (t = –0.04; df = 38; P = 0.48). These results provide strong evidence that linking the magnitude estimates of the intensity-only and the profile groups with the QHCl modulus was successful. We cannot directly compare these estimates to those of the salty-only and sour-only groups since those subjects did not estimate bitterness and sweetness. However, the effectiveness of the modulus for the intensity-only and profile groups supports the assumption that the magnitude estimates of all four groups are comparable.

The data were analysed using repeated measures ANOVA (SPSS v. 6.1). Two-way ANOVAs and Bonferroni paired t-tests were used for post-hoc analyses. Degrees of freedom and P values were corrected with the Huynh–Feldt correction (Huynh and Feldt, 1976) to ensure that significance values were not overestimated due to correlations among observations in the repeated measures design. Although these degrees of freedom were corrected before establishing the P values, only the uncorrected degrees of freedom are reported. Information on significance values is partly provided in the figure captions.

An overall ANOVA for repeated measures was performed with group (intensity-only, salty-only, sour-only and profile) as a between-subjects factor, and adaptation (water and amiloride) and LiCl concentration (0.02, 0.05, 0.10, 0.21 and 0.45 M) as within-subjects factors. Saltiness was the dependent variable for the profile group. The results show a significant group effect [$F(3,66)$ = 42.31, P < 0.001], a significant adaptation effect [$F(1,66)$ = 93.72, P < 0.001] and a significant group × adaptation interaction [$F(3,66)$ = 7.43, P < 0.001]. Additional two-way ANOVAs were performed over each of the groups.

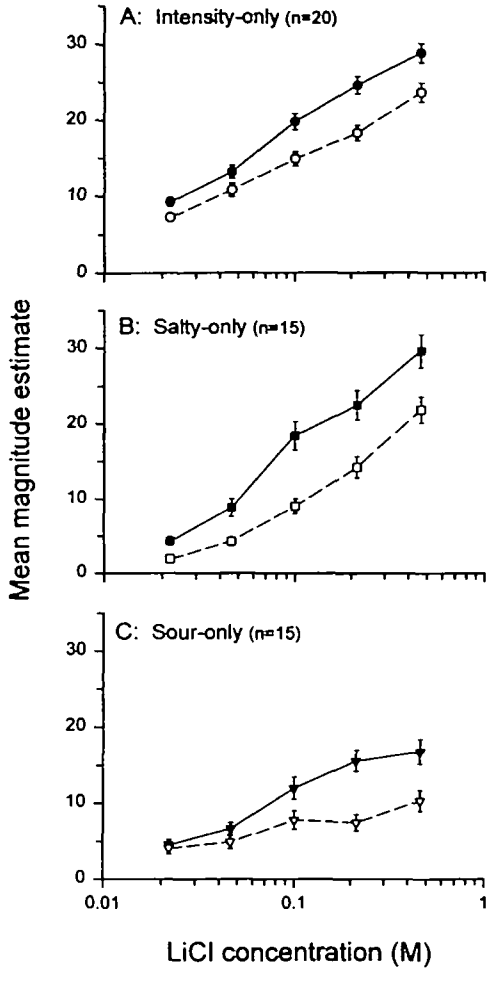

Figure 1 Mean magnitude estimates of the intensity of LiCl for the intensity-only group **(A)**, the saltiness of LiCl for the salty-only group **(B)**, and the sourness of LiCl for the sour-only group **(C)** after water adaptation (solid symbols) and after 10 μM amiloride treatment (open symbols). Amiloride significantly suppressed LiCl intensity [$F(1,19) = 45.77$, $P < 0.001$], saltiness [$F(1,14) = 35.60$, $P < 0.001$] and sourness [$F(1,14) = 51.34$, $P < 0.001$]. Error bars show ±1 SEM.

Before separate ANOVAs were performed over each of the taste qualities of the profile group, an ANOVA over the qualities (salty, sweet, sour, bitter), adaptation and LiCl concentration showed significant quality × adaptation and quality × adaptation × concentration interactions [$F(3,57) = 13.87$, $P < 0.001$ and $F(12,228) = 3.10$, $P < 0.01$ respectively].

