Anterior Tongue Stimulation with Amiloride Suppresses NaCl Saltiness, but not Citric Acid Sourness in Humans

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Abstract

Suppression of the saltiness of NaCl solutions by amiloride, a sodium channel blocker, has previously been reported a number of times in humans. This suppression was seen with techniques that involved stimulation of small areas of the tongue. It was not certain, however, whether amiloride would suppress saltiness with stimulation of a much larger area of the tongue; one published study, in fact, found negative results with whole mouth stimulation. For this study, eight subjects dipped a large part of the anterior portion of the tongue into a 10-ml sample of NaCl solution, or a NaCl and amiloride solution, and reported its magnitude of saltiness intensity. The results show that amiloride suppressed the saltiness of NaCl when a large area of the anterior tongue was stimulated. Consistent with previous studies, there was individual variability across subjects in this suppressive effect of amiloride. This study also used this method to test the effects of amiloride on the sourness of citric acid, which was not expected to be affected. No suppression of sourness was seen with amiloride. **Chem. Senses 21: 113–120, 1996.**

Introduction

Amiloride, a sodium channel blocker, affects the response to NaCl stimulation. Numerous experiments with animals have demonstrated the suppressive effects of amiloride on NaCl response; recordings from the chorda tympani nerve in the rat, mouse, golden hamster, gerbil and rhesus monkey show a large reduction in response to NaCl (Jakinovich, 1985; Hellekant *et al.*, 1988; Ninomiya and Funakoshi, 1988; Ninomiya *et al.*, 1989; Hettinger and Frank, 1990). In the dog, amiloride reduced chorda tympani response to NaCl and to KCl (Mierson *et al.*, 1988). BALB and DBA mice strains do not show amiloride reduction of neural taste activity, while C57 and C3H mice do (Ninomiya *et al.*, 1989). Amiloride blocks transepithelial transport of Na and Li in the frog (Hamilton and Eaton, 1985). However, no suppressive effect of amiloride in response to NaCl was seen in the mudpuppy (McPheeters and Roper, 1985). At the single unit level, Schiffman *et al.* (1983) showed that amiloride reduced the electrophysiological responses to NaCl in the nucleus tractus solitarius of the rat. Scott and Giza (1990) reported the response profiles of four groups of neurons in the same area of the rat and found that amiloride application suppressed the response of two of the four groups of neurons that normally responded to NaCl; the groups classified as salt-sweet and salt sensitive. Changes in behavioral response to NaCl in rats have also been reported. In a conditioned aversion experiment, rats failed to avoid ingesting a 0.1 M concentration of NaCl that was previously associated with gastric distress if their tongue was exposed to amiloride (Hill *et al.*, 1990). Other studies (Bernstein and Hennessy, 1987; McCutcheon, 1991) demonstrated that sodium deficient rats did not increase ingestion of hypertonic NaCl solutions after exposure to amiloride. Amiloride can also inhibit the larval chemotactic responses of *Drosophila* to 0.1 M NaCl (Jenkins and Tomkins, 1990).

In human experiments, where small areas of the anterior surface of the tongue were exposed to amiloride, subjects reported a decrease in the perceived intensity of NaCl solutions. Using filter paper discs to apply the stimuli to the tongue, Schiffman *et al.* (1983, 1986) reported that subjects experienced a reduction in intensity as measured by the method of stimulus matching. In two other human studies (McCutcheon, 1992; Tennissen, 1992), which compared NaCl stimulation of anterior tongue fungiform papillae to stimulation with a mixture of NaCl plus amiloride, subjects reported that amiloride decreased the perceived intensity of the saltiness of NaCl. Halpern *et al.* (1992) used mixtures of NaCl and amiloride in a flow method, and reported that some subjects experienced a change in the taste quality of NaCl; namely, the description of NaCl changed from 'salty' to 'odd/indescribable' after exposure to amiloride. Another study by Halpern *et al.* (1993) reported decreases in the use of the descriptor 'saltiness' across subjects when exposed to amiloride. However, not all subjects showed differences in time-intensity measurements of saltiness. Smith and Van Der Klaauw (1993) found that flowing amiloride over the anterior tongue produced a partial reduction in the saltiness of NaCl. In a study using stimulation of the whole mouth, however, Desor and Finn (1989) reported no suppression of NaCl saltiness by amiloride. They asked subjects to rate NaCl solutions on a 7-point category scale for perceived saltiness, sweetness, sourness and bitterness. The results showed that amiloride made no difference in the ratings for saltiness or any other quality category. It is suprising that stimulation with amiloride in the Desor and Finn (1989) study did not yield results that are consistent with the body of information now known about amiloride's suppressive effects. The purpose of the current study, therefore, was to investigate the amiloride suppression of saltiness using stimulation of the anterior portion of tongue, an area smaller than the whole-mouth technique of Desor and Finn (1989), which could involve input from the glossopharyngeal nerve and larger than sets of fungiform papillae. Additionally, this study was done to examine the specificity if amiloride's effects on saltiness as compared to sourness.

