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Effects of Amiloride on Gustatory Quality Descriptions and Temporal Patterns Produced by NaCl

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Abstract

The amiloride-sensitivity of perceived taste qualities and time–intensity patterns for NaCl, and interactions between amiloride and NaCl as taste stimuli, were explored using caffeine as the control treatment. NaCl at 100, 250 and 500 mM, dissolved in 10 or 100 μM amiloride, or in caffeine concentrations matched to the amiloride taste, was flowed over 39.3 mm^2 of the anterodorsal tongue for 4 s using a closed stimulus delivery system. Amiloride, caffeine and NaCl in H_2O were also presented. It was found that NaCl–amiloride mixtures were most frequently described as salty, with the incidence of salty descriptions directly associated with NaCl concentration but not significantly associated with the presence or concentration of amiloride. Amiloride in H_2O was called 'bitter', and the incidence of bitter descriptions was significantly associated with the presence of amiloride. The perceived temporal patterns varied with NaCl concentration but did not change with the presence of amiloride, except for an increase in perceived duration. No evidence was found for a dependence upon specific amiloride-sensitive mechanisms of human description of NaCl as salty or of gustatory temporal patterns evoked by NaCl.

Introduction

Sodium chloride is an important gustatory stimulus for many vertebrates, and a significant component of human food systems (e.g. Denton, 1982; Friedman *et al.*, 1991). The sensory processes that permit taste responses to NaCl and discriminative behavior between NaCl and other tastants have been of considerable interest. Gustatory transduction of Na^+ has been conceptualized from various points of view (see Schiffman, 1988; Brand *et al.*, 1989; Margolskee, 1993; Simon and Roper, 1993; Lindemann, 1995; Kinnamon, 1996). The possibility that taste bud receptor cells, being of epithelial origin, might utilize for transduction of Na^+ the amiloride-sensitive epithelial Na^+ channels which are found in many epithelia was examined in germinal biophysical (DeSimone *et al.*, 1981) and psychophysical (Schiffman *et al.*, 1983) studies which applied amiloride to lingual epithelia and observed effects on responses to NaCl (for a review of the psychophysical studies see Halpern, 1998).

Schiffman *et al.* (1983) reported that the concentrations of NaCl, LiCl or stevioside needed to match the perceived taste intensity of a solution containing NaCl, LiCl or stevioside were reduced by ~50% if the to-be-matched solution either was a mixture that also contained 50 μM amiloride or was preceded by a 5 min lingual application of 50 μM amiloride. From these observations, Schiffman *et al.* (1983) suggested that human gustatory responses to sodium and lithium salts involved amiloride-sensitive sodium transport pathways. A number of studies on gustatory aspects of amiloride effects followed these initial reports. They included biophysical, neurophysiological, behavioral and human psychophysical investigations (for a review see Halpern, 1998).

Many subsequent psychophysical studies identified amiloride itself as a bitter tastant, and consequently introduced control treatments with caffeine, quinine hydrochloride (QHCl) or amiloride (Desor and Finn, 1989; Ossebaard and Smith, 1995, 1996; Smith and Ossebaard, 1995; Tennissen and McCutcheon, 1996; Ossebaard *et al.*, 1997). Most of the investigations that provided a control for the taste of amiloride and used amiloride at concentrations of ≤500 μM found little or no effects on the saltiness of NaCl or LiCl solutions (Desor and Finn, 1989; Breslin and Beauchamp, 1995; Ossebaard and Smith, 1995, 1996; Smith and Ossebaard, 1995; Ossebaard *et al.*, 1997) but one study (Tennissen and McCutcheon, 1996) reported substantial suppression of saltiness in some subjects. Higher amiloride concentrations, ranging from 700 μM to 1 mM, were associated with decreases in the incidence of reports of saltiness in some subjects (McCutcheon, 1992) or with decreased saltiness intensity (Tennissen, 1992; Breslin and Beauchamp, 1995), but in the Breslin and Beauchamp (1995) investigation, QHCl, $MgSO_4$, urea or caffeine, at concentrations matched to the bitterness of 700 μM

amiloride, produced even greater reductions in saltiness intensity.

Small decreases in the total taste intensity of NaCl or LiCl solutions with amiloride treatment were generally observed (Ossebaard and Smith, 1995, 1996; Smith and Ossebaard, 1995; Anand and Zuniga, 1997; Ossebaard *et al.*, 1997), with differences between subjects ranging from no effect to substantial decrements (Anand and Zuniga, 1997).

Overall, the available studies on effects of amiloride upon human judgements of NaCl contained several consistent findings: (i) amiloride concentrations of ≥10 μM were perceived as taste stimuli; (ii) with sufficiently high concentrations of amiloride, reductions of saltiness could be produced; and (iii) when effects of amiloride on the taste of NaCl were observed, substantial individual differences were often noted.

On the other hand, a crucial and explicit disagreement existed: some investigations found no changes in saltiness intensity when the taste of amiloride was controlled for by preadaptation, use of another bitter compound in a mixture with NaCl, or both. Other studies, especially those using high amiloride concentrations, often without control treatments, observed decreased saltiness.

Independently of the taste psychophysics data outlined above, much information regarding the range of effects of amiloride on cationic transport mechanisms in cell membranes, and on intracellular processes, has become available since the early 1980s (Halpern, 1998). Briefly, amiloride at concentrations <1 μM selectively blocked amiloride-sensitive Na^+ channels, while Na^+/K^+ exchangers were also blocked at amiloride concentrations of 3 μM to 1 mM, and, in addition, Na^+/Ca^{2+} antiporters were blocked at 300 μM to 1.1 mM amiloride. Furthermore, if amiloride concentrations >100 μM were used, intracellular protein synthesis and enzyme function could be inhibited, since amiloride is able to penetrate cell membranes (Garty and Benos, 1988; Smith and Benos, 1991; Luciani *et al.*, 1992). These characteristics of amiloride effects on cell membrane cationic transport mechanisms and intracellular processes suggest that studies of gustatory actions of amiloride should avoid concentrations >100 μM, should use appreciably lower amiloride concentrations if possible and should minimize the duration of contact between taste receptor organs and amiloride-containing solutions.

The conflicting available data on effects of amiloride on human taste perception, and the methods that have been used to obtain those results, leave a number of questions unresolved. They include: (i) do moderate concentrations of amiloride, i.e. not greater than 100 μM, limited to the anterodorsal tongue region and applied for a duration that does not exceed the ~4 s of a normal sip (see Halpern, 1985; Delconte *et al.*, 1992) alter the incidence with which NaCl solutions are perceived as salty? (ii) Is human taste function strongly affected by amiloride in some individuals but not in others? (iii) Might the previous studies' measurement procedures, i.e. total taste intensity or assignment of taste intensity to one of five specified taste quality categories, have missed effects of amiloride on taste perception of NaCl that could be revealed by other psychophysical approaches?