Results

Figure 1 shows the mean intensity estimates of LiCl for the intensity-only group (A), the mean saltiness estimates for the salty-only group (B) and the mean sourness estimates for the sour-only group (C) after water (solid symbols) and after 10 μM amiloride (open symbols). In these groups, in which only a single response category was available to the subjects, amiloride produced an effect in each case: it suppressed the overall intensity (Figure 1A), the saltiness (Figure 1B) and the sourness of LiCl (Figure 1C). The amount of suppression was the same for each of these groups; the group × adaptation interaction was not significant [$F(2,52) = 1.04$, $P = 0.36$].

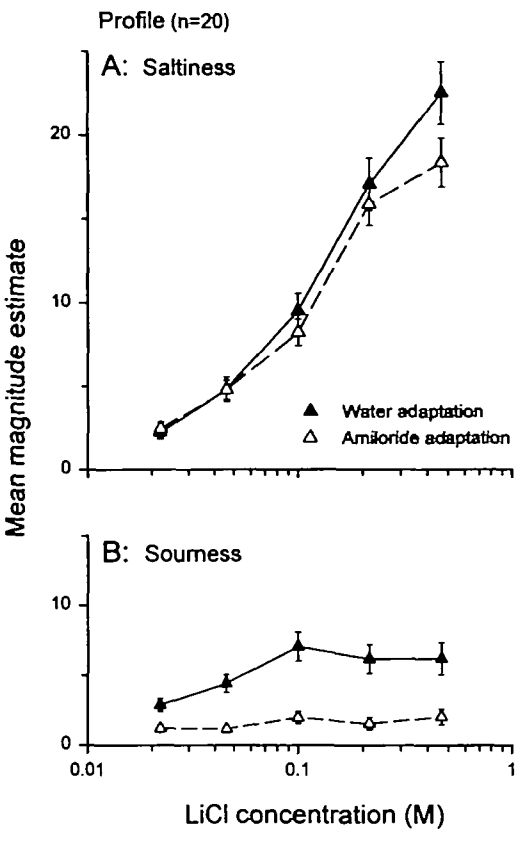

Figure 2 Mean magnitude estimates of the saltiness **(A)** and sourness **(B)** of LiCl after water adaptation (solid symbols) and after 10 μM amiloride treatment (open symbols) for the profile group. Sourness was significantly reduced by amiloride [$F(1,19) = 14.43$, $P < 0.001$]. Saltiness was unaffected (adaptation main effect not significant [$F(1,19) = 1.09$, $P = 0.31$]; adaptation × concentration interaction was significant [$F(4,76) = 3.03$, $P < 0.05$] but none of the Bonferroni adjusted paired t-tests for the individual concentrations reached significance [$2.25 < t < 0.87$; $df = 19$; $0.04 < P < 0.69$]. Error bars show ±1 SEM.

The mean magnitude estimates of LiCl saltiness (A) and sourness (B) for the profile group are shown in Figure 2. Sweetness, bitterness and 'other' estimates were very small and are therefore not presented in this figure. There was no significant effect of amiloride on the saltiness of LiCl for this group; sourness, however, was significantly reduced.

Comparison of the LiCl saltiness estimates of the profile and salty-only groups revealed that after water adaptation,