Experiment 1a

Methods

Subjects

Eight subjects from the State University of New York at Albany (five females and three males) participated in the study. They ranged in age from 20 to 36 and were paid for their participation.

Stimuli

Stimuli were three concentrations of NaCl (0.05, 0.15 and 0.30 M) for the salty taste, and three concentrations of citric acid (0.02, 0.05 and 0.08 M) for the sour taste. QHCl (0.00025 M, pH 7.1) was used in some conditions as a control for the bitter taste of amiloride. QHCl was used because it match the bitter quality of the amiloride and has not been reported to influence the perception of saltiness when mixed with NaCl, unlike the use of bitter-tasting caffeine which can affect the perception of saltiness (Brosvic and Rowe, 1992). The QHCl and amiloride were matched for iso-intensity of bitterness. Amiloride (500 μM, pH 6.4) was presented alone and mixed with the stimuli. This concentration of amiloride was shown to be effective in previous human work (McCutcheon, 1992; Schiffman *et al.*, 1983). De-ionized water was used as the solvent for all chemicals.

Procedure

Training

Each subject was given a 40-min training session in the use of magnitude estimation, a proportional ratio judgment of intensity of the test stimulus. Citric acid was chosen as a training stimulus in order to avoid setting a bias to subsequent testing with NaCl and to allow the subjects to feel comfortable with the procedure. The standard, 0.05 M citric acid, was assigned a value of '10' and all taste intensities were judged relative to that standard. If the taste intensity was twice as strong as the standard, the subject was to assign it a '20'; if the intensity was half as strong as the standard, then it was assigned a '5'; and so forth. Half of the practice trials were with citric acid (0.02, 0.05 and 0.08 M) and the other half were of the same concentrations, but mixed with 0.00025 M QHCl. Quinine was used as the control for the bitter taste of amiloride, which would later be presented during the test trials. Subjects were told they might taste something else in addition to the citric acid taste and that they were to assign a magnitude estimation number only to the perceived sourness of the stimuli. Stimuli were presented in 10-ml amounts in small paper cups cut down to the depth of 2.5 cm so that the tongue could be comfortably dunked into the stimulus solution. Approximately 1.5–2.0 cm of the front of the tongue was submerged by this technique. Subjects dipped the anterior tongue into the 10-ml solutions and, after a few seconds, wrote down their magnitude estimation.

Test procedure

The test session used three concentrations of NaCl (0.05, 0.15 and 0.30 M) dissolved in water, 0.00025 M QHCl or 500 μM amiloride as test stimuli. Subjects were asked to rate the perceived saltiness of the stimuli in the same manner as they had been taught to judge sourness magnitudes during training. That is, 0.15 M was presented at the beginning of the test session and assigned a magnitude of '10'; all stimuli were judged against that standard. Again, they were told to expect some added tastes in some of the stimuli, but to judge only the magnitude of the salty taste. Following each trial, they rinsed their mouth with water and rested 30 s before the next trial began. Stimuli were presented as in the training session; the anterior tongue was dipped into 10-ml samples and magnitude estimations given.