Consequently, further examination of human gustatory responses to NaCl in the presence of amiloride appeared to be necessary. The experiments were designed to avoid amiloride concentrations that would be likely to have effects other than on membrane transport of Na^+, to control for the taste of amiloride, to obtain a measure of possible effects on the temporal pattern of perceived taste, to directly assess effects of amiloride and a control treatment on unrestricted taste quality descriptions of NaCl, and to present solutions not only at a flow rate and duration comparable to those of a normal sip but also to a consistent area of the anterodorsal tongue. To accomplish these goals, a range of NaCl concentrations dissolved in 10 or 100 μM amiloride, and in caffeine control solutions, was flowed over a small region of the anterodorsal tongue by a closed stimulus delivery system. The temporal pattern of perceived taste intensity was measured throughout each trial using a high-resolution time–intensity method, and taste quality was directly described at the end of every trial. Brief reports of these experiments have been made previously (Halpern *et al.*, 1995, 1996).

Materials and methods

Subjects and screening

Subjects were non-pregnant, non-lactating, paid 18- to 38-year-old adult volunteers associated with Cornell University. They were screened in a two-step procedure, with at least 2 h between the two steps, before participating in the main experiment. In step 1, in which screening was for consistency of judgements, no suggestions were made as to correct taste descriptions. Any consistent (≥4 identical descriptions) but unique (not given for more than one screening solution) taste quality descriptors were accepted for each of the screening solutions, as in Halpern (1987). Screening solutions for step 1 were 40 mM NaCl, 2 mM HCl, 25 mM sucrose and 8 μM quinine sulfate, presented seven times each in random order using whole-mouth, sip-and-spit presentations.

Subjects who passed screening step 1 were next tested in screening step 2 for responses of 'salt' or 'salty' to aqueous 100, 250 and 500 mM NaCl, presented seven times each in random order. A fourth solution, 10 μM amiloride in 100 mM NaCl, was also presented, but descriptions of this mixture were not used for acceptance decisions. Solutions were applied to only the anterior portion of the tongue by protruding the tongue, through closed lips, into a 10 ml disposable polystyrene microbeaker containing ~5 ml of solution (Delwiche *et al.*, 1996). The acceptance criterion for screening step 2 was describing the 100, 250 and 500 mM NaCl as 'salt' or 'salty' at least 4/7 times.

The responses to the 10 μM amiloride in 100 mM NaCl during screening step 2 were not used for subject acceptance decisions. Instead, these responses to 10 μM amiloride in 100 mM NaCl were obtained to permit post-hoc comparisons with effects of amiloride treatment observed during the main experiment (see Table 2).

Eight subjects (three male, five female) passed both steps of the screening procedure. Two withdrew during the experiment while six (one male, five females, age range 18–22; median age 19.5, mean 19.7) participated in all 11 sessions of the main experiment.

Main experiment

Task

Subjects tracked taste intensity using a single axis joystick to control the vertical position of a bright line image on a monochrome computer display (8 cm wide, 4.5 cm high) positioned directly in front of them, which they viewed at eye level from a distance of 13.5 cm (Halpern, 1991, 1994). Horizontal movement of the bright line image occurred every 100 ms across the 80 'columns' of the computer display, with the previous horizontal and vertical positions of the graphic display retained and visible to the subject during an intensity tracking trial. The angular position of the joystick was digitized from the start of the stimulus solution delivery portion of a trial every 100 ms for 8 s, and was automatically stored by a digital computer, together with the successive times of each position.

Subjects were instructed that their task was to track any change in intensity of the taste of a liquid flowing over their tongue, using the joystick. They were told that their responses should be made in reference to the intensity of a standard which would be given six times during a session, and that they would be notified before each such presentation. They were informed that during a standard stimulus trial they should indicate the maximum taste intensity that they would perceive by moving the joystick so that the bright line image on the screen arrived at the standard position, which was represented on the screen by a fixed, constantly visible horizontal white line 3.6 cm above the bottom of the viewing area (69% of the available vertical excursion). Subjects were instructed that if the taste became less intense they should show this on the screen by moving the bright line back toward the bottom of the display, which represented minimum taste intensity. They were asked to try to make their graph of the intensity of the taste as accurate as possible, tracking the taste during its entire duration. With reference to all trials other than the standard trials, subjects were instructed to track the intensity of the stimulus during its entire duration in proportion to the standard. They were told that if the intensity of the liquid flowing over their tongue at any moment was half of the standard, they should position the bright line image on the screen equally between the standard line and the bottom of the display. Subjects were informed that the perceived intensity of stimuli other than the standard might fall anywhere on the graph, above or below the standard line, and that if they did not notice a change in the intensity of the taste, the bright line image should remain positioned along the bottom of the display, which corresponded to the lowest position of the joystick. They were also told that at the end of each trial they would be asked the question 'What did it taste like?'. This provided free choice profiling of taste quality (Rubico and McDaniel, 1992; Halpern, 1997). Trials were separated from each other by ~90 s.

Stimuli

Stimuli were aqueous solutions flowed at 10 ml/s over 39.3 mm^2 of the anterodorsal tongue surface by a closed stimulus delivery system (Kelling and Halpern, 1986). The solvent and the pre-solution and post-solution rinse liquid was polished reverse-osmosis water (H_2O), pH ~6 (< 7 > 5), conductivity <1.3 μS, refractive index = 1.3330. The conductivity and refractive index of all solutions were tested before each was used. Solutions that differed by >10% from established values were discarded.

A 10 s H_2O flow over the tongue preceded each solution presentation; a 5 s H_2O flow immediately followed each solution presentation (Kelling and Halpern, 1988). A subject positioned their head and tongue in the apparatus upon hearing a signal that preceded the initial H_2O flow by 5 s. The subject remained positioned in the apparatus, with the anterodorsal area of their tongue sealing the 10 × 5 mm opening in the liquid delivery tube, until a second auditory signal indicated the end of the liquid flow duration of each trial. The subject's tongue and head were outside of the apparatus for ~66 s between trials.

The solutes were NaCl (analytical reagent grade), amiloride (Sigma Chemical), caffeine (Aldrich or Sigma Chemical), or both NaCl and amiloride or caffeine. Each solution flowed through the stimulus delivery system for ~100 s before presentation to the tongue began. This was sufficient to completely remove the preceding solution, based upon measurements from the flow-through conductivity cells in the delivery system (Kelling and Halpern, 1986).

The order in which the stimulus solutions were presented within a session was randomized, as was which stimulus solutions were presented for which session. However, a 500 mM NaCl in H_2O standard solution was used on the first and second trials of each session and after every four stimulus solutions thereafter. Each subject was told 'the next one is a standard' immediately before any standard trial. No other stimulus information whatsoever was provided.

The first three sessions of the main experiment were practice sessions. Sessions 1–10 presented five different solutions, each of which was presented four times. Session 11, which was shorter, presented three solutions, four times each. Solution duration was always 4.0 s. A particular

solution was presented eight times over the eight data collection sessions.

The stimulus solutions were 100, 250 and 500 mM NaCl, 10 and 100 μM amiloride, caffeine solutions which were selected by each subject to match the taste of these two concentrations of amiloride (see below) and are herein designated 10× and 100× caffeine, and the two amiloride and the two matched caffeine concentrations in each of the three concentrations of NaCl. This provided a total of 19 stimulus solutions. The amiloride in NaCl solutions were the experimental treatments; the caffeine in NaCl solutions, the control treatments.