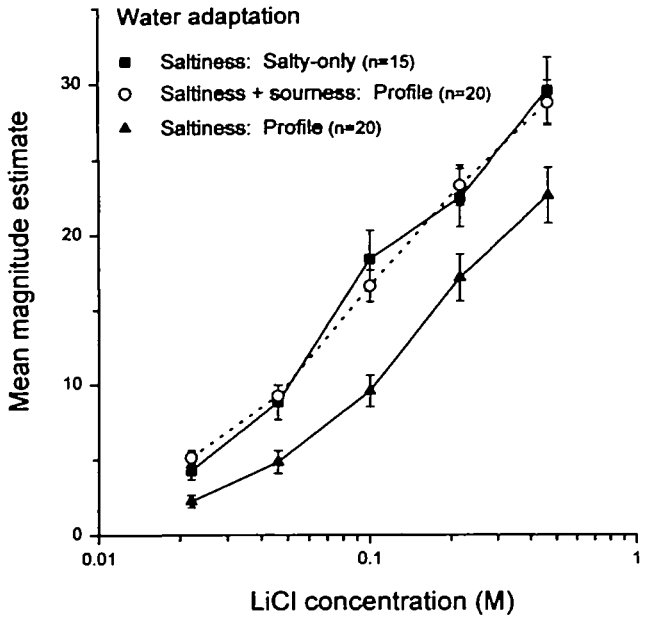

Figure 3 Mean magnitude estimates of the saltiness of LiCl after water adaptation for the profile group (solid triangles) and for the salty-only group (solid squares), and the summed magnitude estimates of saltiness and sourness for the profile group (dotted line, open circles). Error bars show ±1 SEM.

saltiness for the profile group was significantly less than saltiness for the salty-only group (mean over concentrations =11.10 and 16.66 respectively, Bonferroni modified LSD). However, after amiloride treatment saltiness was equal for both groups (profile = 10.19; salty-only = 10.75). Thus, the saltiness estimates of the salty-only group after amiloride were equal to the saltiness estimates of the profile group after amiloride or after water.

The larger saltiness estimates of the salty-only group after water may have resulted from the subjects' combining of saltiness and sourness into one estimate when they were asked to rate only saltiness. If this is true, then the summed saltiness and sourness estimates of the profile group should be equal to the saltiness estimates of the salty-only group. Figure 3 shows the saltiness of LiCl after water for the profile group (solid triangles) and for the salty-only group (solid squares), and the summed saltiness and sourness estimates for the profile group after water (dotted line, open circles). The data in this figure support the hypothesis that the salty-only subjects are combining saltiness and sourness: the sum of saltiness and sourness for the profile group equals saltiness for the salty-only group.

The results of the profile group further show that amiloride adaptation reduced the bitterness of 10 μM amiloride and 0.1 mM QHCl, but had no effect on the sweetness of 0.1 M sucrose or on the sourness of 3.2 mM citric acid. These results (not shown) are similar to our previous findings (Ossebaard and Smith, 1995, 1996). The decrease in the bitterness of QHCl after amiloride may be the result of cross adaptation (cf. McBurney *et al.*, 1972). Amiloride also had no effect on the sourness of 3.2 mM citric acid for the sour-only group, and these sourness estimates were approximately equal to those of the profile group [mean sourness estimate after water 10.91 ± 0.98 (SEM) and 11.33 ± 0.96 respectively].

Discussion

The evidence that amiloride has an effect on the taste of Na^+ and Li^+ salts in humans is compelling (Schiffman *et al.*, 1983; McCutcheon, 1992; Tennissen, 1992; Ossebaard and Smith, 1995, 1996; Smith and Ossebaard, 1995; Tennissen and McCutcheon, 1996). Only one human psychophysical study (Desor and Finn, 1989) has failed to show an effect of amiloride. However, in that study the mixed presentation of amiloride and non-amiloride trials might have been responsible for the lack of an effect, as recently suggested (Tennissen and McCutcheon, 1996). The current debate concerns whether it is the saltiness or the sourness of Na^+ and Li^+ salts that is reduced by amiloride.

Previous studies either showed suppression of the saltiness of Na^+ and Li^+ salts by amiloride (McCutcheon, 1992; Tennissen, 1992; Tennissen and McCutcheon, 1996) or no effect on their saltiness but a specific effect on their sourness (Ossebaard and Smith, 1995, 1996). One of the differences between these studies was the number of response categories available: those showing an effect on saltiness used one response category (saltiness), and those demonstrating an effect only on sourness assessed all taste qualities (saltiness, sweetness, sourness and bitterness). Tennissen and McCutcheon (1996), who had subjects estimate only saltiness, concluded that '...amiloride's action on the human taste system appears, therefore, to be specific to those structures mediating the salty taste'. Although these authors stated that our conclusion (that the sour side taste and not the saltiness is affected by amiloride; Ossebaard and Smith, 1995) might be another valid interpretation of their data, they also suggested that the differences in procedure and amiloride concentration between the studies were too large to rule out other possibilities. However, the present data strongly support the hypothesis that the number of

response categories is responsible for the differences in the amiloride effects. Using the same experimental procedures (amiloride concentration, stimulation method, exposed area of the tongue, flow rate of the solution, stimulus set, stimulus duration, interstimulus interval, and adaptation duration), amiloride suppressed *saltiness* only if saltiness alone was rated (Figure 1B). *Sourness* (but not saltiness) was suppressed if all taste qualities were rated (Figure 2B), or if sourness alone was rated (Figure 1C).