The whole test session consisted of 24 trials, divided into three blocks of eight trials each. The blocks of trials were always presented in the same order: NaCl, NaCl plus QHCl, NaCl plus amiloride. There were several reasons for keeping a fixed order rather than balancing conditions. First, we were convinced from our considerable experience with the procedure that saltiness judgements are stable over 24 trials and that having quinine plus NaCl trials preceding NaCl only trials does not affect this stability. [The question of order effects was evaluated in a control study (Experiment 1b) and found to be not of concern.] Secondly, amiloride can produce lingering taste insensitivity lasting 30 min or more, so it can only be used at the end of a test series. Finally, having NaCl-alone trials first is helpful in establishing a clear baseline experience against which to judge any changes. Within each block of eight trials the three stimulus concentrations and the water stimulus trials, two per stimulus, were randomly presented. Prior to each block of eight trials, the tongue was exposed, by dunking for 30 s, to water before NaCl trials, to 0.00025 M QHCl before NaCl plus QHCl trials, and to 500 μM amiloride before NaCl plus amiloride

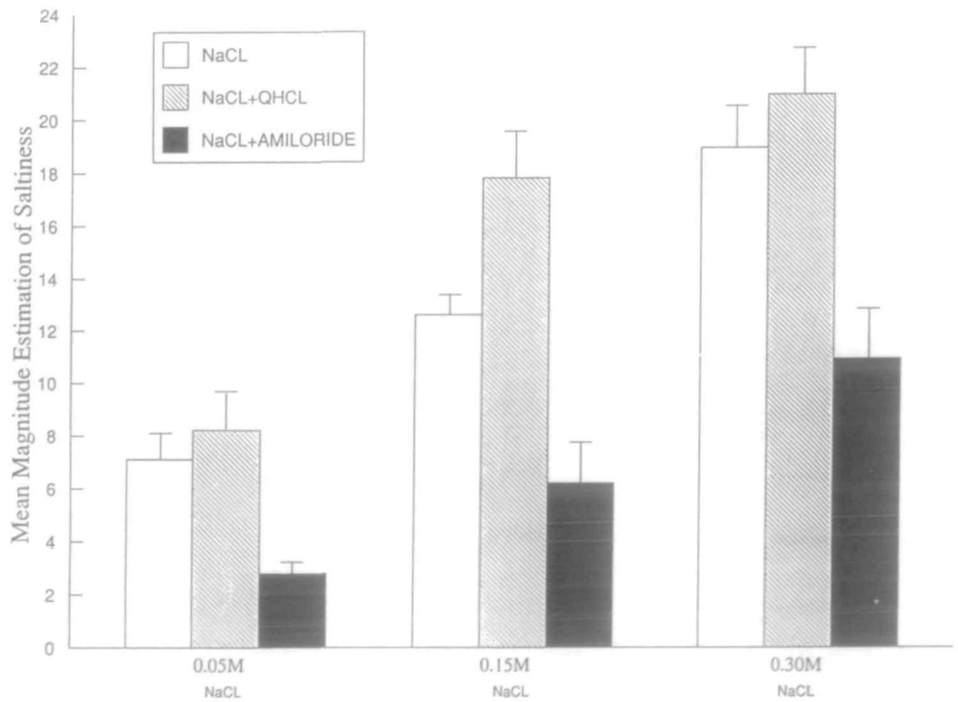

Figure 1 Magnitude estimation ratings for saltiness across all eight SUNY subjects, for NaCl alone (0.05, 0.15 and 0.30 M), NaCl mixed with quinine hydrochloride (QHCl, 0 00025 M) and NaCl mixed with amiloride (500 μM). White bars indicate ratings for NaCl solutions, hatched bars show ratings for NaCl mixed with QHCl; dark bars show ratings for NaCl mixed with amiloride. The vertical bars are standard error bars. Saltiness ratings for trials of water, QHCl, and amiloride alone were negligible; mean values are 0.123, 0.50, 0 06, respectively.

trials. The point of this was to increase exposure time to amiloride in order to strengthen the anticipated suppression effect on NaCl saltiness; the other pre-exposure conditions were simply procedural controls.