Caffeine control treatment concentrations

The caffeine control solution concentration for 10 μM amiloride was established for each subject using the following procedure. First a 10 ml quantity of 10 μM amiloride was presented to the subject to be sipped and then spit out. Then 100, 50 and 33 μM caffeine were presented as three 'unknown' solutions, to be sipped and spit. The subject was then asked to identify the closest match to the first solution among the three 'unknown' solutions. The caffeine concentration identified as the closest match was used as the caffeine control solution for that subject, and was designated as their 10× caffeine control solution. A similar procedure was used to determine the caffeine control solution concentration for 100 μM amiloride for each subject, but with 10 ml of 100 μM amiloride as the first solution, and 25, 12.5 and 8.33 mM caffeine as the three comparison liquids. The caffeine concentration identified as the closest match to 100 μM amiloride was used as the caffeine control solution for that subject, and was designated as their 100× caffeine control solution. No information whatsoever was provided on the taste of the solutions or the purpose for which the comparisons were being made. The matched caffeine concentrations ranged from 33 to 100 μM caffeine for the 10× caffeine, and from 8.3 to 12.5 mM for the 100× caffeine (Table 1).

This procedure for allowing each subject to select the caffeine control treatment concentrations which they perceived to most closely match the 10 or the 100 μM amiloride was adopted because in preliminary experiments (Halpern *et al.*, 1992, 1993) concentrations of caffeine predetermined to match for all subjects various concentrations of amiloride had proven to be too strong for some subjects and too weak for others.

Statistical analysis

Results were analyzed using analysis of variance (ANOVA) general linear models. Those F or t outcomes associated with a $P \leq 0.05$ were considered significant. Whenever multiple comparisons were made, P-values were corrected using Bonferroni layering (Darlington, 1990). The quantitative independent variables NaCl concentration and treatment concentration were coded such that their means were zero.

Table 1 Concentrations of aqueous caffeine solutions selected by each subject as the closet match, of three available caffeine solutions (33, 50 and 100 μM caffeine for 10 μM amiloride; 8.3, 12.5 and 25 mM caffeine for 100 μM amiloride) to either 10 or 100 μM amiloride in H_2O

Subject	Caffeine concentration matched to amiloride	
	10× caffeine, matched to 10 μM amiloride	100× caffeine, matched to 100 μM amiloride
JD	50 μM	12.5 mM
KM	100 μM	12.5 mM
LG	33 μM	8.3 mM
SC	33 μM	12.5 mM
SS	100 μM	12.5 mM
TT	100 μM	12.5 mM

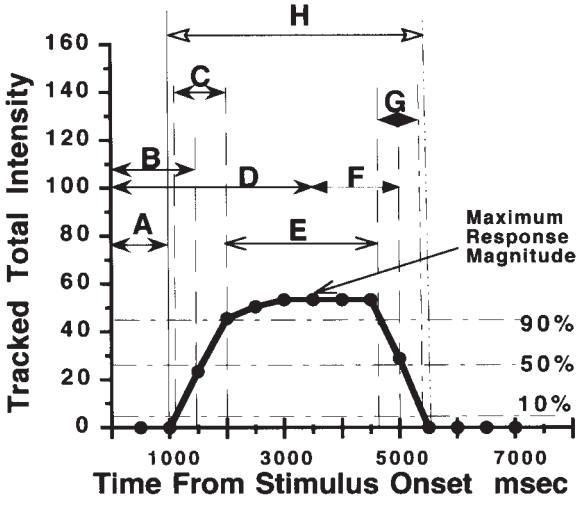


Figure 1 Eight measures of the temporal pattern of time–intensity data. Here and in all subsequent line graphs, the horizontal axis is the time from stimulus liquid onset at the tongue, in ms; the vertical axis is tracked total taste intensity, measured every 100 ms. **(A)** Latency. **(B)** Time up to 50% of maximum response magnitude. **(C)** Rise time (time from 10 up to 90% of maximum response magnitude). **(D)** Time to maximum response magnitude. **(E)** Time within 90% of maximum response magnitude. **(F)** Time down to 50% of maximum response magnitude. **(G)** Fall time (time from 90 down to 10% of maximum response magnitude). **(H)** Duration. Temporal measures are shown in relation to the mean of eight time–intensity responses by subject JD to 4 s stimulation by 100 mM NaCl in H_2O, represented by a heavy continuous line and filled circle symbols.

For the temporal aspects of the time–intensity data as well as the taste quality descriptions, relationships between the three independent variables, namely NaCl concentration, treatment concentration and treatment type (amiloride or caffeine), and each of the dependent temporal variables (Figure 1) or taste quality descriptor variables, were assessed both for individual subjects and across subjects, as follows. First, separate ANOVAs were performed for each subject, testing the nature of the relationships within the responses

of that one subject. Next, for each of the dependent variables, the significant (t with $P \leq 0.05$) ANOVA outcomes for that dependent variable and one of the independent variables were examined across subjects. If the relationship of an independent variable to a particular dependent variable was significantly ($P < 0.05$) positive for some subjects and significantly negative for others, it was concluded that a consistent effect across subjects had not been found for that dependent variable, and no further analysis was done. Otherwise, a meta-analytic technique was used to combine the significance levels from different subjects, in order to test whether the combined significance level was beyond that expected by chance. The technique consisted of multiplying independent significance levels (Ps) together, and comparing the product of Ps to a table developed by Monte Carlo methods (Darlington, 1998). The significant temporal and taste quality outcomes for all subjects are shown in Figures 3 and 4.

The treatment type (amiloride or caffeine) was coded such that the sign of the resulting t values indicated whether the amiloride or the caffeine treatment was associated with the larger mean. In Results and Discussion, instances in which a statistically significant relationship was found between treatment type and a dependent variable will be referred to as 'due to', 'associated with' or having 'changed with' the treatment type that had the larger mean.

Temporal pattern

Temporal aspects of the time–intensity data were analyzed using eight derived measurements, each of which produced a single value, in milliseconds, for each trial with each solution and subject. The eight derived time pattern measures were response latency, time to 50% of maximum response magnitude, rise time (time from 10% of maximum to 90% of maximum), time to maximum, time within 10% of maximum, time down to 50% of maximum, fall time (time from 90% of maximum down to 10% of maximum) and total response duration (Figure 1). ANOVAs were done individually in each subject for each of the derived temporal measures. The model was: derived temporal measure = constant + the three independent variables + the three two-way interactions between the independent variables + the one three-way interaction. The independent variables were NaCl concentration, treatment concentration and treatment type [experimental (amiloride) or control (caffeine)].

Taste quality

Taste quality descriptions were recorded as reported by subjects, with no constraints imposed on permissible words (see O'Mahony *et al.*, 1990). A single descriptor was obtained at the end of each trial. For ANOVA analysis, all descriptors were placed into one or more of five categories by the following scheme: a bitter category if the word 'bitter' was used in the description; a salty category if 'salt' or 'salty' was used; a sour category if 'sour' was used; a sweet category if 'sweet' was used; and a no taste category if the description was 'no taste'. These categories accommodated all the descriptions made by subjects. When taste quality descriptions occurred that contained two of the specified words, e.g. 'salty and bitter', the occurrence of both descriptors was encoded. ANOVAs for each of these five taste quality categories were done across trials for the several solution parameters individually in each subject.

