Chem. Senses 34: 373–382, 2009
doi:10.1093/chemse/bjp009
Advance Access publication March 12, 2009

Human Salt Taste and the Lingual Surface Potential Correlate

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Abstract

We have demonstrated in humans that Na^+ evokes changes in the lingual surface potential (LSP) using a custom chamber. To assess whether a relationship exists between the Na^+-evoked changes in the LSP and the intensity of salt taste, we measured the LSP and the intensity of salt taste simultaneously in 7 subjects using test solutions (50, 100, 300, and 1000 mM NaCl) presented in random order. The evoked LSPs and intensity scores correlated with one another well ($r^2 = 0.992$, $P < 0.01$). We then screened 14 subjects for their ability to discriminate between 100 and 300 mM NaCl using the chamber. Three subjects were consistently capable of distinguishing the salt concentrations. In these 3 subjects, an inhibitor of the epithelial sodium channel, amiloride (10 μM), blocked the ability to distinguish salt concentrations and affected the LSP. These data suggest that the LSP may be a component of the signal transduction system involved in human salt taste. In adept salt tasters, an amiloride-sensitive mechanism appears to have a role in distinguishing salt concentrations.

Key words: amiloride, electrophysiology, salt taste

Introduction

Psychophysical investigations indicate that salt taste in humans and animals is determined by the chemical composition (NaCl being the saltiest) and the concentration of the salt. A cellular model for salt taste occurring in taste receptor cells (TRCs) has been constructed from electrophysiological studies performed in animals. In this construct, salt taste is attributed to Na^+ moving through transcellular and paracellular pathways (Ye et al. 1991, 1993, 1994). Na^+ movement is coupled to the basolateral Na^+–K^+ ATPase of TRCs (DeSimone et al. 1984; Simon and Garvin 1985; Mierson et al. 1996; Gilbertson and Zhang 1998). Influx of Na^+ into TRCs depolarizes cells and ultimately results in neurotransmitter release activating gustatory nerve fibers. The influx of Na^+ happens at least in part through amiloride-inhibitable epithelial sodium channels (ENaCs) that are considered the specific Na^+ "receptors" and are predominantly located on the apical membranes of TRCs in the fungiform papillae (DeSimone et al. 1981; Simon and Garvin 1985; Avenet and Lindemann 1988; Gilbertson et al. 1993). In addition, part of Na^+ influx and taste is amiloride insensitive (Ye et al. 1991). This component may occur through permeation of the tight junction by Na^+ with a subsequent entry into TRCs through either a putative submucosal sodium transport system, which might be basolateral ENaCs (Mierson et al. 1996), or a nonselective cation channel such as Vr1, a member of the transient receptor potential family of channels (Lyall et al. 2004, 2005). By virtue of their transepithelial movement and by influencing paracellular Na^+ flux, anions also contribute to both chorda tympanii (CT) response as well as to the local lingual short circuit current and open circuit voltage (surface potential) (Elliott and Simon 1990; Ye et al. 1991; Simon et al. 1993).

Whereas a mechanistic relationship between salt taste and the likely target of amiloride, ENaC, exists in many animal species, that relationship has not been established in humans. In the first study using amiloride in humans, Schiffman and coworkers indicated amiloride-inhibited salt taste significantly (Schiffman et al. 1983). However, subsequent studies have been more impressive in their inability to document a strong relationship between salt taste and an amiloride-sensitive pathway (Halpern et al. 1992; McCutcheon 1992; Tennissen 1992; Ossebaard and Smith 1995, 1996; Smith and Ossebaard 1995; Tennissen and McCutcheon 1996;

Anand and Zuniga 1997; Ossebaard et al. 1997; Halpern 1998; Halpern and Darlington 1998).

Because the lingual surface is easily inspected, we realized that it was also possible to employ an electrophysiological approach in humans to study the mechanisms responsible for salt taste. We have reported that the potential at the lingual surface can be measured, that the application of salt to the lingual surface caused the lingual surface potential (LSP) to change, and that amiloride affects the LSP in some individuals (Feldman et al. 2003). In the present study, we have sought to evaluate whether the changes in the LSP evoked by salt correlate with the human intensity of salt taste and whether amiloride, a blocker of the ENaC, affects the salt taste and the LSP in the same subjects.