Results

Magnitude estimation of saltiness significantly decreased by approximately 50% when the NaCl stimuli contained 500 μM amiloride, but not when they contained an equally bitter substance, 0.00025 M QHCl [$F(1,7) = 15.4, P < 0.01$; see Figure 1]. This decrease was seen in all three concentrations, and was reliable at 0.15 and 0.30 M NaCl concentrations that are unequivocally salty to most subjects (Tukey test, $P < 0.01$). The strength of the suppressive effect was not the same in all subjects; two of the subjects (nos 6 and 8) failed to exhibit saltiness magnitude changes (see Figure 2). No subject showed suppression with QHCl. If anything, QHCl might have slightly enhanced saltiness of the 0.15 M NaCl for some subjects (nos 1, 2, 3, 5, 6 and 7).

Discussion

The results of this study show that amiloride exerts its suppressive effect on NaCl saltiness when a large part of

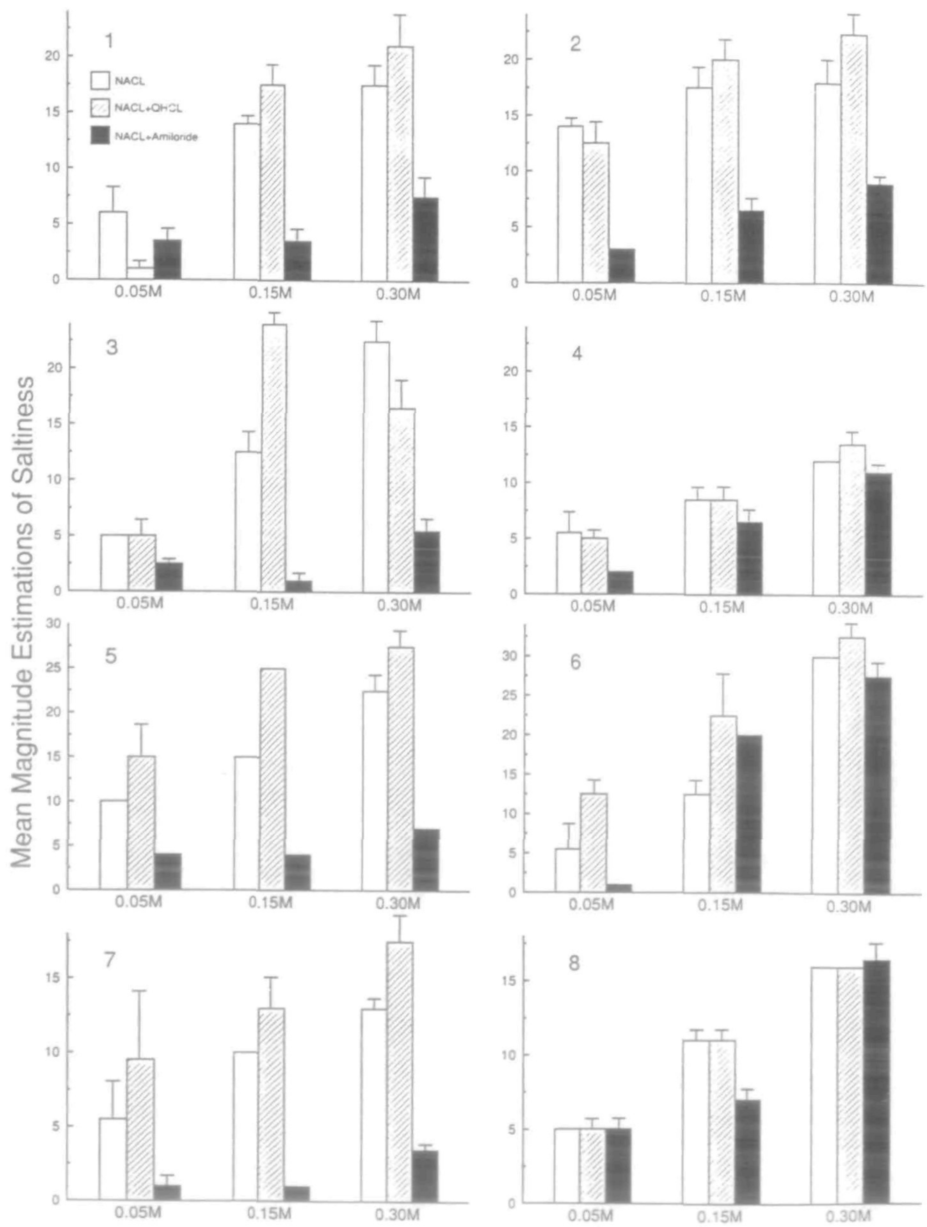

Figure 2 Magnitude estimation ratings of saltiness for each of the eight SUNY subjects. Arithmetic means are shown for stimulation with NaCl alone (0.05, 0.15 and 0.30 M), NaCl mixed with QHCl (0.00025 M), and NaCl mixed with amiloride (500 μM). The vertical bars are standard error bars.

the anterior tongue is stimulated. Thus, for both small area and large area tongue exposure, amiloride is effective.

That the study by Desor and Finn (1989) failed to find effects of amiloride in humans may be due to several factors. First, and possibly the more major problem, was that the amiloride trials were mixed throughout the session along with the non-amiloride trials. Previous work (McCutcheon, 1992; Tennissen, 1992) showed that the effect of amiloride remains active during the testing session; that is, the blocking by amiloride in one trial does not disappear by the next trial. Secondly, the stimuli in the Desor and Finn (1989) study were mixed with spring water, which may have lowered the pH of the solutions and possibly resulted in no suppressive effect being seen. Interestingly, the Desor and Finn (1989) study used an amiloride concentration of 100 μM, as did the Halpern *et al.* studies (1992, 1993), yet only the latter studies found a suppression of saltiness. Again, it is possible that this could be due to procedural differences in these studies. The data from the current study support nearly all of the studies so far, both from the animal neural recording and behavioral testing as well as from psychophysical testing of humans, in concluding that amiloride interferes with the activation of an important part of the taste message that generates NaCl's distinctive salty taste. A matter that remains to be resolved, however, is the individual variability across human subjects in showing this suppression. The present study, along with other published human data on amiloride taste suppression (Schiffman, 1987; McCutcheon, 1991; Tennissen, 1991; Halpern *et al.