In addition, in order to directly represent the frequency of taste quality descriptor use across subjects, the incidence of both single descriptive words, e.g. 'bitter' as a one word description, and of multiple word descriptions, e.g. 'bitter, salt', were calculated for each stimulus solution. For these across-subjects computations of taste quality descriptor frequency, a Salty category was established for occurrences of the word 'salt' or the word 'salty' as the entire descriptor;a Salt/Bitter category for 'salty and bitter' or 'bitter, salt'; a Salt/Sour category for 'salty, sour' or 'salt, sour'; and a Salt/Sweet category for instances in which the taste quality description was 'salt, sweet' or 'sweet and salty'. Occurrences of the single word descriptors 'bitter', 'sour' or 'sweet', and of 'no taste', were each treated separately in calculations of taste quality descriptor frequency (see Figure 2).

Results

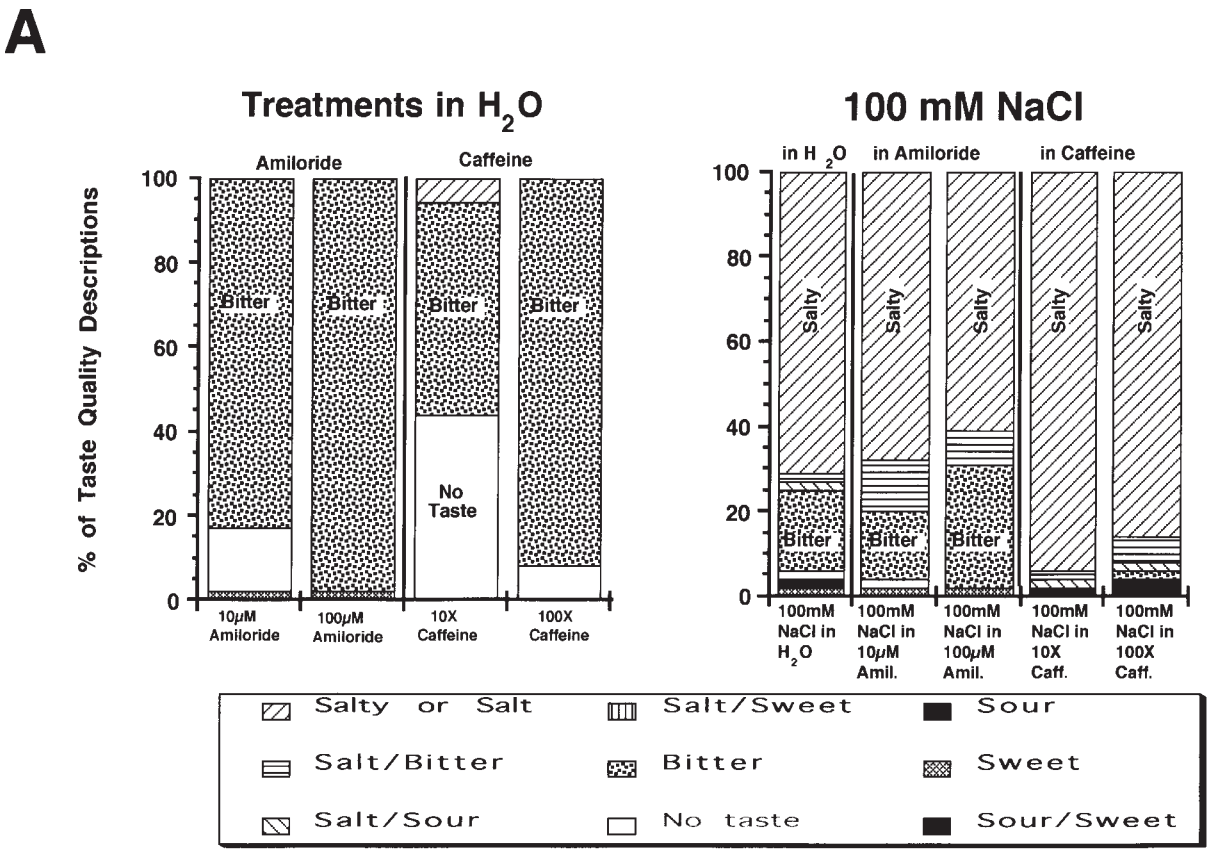
Taste quality

Across subjects

The words 'salt' and 'salty' were the majority of the taste quality descriptions for every stimulus solution that contained NaCl (Figure 2). For subjects LG and SS, all of their descriptions of 100 mM NaCl in water were the words 'salt' or 'salty', but description of 100 mM NaCl as 'bitter' were provided by subjects JD, KM and SC on 25–50% of their trials. However, for all subjects the bitter descriptions disappeared completely for 250 mM NaCl in water.

The combination of 10 μM amiloride with NaCl had little effect on the incidence of Salty descriptions or on reports of 'bitter', but the percentage of salt/bitter compound terms increased. When the amiloride component of mixtures containing 100 or 250 mM NaCl was 100 μM, the incidence of bitter and Salt/Bitter descriptions rose substantially, while the percentages of salty taste quality reports fell (Figure 2).

In contrast, addition of 10× or 100× caffeine (matched to the taste of 10 or 100 μM amiloride) to 100 mM NaCl was accompanied by obvious increases in the percentages of 'salty' or 'salt' taste quality descriptions across subjects, and decreases in reports of bitterness (Figure 2). All subjects for whom descriptions of 100 mM NaCl in water had been <100% 'salt' or 'salty' increased their percentages of Salty descriptions for the mixture containing 10× caffeine and 100 mM NaCl. Furthermore, combination of 10× caffeine with