But why is saltiness reduced by amiloride if subjects rate only saltiness? Previously we have suggested that 'dumping' of the sour side taste and the salty main taste into a single magnitude estimate could explain this effect (Ossebaard and Smith, 1995, 1996). Any effect of amiloride on the sour side taste would consequently be reflected in the estimate for saltiness. Several psychophysical studies have reported the existence of such a 'dumping' effect: a bias that results in the assigning of a sensation to an inappropriate category, usually in the absence of an appropriate one (Frank and Byram, 1988; Frank *et al.*, 1993; Clark and Lawless, 1994). For example, when subjects are instructed to ignore all attributes except sweetness, the addition of a strawberry odor results in enhanced estimates of the sweetness of sucrose (Frank and Byram, 1988) or aspartame (Clark and Lawless, 1994). That is, the odor perception is 'dumped' into the sweetness category. However, if subjects are instructed to estimate sweetness, sourness and fruitiness in a mixture of sucrose and strawberry odor, sucrose sweetness is unaffected by the addition of the odor (Frank and Byram, 1988). Providing several appropriate response categories eliminates the dumping effect. Similar results have been shown in mixtures of two taste stimuli (Frank *et al.*, 1993).

Providing the opportunity to use more than one response category demonstrates that subjects are able to differentiate between a quality that is not affected by amiloride and one that is virtually eliminated after amiloride treatment. The quality that is eliminated is labeled as 'sourness', whereas the quality that remains unaffected is labeled as 'saltiness'. The single magnitude estimate obtained when only one response category (saltiness) was available reflected both saltiness and sourness (Figure 3). Once sourness was eliminated by amiloride treatment, the magnitude estimates of LiCl saltiness were the same for both the salty-only and the profile group. In addition, the magnitude estimates of the sourness of LiCl by the sour-only group (Figure 1C) were considerably greater than those by the profile group (Figure 2B), suggesting that subjects may also combine sensations when asked to rate only LiCl sourness.

Sourness is usually associated with acids, and therefore with pH and the transduction of hydrogen ions. However, the taste profiles of salts in humans suggest that many salts have a sour side taste (Smith and McBurney, 1969; McBurney and Bartoshuk, 1973; Cardello, 1979; Schiffman *et al.*, 1980; Smith and van der Klaauw, 1995; van der Klaauw and Smith, 1995). Apparently, the transduction of hydrogen ions is not the only mechanism that can result in the sour taste quality. The present data and our previous studies (Ossebaard and Smith, 1995, 1996) indicate that the sourness of Na^+ and Li^+ salts is mediated by amiloride-sensitive channels on the apical membrane of taste receptor cells. The saltiness of these salts may arise from other mechanisms, but it does not appear to depend upon an amiloride-sensitive transduction component.

Even though there is a clear effect of amiloride on the sourness of Na^+ and Li^+ salts, there is no evidence for an amiloride-sensitive transduction component for acids in humans (Ossebaard and Smith, 1995, 1996; Tennissen and McCutcheon, 1996). Recent data suggest that this may be different in the hamster: Gilbertson *et al.* (1992) have reported an amiloride-sensitive proton current in hamster taste cells in response to citric acid. Preliminary data from our own laboratory show that amiloride strongly suppresses the citric acid response in Na^+-best cells of the hamster NST, which may translate into a reduction of citric acid intensity for this species (see also Gilbertson and Gilbertson, 1994). On the other hand, there does not appear to be an amiloride-sensitive proton current in rat taste cells (Harris *et al.*, 1994), suggesting that this transduction pathway may not be universal. Data in the present experiment on the sourness of citric acid show no effect of amiloride, as previously shown in several other experiments (Ossebaard and Smith, 1995, 1996; Tennissen and McCutcheon, 1996).