*, 1992, 1993) presents some subjects who appear unaffected or minimally affected by amiloride (in contrast to animal studies, where no individual differences in amiloride effectiveness have ever been reported, to our knowledge). The reason for these individual differences remains unknown, but probably is not a trivial matter of differences in access of amiloride to taste receptors; those subjects who do not show and amiloride suppression are as sensitive to the bitterness of amiloride as those showing salty suppression (Tennissen, 1991).

Experiment 1b

Methods

Subjects

Five female subjects from the College of Saint Rose were used in a control study to evaluate possible order effects of a fixed order of trials. The subjects ranged in age from 18 to 23 and were paid for their participation.

Stimuli, training and procedures

Stimuli, training and procedures were the same as in Experiment 1a except that citric acid and amiloride were not used.

Therefore, during the 24 trials test procedure the first block of eight trials was with the three NaCl concentrations and water, the second block of eight trials with QHCl plus NaCl and the third block of eight trials with the same stimuli as the first block of eight trials.

Results

Analysis of variance results were significant [$F(1,4) = 23.8$. $P < 0.01$], indicating an enhancement of saltiness ratings after exposure to QHCl. This is an effect which is opposite to the treatment effect.

Discussion

This control study shows that the order of conditions used in Experiment 1a—NaCl alone first, NaCl plus QHCl second—resulted in an enhancement of the perceived saltiness of NaCl. However, this effect does not complicate the results of amiloride suppression; the enhancement is in the opposite direction to the treatment effect. This enhancement of NaCl saltiness may actually result in seeing less of the decrease of saltiness with amiloride and is probably a contrast effect. It is possible that the increase in magnitude estimation here may be due to the concentration of QHCl (0.00025 M) which was needed to match the bitterness of amiloride. Other work in progress seems to support this idea; using a lower concentration of QHCl does not show an enhancement of saltiness. Additionally, Schifferstein and Frijters (1992) showed that in mixtures of QHCl and NaCl the intensity of the saltiness of NaCl was not changed by its presence in the mixture. However, this possible enhancement effect would be an interesting question to investigate.