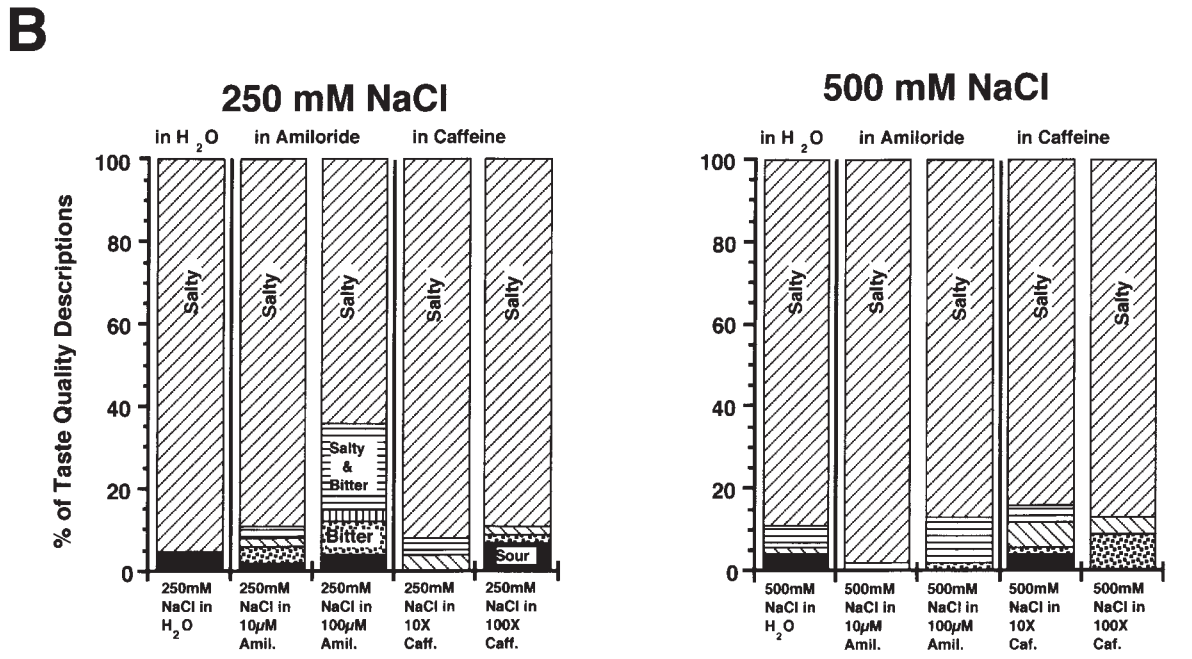


Figure 2 Taste quality descriptions across all subjects. The vertical axis of the four stacked bar diagrams is percent of total taste quality judgements accounted for by each of nine taste quality description categories. The Salty or Salt category represents those descriptions that consisted only of the words 'salt' or 'salty'; the Salt/Bitter category, the words 'salty and bitter' or 'bitter, salt'; Salt/Sour, 'salty, sour' or 'salt, sour'; Salt/Sweet, 'salt, sweet' or 'sweet and salty'; Bitter, 'bitter'; No Taste, 'no taste'; Sour, 'sour'; Sweet, 'sweet'; Sour/Sweet, 'sour, sweet'. **(A)** Taste quality description for 10 and 100 μM amiloride (Amil.) and 10× and 100× caffeine (Caff.) in H_2O ('Treatments in H_2O') and for 100 mM NaCl in H_2O, in 10 and 100 μM amiloride, and in 10× and 100× caffeine ('100 mM NaCl'). Taste quality description categories are represented by different patterns, shown in the key located in the lower portion of the figure. **(B)** Taste quality description for 250 and 500 mM NaCl in H_2O, in 10 and 100 μM amiloride, and in 10× and 100× caffeine.

100 mM NaCl was accompanied by disappearance of 'bitter' descriptions for all subjects who had provided them for 100 mM NaCl in water. At the higher NaCl concentrations, which had essentially received only 'salt' or 'salty' descriptions when dissolved in water, there was little effect of adding caffeine.

'Bitter' was the taste quality description given on the majority of trials of amiloride or caffeine in water. The 10 μM amiloride in water and, to a greater extent, its 10× caffeine control were also perceived as tasteless on substantial numbers of trials, but descriptions of 'no taste' were uncommon for the 100 μM amiloride and the 100× caffeine control (Figure 2). However, the incidence of 'bitter' taste quality descriptions decreased markedly when amiloride or caffeine were mixed with NaCl. Combining amiloride with NaCl resulted in reports of 'bitter' on less than one-third of the trials when 100 mM NaCl was present, and on fewer than 10% of the trials for 250 and 500 mM NaCl. On the other hand, salty-bitter compound terms, which had never been used for amiloride in H_2O, appeared for the NaCl–amiloride mixtures (Figure 2). The percentages of 'bitter' descriptions for mixtures of caffeine and NaCl were always smaller than those for comparable amiloride and NaCl mixtures; this was generally also the case for the incidence of salty-bitter compound terms.

Individual subjects

There was a direct relationship between the concentration of NaCl present in stimulus solutions and the incidence of Salty descriptions, and an inverse relationship with the incidence of Bitter descriptions (Figure 3): as NaCl concentration increased, every subject showed a significant increase in Salty descriptions and a significant decrease in Bitter descriptions. Reports of No Taste also decreased significantly as NaCl concentration rose for the four subjects who used this descriptor. These decreases and increases with NaCl concentration were all significant across subjects (Figure 3).

In contrast, the frequency of use of Salty taste quality descriptions never varied with the amiloride or caffeine concentration for any subject. On the other hand, more frequent use of Bitter taste quality descriptions was both associated with increase in treatment concentration for two subjects and significant across these subjects (Figure 3).

Amiloride treatment never had a significant association with Salty taste descriptions, but was significantly associated with the use of Bitter taste descriptions within and between four subjects (Figure 3).

Every subject had significant F values (df = 7, 120) for Bitter ($P < 7.0 \times 10^{-5}$) and for Salty ($P < 2.3 \times 10^{-7}$) taste quality descriptions, while four subjects had significant F values for No Taste ($P < 1.8 \times 10^{-4}$). Descriptions of No Taste occurred on 3–8% of these four subjects' trials. Most of the No Taste descriptions, 83%, were made in response to either 10 μM amiloride or its caffeine control (Figure 2).

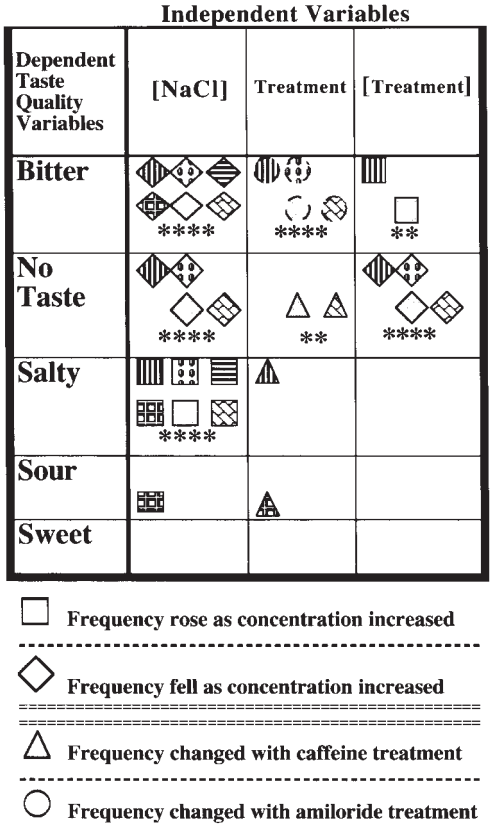


Figure 3 Taste quality description main effects. Individual subject's statistically significant general linear model main effects for taste quality descriptions (patterned shapes), and outcomes of a products-of-*P* (Darlington, 1998) meta-analysis of those effects (asterisks). [NaCl] = NaCl concentration independent variable, Treatment = caffeine (triangles) or amiloride solution (circles) as solvent, [Treatment] = treatment concentration. Each of the six subjects is represented by a different pattern in filled geometric shapes; the location of a subject's filled shape is consistent across all cells. Patterned shapes are shown for a subject only when the associated *P* for that subject and that condition is ≤0.05. Squares are shown for the concentration variables [NaCl] and [Treatment], when the associated *t* value is positive; diamonds, when the associated *t* is negative. For the treatment type variable, circles denote an effect associated with amiloride treatment; triangles, caffeine control treatment. Empty cells denote conditions for which no subjects had a *t* that corresponded to a $P \leq 0.05$; empty locations within cells, conditions for which a subject did not have a *t* that corresponded to a $P \leq 0.05$. No asterisks denote a Bonferroni-corrected products-of-*P* value with a $P > 0.05$; two asterisks, $P \leq 0.01$; three asterisks, $P \leq 0.004$, four asterisks, $P \leq 0.0004$. Products-of-*P* were calculated only when all *t* for a condition were of the same sign.