Studies using other species have linked the suppressive effect of amiloride on neurophysiological responses to Na^+ and Li^+ salts to a suppression of saltiness (Scott and Giza, 1990; Giza and Scott, 1991), most likely because saltiness is the main quality of these salts for humans. However, physiological measures obtained from animal preparations in response to Na^+ and Li^+ salts should not be confused with the salty sensation elicited by these salts in humans. Clearly, the amiloride-sensitive transduction component is a major contributor to the distinctive

taste of NaCl in other species (see Hill *et al.*, 1990). The present data demonstrate, however, that in humans it is the *sourness* and not the saltiness of these salts that is affected by amiloride. Because taste quality is a human psychological concept, it is misleading to draw conclusions about quality on the basis of physiological or behavioral measures in other species that do not directly assess this level of information.

Conclusion

Salts are not just salty. LiCl, for instance, has a large sour component in addition to its salty taste. The present study shows that amiloride suppresses LiCl saltiness if only saltiness is rated, and LiCl sourness if only sourness is rated. However, when all taste qualities are available as response categories, amiloride strongly suppresses sourness but not saltiness. Furthermore, these results indicate that subjects provided with the single response category 'saltiness' combine saltiness and sourness into one magnitude estimate. We conclude that when subjects are asked to rate only saltiness, amiloride's effect on those ratings reflects the suppression of the salts' sour component. The action of amiloride on the human taste system appears to be on those mechanisms mediating the sour component of Na^+ and Li^+ salts. The saltiness of these stimuli may be mediated by other transduction mechanisms.

ACKNOWLEDGEMENTS

This research was supported by National Institute on Deafness and Other Communication Disorders Grant DC00353 to D.V.S. A portion of these data was presented at the 1996 meeting of the Association for Chemoreception Sciences, Sarasota, Florida. Thanks are due to Dr Mark B. Vogt for valuable comments on the manuscript.

REFERENCES

Avenet, P.P. and Lindemann, B. (1988) Amiloride-blockable sodium currents in isolated taste receptor cells. *J. Membrane Biol.*, **105**, 245–255.

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.

Cardello, A.V. (1979) Taste quality changes as a function of salt concentration in single human taste papillae. *Chem. Sens. Flav.*, **4**, 1–12.

Clark, C.C. and Lawless, H.T. (1994) Limiting response alternatives in time-intensity scaling: an examination of the halo-dumping effect. *Chem. Senses*, **19**, 583–594.

Desor, J.A. and Finn, J. (1989) Effects of amiloride on salt taste in humans. *Chem. Senses*, **14**, 793–803.

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

Frank, R.A. and Byram, J. (1988) Taste–smell interactions are tastant and odorant dependent. *Chem. Senses*, **13**, 445–455.

Frank, R.A., van der Klaauw, N.J. and Schifferstein, H.N.J. (1993) Both perceptual and conceptual factors influence taste–odor and taste–taste interactions. *Percept. Psychophys.*, **54**, 343–354.

Gilbertson, D.M. and Gilbertson, T.A. (1994) Amiloride reduces the aversiveness of acids in preference tests. *Physiol. Behav.*, **56**, 649–654.

Gilbertson, T.A., Avenet, P., Kinnamon, S.C. and Roper, S.D. (1992) Proton currents through amiloride-sensitive Na^+ channels in hamster taste cells: role in acid transduction. *J. Gen. Physiol.*, **100**, 803–824.

Giza, B.K. and Scott, T.R. (1991) The effect of amiloride on taste-evoked activity in the nucleus tractus solitarius of the rat. *Brain Res.*, **550**, 247–256.

Harris, D.E., Gilbertson, D.M., Monroe, W.T., Kinnamon, S.C. and Gilbertson, T.A. (1994) Contribution of amiloride-sensitive pathways to acid transduction in rats. *Chem. Senses*, **19**, 481–482.

Heck, G. L., Mierson, S. and DeSimone, J.A. (1984) Salt taste transduction occurs through an amiloride-sensitive sodium transport pathway. *Science*, **233**, 403–405.