The advantages of the fixed order used in Experiment 1a do not seem to be vitiated by order effects.

Experiment 2

There are no psychophysical data to suggest that amiloride has a suppressive effect on acid sourness; when acidic stimuli have been used as a control for the specificity of amiloride's action, the response outcome is almost always unchanged by amiloride (e.g. see McCutcheon, 1992; Schiffman *et al.*, 1983, 1991) Because we have employed a new stimulus exposure condition, however, we addressed the specificity question by repeating the same conditions

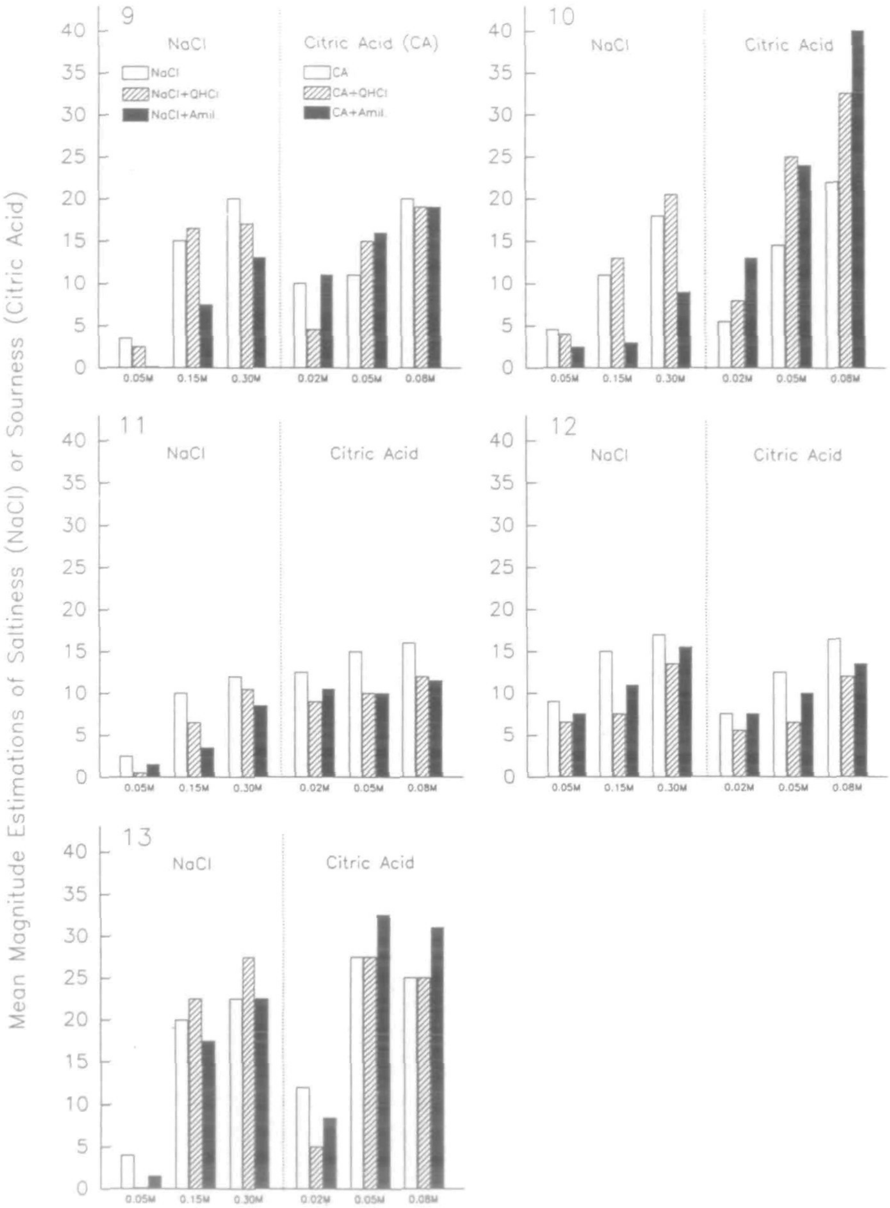

Figure 3 Magnitude estimation ratings of saltiness and sourness for each of the five CSR subjects. Arithmetic means are shown for stimulation with NaCl alone (0 05, 0.15 and 0.30 M), NaCl mixed with QHCl (0.00025 M), and NaCl mixed with amiloride (500 μM), citric acid alone (0.02, 0 05 and 0.08 M), citric acid mixed with QHCl and citric acid mixed with amiloride.

described in Experiment 1a, but with added citric acid sessions.

Methods

Subjects

Five female undergraduate, aged 21–55, from the College of Saint Rose, volunteered as paid subjects for this experiment.

Stimuli

Stimuli were three concentrations of NaCl (0.05, 0.15 and 0.30 M), citric acid (0.02, 0.05 and 0.08 M), QHCl (0.00025 M), amiloride (500 μM, pH 6.4) and deionized water.

Training and test procedure

Training and test procedure were done exactly as described for the first experiment. For three subjects NaCl was tested in the first session and in the second session; several days later, citric acid replaced NaCl in the test session. For the other two subjects, the order was reversed.

Results

The results for these five subjects indicated a suppressive effect of amiloride on saltiness intensity [$F(1,4) = 18.0$, $P < 0.05$]. The data for the citric acid test indicate a significant effect of concentration [$F(1,4) = 7.7$, $P <$

0.05], but no significant reduction of sourness by amiloride [$F(1,4) = 0.331$). Two subjects (nos 9 and 10) showed a large (approximately 50%) reduction of NaCl saltiness with amiloride for some of the concentrations, and some reduction at all concentrations, but no suppression of citric acid sourness (see Figure 3). The other three subjects showed some amiloride suppression of NaCl, but none for citric acid taste magnitudes. None of the subjects showed any taste suppression due to the presence of QHCl. If anything, QHCl and amiloride may have slightly increased the magnitude of the reported sour taste, perhaps by adding their bitter taste to the citric acid taste.