Materials and methods

Subjects

Thirty subjects (20 females and 10 males) participated in these studies. Their ages ranged from 26 to 61 years and averaged 37 ± 11 years. Six classified themselves as African–American, 2 as Hispanic, and 22 as white. Subjects were screened for their ability to report the tastes of 100 mM NaCl, 3.2 mM citric acid, 100 mM sucrose, and 100 μM quinine hydrochloride as salty, sour, sweet, and bitter, respectively. No subject used tobacco products or medications known to affect taste. Subjects abstained from ingesting food or liquid except water for at least 1 h prior to study. Experiments were conducted between 11 AM and 3 PM. All subjects participated in more than one protocol, but not in more than one experiment in a day. Subjects were reimbursed for their time. The Institutional Review Board of McGuire VA Medical Center approved the protocols and the informed consent form signed by every subject.

LSP measurements

The voltage across the lingual surface was detected with an Ag/AgCl electrode embedded in a chamber constructed of molded resin (Feldman et al. 2003). The chamber was placed on the dorsal lingual surface as near the tip as possible, where it adhered by suction. It exposed 55.4 mm^2 of the surface to solution. The reference Ag/AgCl electrode was attached to abraded skin (Red Dot Abrasive Tape, 3M, St Paul, MN) near the angle of the jaw. In this arrangement, the voltage is negative-going when cations are the predominantly conducted ion from the perfusion solution across the lingual surface. The voltage signal was conditioned by an optically isolated and battery-operated amplifier (DAM 50, World Precision Instruments, Sarasota, FL), digitized (Personal Daq 55, Iotech, Bedford Heights, OH), and recorded by computer at 1.3 Hz. Computer-controlled pumps (PHD 2000 Syringe Pump, Harvard Apparatus, Holliston, MA) propelled solutions at 333 μl/s through a 0.5-mm diameter entrance hole into the measuring chamber generating a stream with a linear flow velocity of 170 cm/s directed at the lingual surface. A program written in LabVIEW® (National Instruments, Austin, TX) controlled the pumps and recorded the voltage signal as an array.

When a solution contacts an electrode, a junction potential is generated, and its magnitude is influenced by the solution composition. To account for the varying junction potentials encountered during an experiment, chamber potentials were recorded before and after each experiment using a reference calomel electrode in the effluent path and the same protocol as the experiment. The ''before'' and ''after'' arrays of data were averaged and then subtracted from the ''experimental'' array using a template created in Mathcad® (Parametric Technology Corporation, Needham, MA). The change in the LSP evoked by changes in NaCl concentration was the LSP just prior to the exposure (pulse) of NaCl subtracted from the LSP at the end of the exposure.

To maintain stable function of the Ag/AgCl electrode, rinse solutions had to contain a chloride salt. In the earliest studies, 30 mM NaCl was rinsed. After some experimentation, 10 mM NaCl was found to provide stable electrode function and to maximize the concentration difference between rinse and test solutions. Ten millimolar NaCl was the rinse solution in all experiments unless noted otherwise.

The amiloride effect on the LSP was quantified by measuring the change in the LSP over time, which is the slope expressed in millivolts/second. The lingual surface was superfused with 150 mM NaCl for at least 45 s and then replaced with 150 mM NaCl containing 100 μM amiloride for at least 15 s. The amiloride-induced change in LSP slope or Δ slope is the difference between the slope of the 10-s segment after amiloride minus the slope of the 10-s segment prior to amiloride (Feldman et al. 2003).

Psychophysical measurements

In the first protocol, subjects were asked to give magnitude estimates of the intensity of saltiness while the LSP was monitored. After placement of the chamber on the tongue and initialization of the pumps, rinse solution (10 mM NaCl) continuously superfused the lingual surface except during the 5-s presentations of reference solution (100 mM NaCl) and test solution (50, 100, 300, or 1000 mM NaCl). The reference solution (modulus) was presented 5 times during the session. The initial modulus presentation occurred 90 s after chamber placement, and the subject was advised to assign a value to the salt taste intensity. At the subsequent modulus presentations, the subject was reminded of the assigned value. One minute after the initial modulus presentation, the first of 4 cycles of modulus and test solution began. In each cycle, the modulus was presented 45 s prior to the test solution and 90 s separated each cycle as illustrated in Figure 1. After each test solution, the subject recorded the intensity judgment with pen and paper. The test NaCl solutions were presented in random order. The timing of this protocol was