Hellekant, G., DuBois, G.E., Roberts, T.W. and van der Wel, H. (1988) On the gustatory effect of amiloride in the monkey (*Macaca mulatta*). *Chem. Senses*, **13**, 89–93.

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

Hill, D.L. and Bour, T.C. (1985) Addition of functional amiloride-sensitive components to the receptor membrane: a

possible mechanism for altered taste responses during development. *Dev. Brain Res.*, **20**, 310–313.

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.

Huynh, H. and Feldt, L.S. (1976) Estimation of the Box correction for degrees of freedom from sample data in the randomized block and split-plot designs. *J. Educ. Stat.*, **1**, 69–82.

Marks, L.E., Stevens, J.C., Bartoshuk, L.M., Gent, J.F., Rifkin, B. and Stone, V.K. (1988) Magnitude-matching: the measurement of taste and smell. *Chem. Senses*, **13**, 63–87.

McBurney, D.H. (1966) Magnitude estimation of the taste of sodium chloride after adaptation to sodium chloride. *J. Exp. Psychol.*, **72**, 869–873.

McBurney, D.H. and Bartoshuk, L.M. (1973) Interactions between stimuli with different taste qualities. *Physiol. Behav.*, **10**, 1101–1106.

McBurney, D.H., Smith, D.V. and Shick, T.R. (1972) Gustatory cross adaptation: sourness and bitterness. *Percept. Psychophys.*, **11**, 228–232.

McCutcheon, N.B. (1992) Human psychophysical studies of saltiness suppression by amiloride. *Physiol. Behav.*, **51**, 1069–1074.

Nakamura, M. and Kurihara, K. (1990) Nonspecific 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.

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

Ossebaard, C.A. and Smith, D.V. (1995) Effect of amiloride on the taste of NaCl, Na-gluconate and KCl in humans: implications for Na^+ receptor mechanisms. *Chem. Senses*, **20**, 37–46.

Ossebaard, C.A. and Smith, D.V. (1996) Amiloride suppresses the sourness of NaCl and LiCl. *Physiol. Behav.*, **60**, 1317–1322.

Schiffman, S.S., McElroy, A.E. and Erickson, R.P. (1980) The range of taste quality of sodium salts. *Physiol. Behav.*, **24**, 217–224.

Schiffman, S.S., Lockhead, E. and Maes, F.W. (1983) Amiloride reduces the taste intensity of Na^+ and Li^+ salts and sweeteners. *Proc. Natl Acad. Sci. USA*, **80**, 6136–6140.

Scott, T.R. and Giza, B.K. (1990) Coding channels in the taste system of the rat. *Science*, **249**, 1585–1587.

Smith, D.V. and McBurney, D.H. (1969) Gustatory cross-adaptation: does a single mechanism code the salty taste? *J. Exp. Psychol.*, **80**, 101–105.

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

Smith, D.V. and van der Klaauw, N.J. (1995) The perception of saltiness is eliminated by NaCl adaptation: implications for gustatory transduction and coding. *Chem. Senses*, **20**, 545–557.

Smith, D.V., Liu, H. and Vogt, M.B. (1996) Responses of gustatory cells in the nucleus of the solitary tract of the hamster after NaCl or amiloride adaptation. *J. Neurophysiol.*, **76**, 47–58.

Stevens, J.C. and Marks, L.E. (1980) Cross-modality matching functions generated by magnitude estimation. *Percept. Psychophys.*, **27**, 379–389.

Tennissen, A.M. (1992) Amiloride reduces intensity responses of human fungiform papillae. *Physiol. Behav.*, **51**, 1061–1068.

Tennissen, A.M. and McCutcheon, N.B. (1996) Anterior tongue stimulation with amiloride suppresses NaCl saltiness, but not citric acid sourness in humans. *Chem. Senses*, **21**, 113–120.

van der Klaauw, N.J. and Smith, D.V. (1995) Taste quality profiles for fifteen organic and inorganic salts. *Physiol. Behav.*, **58**, 295–306.

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.

Received on July 20, 1996; accepted on November 11, 1996