Discussion

Of the five subjects, two were clearly amiloride sensitive in terms of saltiness suppression, yet failed to show any effect of amiloride on citric acid sourness. These data join all other published reports on amiloride sensitive subjects of failure to see a reliable effect of amiloride on citric acid sourness (Schiffman *et al.*, 1983, 1991; McCutcheon, 1992). Recently, it has been reported that hamster taste bud response to citric acid is suppressed by amiloride (Gilbertson *et al.*, 1992). This neural effect supposedly would translate to reduced taste intensity of acids for the hamster. What may be happening in the hamster may not be occurring in humans, however. Apart from an unresolved question about sugars (Schiffman *et al.*, 1983; Tennissen, 1991), amiloride's action on the human taste system appears, therefore, to be specific to those structures mediating the salty taste.

However, in other recent work (Ossebaard and Smith, 1995) amiloride is reported to decrease sourness, but not the saltiness of NaCl stimuli when subjects divided the magnitude estimate of the stimulus into percentages of saltiness, sourness, sweetness and bitterness. We and others (Halpern *et al.*, 1992) have reported that descriptions of NaCl saltiness changed in the presence of amiloride. These subjects reported that the stimuli were still somewhat salty, but that the saltiness had changed along a dimension that included descriptions such as a change from 'sharp' to 'mild' saltiness. Our method of measurment did not ascertain sourness information and the conclusion of Ossebaard and Smith (1994) may be another valid interpretation of the data. Differences in procedure and amiloride concentration between their study and the present study preclude any facile resolution without further investigation. Ossebaard and Smith's (1994) claim that amiloride does not alter the perceptual experience of saltiness of NaCl stimuli is provocative. Given the wide acceptance of the assertion that amiloride does affect the saltiness of NaCl, and is not just a side-taste of sour, it is important to resolve this controversy with appropriate data.

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