'Sour' as a taste quality description was entirely due to one subject, representing 11% of all her responses, and producing a significant F ($P < 2.4 \times 10^{-4}$). She was also the sole subject to use 'salty, sour' as a descriptor; it accounted for 9% of her responses. Her use of Sour as a taste quality descriptor increased significantly as NaCl concentration rose, and was more associated with the caffeine than with the amiloride Treatment type.

Significant two- or three-way interactions for reports of a Salty taste were not found ($P > 0.05$). Two-way interactions

Table 2 Subjects' descriptions of 100 mM NaCl in 10 μM amiloride, or in water, during the second screening session and during the data collection sessions of the main experiment

Subject	Second screening session					Data collection sessions	
	In 10 μM amiloride				In water	In 10 μM amiloride	In water
	Salty, bitter	Bitter	Salty or salt	No taste	% salty[b]	% salty[c]	% salty[c]
JD	1	6	0 (14%[a])	0	71	50	63
KM	0	3	2 (29%)	2	71	75	75
LG	0	2	1 (14%)	4	71	100	100
SC	0	0	7 (100%)	0	100	75	38
SS	0	0	2 (29%)	5	57	75	100
TT	0	0	7 (100%)	0	100	100	75

Unless indicated, numerals are the frequencies with which subjects gave the listed descriptors during the second screening session, in which aqueous 100, 250 and 500 mM NaCl and 100 mM NaCl in 10 μM amiloride were each presented to the anterior tongue region seven times, in random order.

[a]Numbers in parentheses are the total percentages of Salty descriptions (descriptions that included the words 'salt' or 'salty') for 100 mM NaCl in 10 μM amiloride during the second screening.

[b]Values are the total percentages of Salty descriptions for 100 mM NaCl in water during the second screening session.

[c]Values are the total percentages of Salty description for 100 mM NaCl in amiloride, or in water for the eight data collection sessions.

for Bitter responses between NaCl concentration and treatment concentration or type occurred for two subjects ($t < -2.577$, $P < 0.012$). Interactions for No Taste were more common ($t > 2.42$, $P < 0.018$), and produced, for one of these subjects, the only significant three-way taste quality description interaction ($t = -3.341$, $P = 0.001$).

Responses to NaCl in amiloride during screening and in the main experiment

During step 2 of the screening that preceded the main experiment, 100, 250 and 500 mM NaCl and 100 mM NaCl in 10 μM amiloride were delivered to the anterior region of the tongue. The responses to the 100 mM NaCl in 10 μM amiloride were not used for acceptance decisions, but rather to permit a post-hoc analysis. Comparison of the screening session responses to 100 mM NaCl in 10 μM amiloride with those during the main experiment indicated that the perceived taste quality of 100 mM NaCl in 10 μM amiloride over the eight sessions of the main experiment was not well predicted by the screening session descriptions (Table 2). The four subjects who had described 100 mM NaCl in 10 μM amiloride as salty on less than one-third of the screening trials all gave the same solution salty descriptions on 50–100% of the main experiment trials.

The primary goal of step 2 of the screening was to obtain subjects who would describe NaCl solutions in water using the terms 'salt' or 'salty' when the solutions were applied to the anterior region of the tongue, so that possible effects of amiloride treatment on the incidence of such descriptions could subsequently be detected. This was accomplished in that all but one of the main experiment subjects used descriptions containing 'salt' or 'salty' on >60% of the main experimental trials for NaCl solutions in water (Table 2). It is interesting to note that the proportion of such salty descriptions for 100 mM NaCl in 10 μM amiloride versus 100 mM NaCl in water during the main experiment was equal for two subjects, greater for NaCl in water for two subjects and greater for NaCl in amiloride for two subjects, again indicating the absence of any tendency in the present experiment for description of saltiness to be suppressed when 10 μM amiloride and 100 mM NaCl were combined.

Temporal patterns

Neither treatment concentration nor treatment type had extensive associations with the temporal measures of the time–intensity tracking patterns (Figure 4). For treatment concentration, only Rise Time (for two subjects) was significant across subjects. For treatment type, change in the magnitude of response duration, in association with amiloride treatment, was significant within and across three subjects.

In contrast to the rather few temporal pattern effects of treatment type and concentration, there were many main effects of NaCl concentration upon the tracked temporal pattern. As NaCl concentration increased, rise time, time to maximum, time within 90% of maximum, and duration increased significantly across 3–5 subjects (Figure 4).

The eight temporal measures differed appreciably in the extent to which they demonstrated overall significance. Every subject had significant F values (df = 7, 119 or 120) for time to maximum response magnitude ($F > 2.352$, $P < 0.029$). Five of the six subjects had significant F values for latency ($F > 2.193$, $P < 0.041$), while four subjects had significant values for rise time, time within 90% of

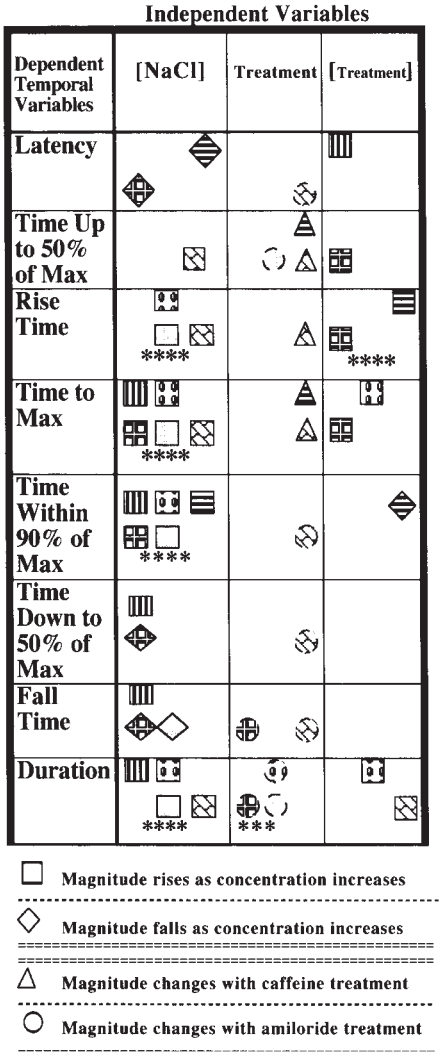


Figure 4 Temporal pattern main effects. Individual subjects' statistically significant general linear model main effects for temporal pattern (patterned shapes), and outcomes of a products-of-*P* (Darlington, 1996) meta-analysis of those effects (asterisks). [NaCl] = NaCl concentration independent variable, Treatment = caffeine (triangles) or amiloride solution (circles) as solvent, [Treatment] = treatment concentration; Max = maximum. Each of the six subjects is represented by a different pattern in filled geometric shapes. Patterned shapes are shown for a subject only when the associated probability value for that subject and that condition is ≤0.05. Squares are shown for the concentration variables [NaCl] and [Treatment], when the associated *t* value is positive; diamonds, when the associated *t* is negative. For the treatment type variable, circles denote an effect associated with amiloride treatment; triangles, caffeine control treatment. Empty cells denote conditions for which no subjects had a *t* that corresponded to a $P \leq 0.05$; empty locations within cells, conditions for which a subject did not have a *t* that corresponded to a $P \leq 0.05$. No asterisks denote a Bonferroni-corrected products-of-*P* value with a $P > 0.05$; three asterisks, $P \leq 0.004$, four asterisks, $P \leq 0.0004$. Products-of-*P* were calculated only when all *t* for a condition were of the same sign.

maximum or duration ($F > 2.278$, $P < 0.034$). Only three subjects had significant *F* values for time up to 50% of maximum or for fall time ($F > 2.216$, $P < 0.038$), and only one for time down to 50% of maximum response magnitude ($F = 3.786$, $P = 0.001$).

Significant two-way interactions for temporal measures never involved more than three subjects. Interactions between NaCl concentration and treatment type occurred for one or two subjects, with only duration having a common direction ($t > -2.584$, $P < 0.012$). Only for the latency measure did the interaction between NaCl concentration and treatment concentration have a consistency across subjects (three subjects, $t > 2.217$, $P < 0.029$), while significant interactions between treatment type and treatment concentration were limited to single subjects (latency or fall time, $t > -2.001$, $P < 0.049$). Three-way interactions between NaCl concentration, treatment concentration and treatment type provided a consistent direction for two subjects only for the latency measure ($t > 2.816$, $P < 0.007$).

Discussion

Taste quality descriptions

Amiloride treatments had no statistically significant across-subject or individual subject effects on the incidence of salty descriptions. This lack of effect of amiloride on the frequency of reports of saltiness is compatible with previous reports of little or no change in the saltiness intensity of NaCl when comparable concentrations of amiloride were employed (Breslin and Beauchamp, 1995; Ossebaard and Smith, 1995; Smith and Ossebaard, 1995).

However, the absence of effects of amiloride on the incidence of salty descriptions in the present data could have resulted from measurement problems. The free choice profiling method (Rubico and McDaniel, 1992; Halpern, 1997) used to obtain taste quality descriptions could have lacked sufficient sensitivity for the conditions of this experiment. This possibility is rejected by the statistically significant direct effects of NaCl concentration for every subject, and across subjects, on the incidence of salty descriptions. These data demonstrated that changes in the frequency of taste quality descriptions of saltiness were sensitively measured in this study. In similar fashion, the absence of significant effects of NaCl concentration on the incidence of sweetness or sourness judgements, and the significant inverse relationship between NaCl concentration and the incidence of 'no taste' descriptions, indicated the precision of the present taste quality description measurements.

Nonetheless, another potential problem could have existed: the amiloride treatment concentrations themselves could have been too low to alter taste quality descriptions. The present data showed that for four of the subjects, and across these subjects, the incidence of bitter judgements varied significantly with amiloride treatment. Thus, effects of amiloride treatment on taste quality descriptions were found, but these changes apparently resulted from amiloride itself acting as a gustatory stimulus. Furthermore, reports of

'no taste' significantly decreased in frequency as treatment concentration increased, again indicating that the treatment concentrations were effective taste stimuli.

Taste quality descriptions were directly reported in the present study of amiloride effects on taste responses to NaCl. Several recent studies (Breslin and Beauchamp, 1995; Ossebaard and Smith, 1995, 1996; Smith and Ossebaard, 1995) found no effects of amiloride on NaCl saltiness by measuring saltiness taste intensity. Consequently, the present negative data, obtained by a different procedure, confirmed previous reports that the saltiness produced by 100–500 mM NaCl is unaffected by 10 or 100 μM amiloride. In addition, the free choice taste quality profiling method of the present study confirmed that amiloride at concentrations of 10 μM and above is a bitter tastant.

Temporal patterns

Many of the temporal measures increased significantly within and between subjects as NaCl concentration increased, but none decreased significantly across subjects with NaCl concentration. An increase in the magnitude of temporal measures is a common effect of increased gustatory stimulus concentration on time–intensity patterns (Halpern, 1991). In similar fashion, amiloride treatment was significantly associated with the total duration of the time course of time–intensity tracked taste responses and, to a lesser extent, fall time. As treatment concentration increased, another parameter of the time–intensity pattern, rise time, also increased significantly across subjects, but no temporal parameters decreased significantly across subjects with treatment concentration and almost none within subjects. This indicates that the amiloride treatments acted as gustatory stimuli. In general, the time–intensity analysis showed only positive effects of the stimuli used in this study, and did not reveal any suppressing effects of amiloride.

Asymmetrical interactions between gustatory perceptions of NaCl and bitter tastants

In common with many previous investigations (for references see Breslin and Beauchamp, 1995; Breslin, 1996), the present study found that the perceived saltiness of NaCl solutions, measured here as the frequency of taste quality descriptions containing the terms salt or salty, showed little or no decrease when bitter caffeine or amiloride was added. Indeed, the reported saltiness frequency of 100 mM NaCl solutions may have actually increased when caffeine was added, as Schiffman *et al.* (1985) had reported. However, such increases in saltiness have not been observed by most investigators (Kamen *et al.*, 1961; Mela, 1989; Brosvic and Rowe, 1992; Breslin and Beauchamp, 1995).

In contrast to the observed slight effect of caffeine or amiloride on saltiness, in the present study the incidence of bitter descriptions for caffeine or amiloride solutions decreased substantially when NaCl was added, again as prior studies have shown (for references see Breslin and Beauchamp, 1995; Breslin, 1996).

Overall, the direct taste quality description technique and the temporal pattern measures of the present study did not reveal any previously undescribed interactions between NaCl and amiloride. The consistent effectiveness of 10 μM amiloride as a gustatory stimulus was confirmed, as was its characterization as bitter. However, the psychophysical measurement procedures that were utilized, which differed somewhat from those used in prior investigations of effects of amiloride on taste responses to NaCl, represented useful convergent approaches. Further support was provided for the concept that amiloride at or below 100 μM concentrations does not suppress perceived saltiness of NaCl (Breslin and Beauchamp, 1995; Ossebaard and Smith, 1995, 1996; Smith and Ossebaard, 1995; Halpern, 1998).

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References

Anand, K.K. and **Zuniga, J.R.** (1997) *Effect of amiloride on suprathreshold NaCl, LiCl, and KCl salt taste in humans*. Physiol. Behav., 62, 925–929.

Brand, J.G., Teeter, J.H., Cagan, R.H. and **Kare, M.R.** (eds) (1989) Chemical Senses. Volume 1. Receptor Events and Transduction in Taste and Olfaction. Marcel Dekker, New York.

Breslin, P.A.S. (1996) *Interactions among salty, sour and bitter compounds*. Food Qual. Pref., 7, 390–399.

Breslin, P.A.S. and **Beauchamp, G.K.** (1995) *Suppression of bitterness by sodium: variation among bitter taste stimuli*. Chem. Senses, 20, 609–623.

Brosvic, G. M. and **Rowe, M.M.** (1992) *Methyl xanthine, adenosine, and human taste responsivity*. Physiol. Behav., 52, 559–563.

Darlington, R.B. (1990) Regression and Linear Models. McGraw-Hill, New York.

Darlington, R.B. (1998) A Meta-Analytic 'p-pooler' with Three Advantages. http://www.psych.cornell.edu/Darlington/pprod1.htm.

Delconte, J.D., Kelling, S.T. and **Halpern, B.P.** (1992) *Speed and consistency of human decisions to swallow or spit sweet and sour solutions*. Experientia, 48, 1106–1109.

Delwiche, J.F., Halpern, B.P. and **Lee, M.Y.** (1996) *A comparison of tip of the tongue and sip & spit screening procedures*. Food Qual. Pref., 7, 293–297.

Denton, D. (1982) The Hunger for Salt. Springer-Verlag, Berlin.

DeSimone, J.A., Heck, G. L. and **DeSimone, S.K.** (1981) *Active ion transport in dog tongue: a possible role in taste*. Science, 214, 1039–1041.

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

Friedman, M.I., Tordoff, M.G. and **Kare, M.R.** (eds) (1991) Chemical Senses. Volume 4, Appetite and Nutrition. Marcel Dekker, New York.

Garty, H. and **Benos, D.J.** (1988) *Characteristics and regulatory mechanisms of the amiloride-blockable Na^+ channel*. Physiol. Rev., 68, 309–373.

Halpern, B.P. (1985) *Time as a factor in gustation: temporal patterns of taste stimulation and response*. In Pfaff, D.W. (ed.), Taste, Olfaction, and the Central Nervous System. The Rockefeller University Press, New York, pp. 181–209.

Halpern, B.P. (1987) *Human judgments of MSG taste: quality and reaction times*. In Kawamura, Y. and Kare, M.R. (eds), Umami: a Basic Taste. Marcel Dekker, New York, pp. 325–354.

Halpern, B.P. (1991) *More than meets the tongue: temporal characteristics of taste intensity and quality*. In Lawless, H. T. and Klein, B.P. (eds), Sensory Science Theory and Applications in Foods. Marcel Dekker, New York, pp. 37–105.

Halpern, B.P. (1994) *Temporal patterns of perceived tastes differ from liquid flow at the tongue*. In Kurihara, K., Suzuki, N. and Ogawa, H. (eds), Olfaction and Taste XI. Springer-Verlag, Tokyo, pp. 297–300.

Halpern, B.P. (1997) *Psychophysics of taste*. In Beauchamp, G.K. and Bartoshuk, L.M. (eds), Tasting and Smelling. Handbook of Perception and Cognition, 2nd edn. Academic Press, San Diego, CA, pp. 77–123.

Halpern, B.P. (1998) *Amiloride and vertebrate gustatory responses to NaCl*. Neurosci. Biobehav. Rev., in press.

Halpern, B.P., Kelling, S.T., Davis, J., Dorries, K.M., Haq, A. and **Meltzer, J.S.** (1992) *Effects of amiloride on human taste responses to NaCl: time–intensity and taste quality descriptor measures*. Chem. Senses, 17, 637 (abstract).

Halpern, B.P., Meltzer, J.S. and **Darlington, R.B.** (1993) *Effects of amiloride on tracked taste intensity and on taste quality descriptions of NaCl: individual differences and dose–response effects*. Chem. Senses, 18, 566 (abstract).

Halpern, B.P., Meltzer, J.S., Lee, M. and **Darlington, R.B.** (1995) *Amiloride and judgments of NaCl taste: no consistent effects on either time course of taste intensity or reports of salty taste*. Chem. Senses, 20, 701–702 (abstract).

Halpern, B.P., Meltzer, J.S., Lee, M. and **Darlington, R.B.** (1996) *Amiloride and judgments of NaCl taste: no effects on tracked taste intensity*. Chem. Senses, 21, 611 (abstract).

Kamen, J.M., Pilgrim, F.J., Gutman, N.J. and **Kroll, B.J.** (1961) *Interactions of suprathreshold taste stimuli*. J. Exp. Psychol., 62, 348–356.

Kelling, S.T. and **Halpern, B.P.** (1986) *Physical characteristics of open flow and closed flow taste delivery apparatus*. Chem. Senses, 11, 89–104.

Kelling, S.T. and **Halpern, B.P.** (1988) *Taste judgments and gustatory stimulus duration: taste quality, taste intensity, and reaction time*. Chem. Senses, 13, 559–586.

Kinnamon, S.C. (1996) *Taste transduction: linkage between molecular mechanisms and psychophysics*. Food Qual. Pref., 7, 153–159.

Lindemann, B. (1995) *Sweet and salty: transduction in taste*. News Physiol. Sci., 10, 166–170.

Luciani, S., Bova, S., Cargnelli, G. and **Debetto, P.** (1992) *Effects of amiloride on the cardiovascular system: role of the Na^+/Ca^{2+} exchange*. Pharmacol. Res., 25, 303–310.

Margolskee, R.F. (1993) *The molecular biology of taste transduction*. BioEssays, 15, 645–650.

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

Mela, D.J. (1989) *Bitter taste intensity: the effect of tastant and thiourea taster status*. Chem. Senses, 14, 131–135.

O'Mahony, M., Rothman, L., Ellison, T., Shaw, D. and **Buteau, L.** (1990) *Taste descriptive analysis: concept formation, alignment and appropriateness*. J. Sensory Stud., 5, 71–103.

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.

Ossebaard, C.A., Polet, I. A. and **Smith, D.V.** (1997) *Amiloride effects on taste quality: comparison of single and multiple response category procedures*. Chem. Senses, 22, 267–275.

Rubico, S.M. and **McDaniel, M.R.** (1992). *Sensory evaluation of acids by free-choice profiling*. Chem. Senses, 17, 273–289.

Schiffman, S.S. (1988) *Taste transduction and modulation*. News Physiol. Sci., 3, 109–112.

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.

Schiffman, S.S., Gill, J.M. and **Diaz, C.** (1985) *Methyl xanthines enhance taste: evidence for modulation of taste by adenosine receptor*. Pharmacol. Biochem. Behav., 22, 195–204.

Simon, S.A. and **Roper, S.D. (eds)** (1993) Mechanisms of Taste Transduction. CRC Press, Boca Raton, FL.

Smith, P.R. and **Benos, D.J.** (1991) *Epithelial Na^+ channels*. Annu. Rev. Physiol., 53, 509–530.

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.

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.

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