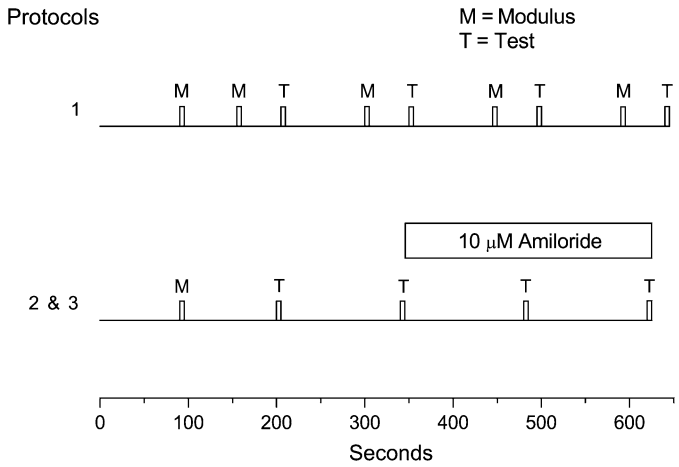

Figure 1 Psychophysical protocols. The timing of the 3 psychophysical protocols is illustrated. Continuous rinse solution intervened between pulses. Each pulse of modulus and test solution was 5 s in duration and is represented by a rectangle in the illustration. In the second protocol, amiloride was not utilized. In the third protocol, amiloride was applied to one side of the tongue only during the psychophysical measurements. The amiloride effect on the LSP was measured after psychophysical measurements on the side of the tongue that was not exposed to amiloride during psychophysical measurements.

based on preliminary experiments in which the recovery time of the LSP following pulses of NaCl was evaluated. Prior to judging the intensity of saltiness, each subject practiced estimating the lengths of cardboard strips.

In the second or screening protocol, following a single modulus pulse of 100 mM NaCl, subjects were asked to report the salt intensity of 4 pulses of test solutions. The test solutions were 100 and 300 mM NaCl, which were presented in a paired fashion, either 100 mM followed by 300 mM or in reverse order. The pairings were random, and the timing of the pulses was identical to the previous protocol except for the absent modulus prior to each test solution, Figure 1. Also the left and right sides of the tongue were tested in random order during the testing session. The criterion for success was that the subject reported a higher score for 300 mM NaCl than for 100 mM for each pair of presentations.

In the third protocol, the effects of amiloride on salt taste and on the LSP were assessed by modifying the previous protocol. Psychophysical measurements were performed in paired fashion as above. On one side of the tongue, 10 μM amiloride was added to rinse and test solutions after the first pair of salt solutions as illustrated in Figure 1. On the other side of the tongue, sensitivity of the LSP to 100 μM amiloride was assessed after the second pair of pulses by measuring the Δ slope.

Preparative procedures and solutions

Prior to each use, the chamber was disinfected with 3.4% glutaraldehyde (Cidex Plus®, Advanced Sterilization Products, Irvine, CA) and its Ag/AgCl electrode was chloridized with 5.25% NaOCl (Clorox®, Clorox Company, Oakland, CA). All solutions were prepared with distilled water and were used at room temperature (20–22 °C). NaCl, KCl, and LiCl were obtained from Fisher Scientific, Pittsburgh, PA. Amiloride, obtained from Sigma-Aldrich Corporation, St Louis, MO, was used at 2 concentrations: 10 μM, which minimized its bitter taste when psychophysical measurements were assessed, and 100 μM, which maximized its inhibitory effect when the LSP only was measured.

Statistical and graphical methods

Data are presented as mean ± standard deviation (SD). Statistical tests were performed using Excel® (Microsoft Corporation, Seattle, WA) and OriginPro® (OriginLab Corporation, Northampton, MA). Significance was accepted if the 2-tailed P value was less than 0.05. When analyzing significance using repeated measures analysis of variance (ANOVA), sphericity violations were corrected using Huynh–Feldt ϵ, and means were compared using the Tukey method. The coefficient of variation (CV) was utilized to compare variation of data in a scale-independent fashion.

Results

In the initial experiments, we evaluated the lingual response to varying salt concentrations. In the illustrative example in Figure 2A, the dorsal surface at the tip of the tongue was continuously superfused with a rinse solution of 30 mM NaCl, and at 60-s intervals, the lingual surface was replaced with higher NaCl concentrations (75, 150, 300, 600, and 1200 mM) for 10 s. Changing the salt concentration evoked rapid negative-going changes in the LSP, and the amplitude of these salt-evoked changes increased with the salt concentration. Such salt-evoked effects on the LSP have been observed in every experiment in which the NaCl concentration was altered. Also illustrated in Figure 2A is the slow negative-going change in the baseline LSP, which appears to decay with continued exposure to the rinse solution and this too was observed in every experiment.

To evaluate whether the lingual location and the type of epithelium influences the NaCl-evoked potential, the potentials evoked by 60 and 300 mM NaCl were recorded at 3 midline locations on the dorsal tongue (at the tip, 3–3.5 cm from the tip and 6–7 cm from the tip), the mucosal surface of the cheek, and the volar surface of the forearm. As shown in Table 1, the 3 sites on the tongue exhibited similar responses to pulses of NaCl, including responding to the changes in NaCl. The mucosal surface of the cheek, however, did not exhibit a statistically significant change in the LSP in response to the differing NaCl pulses. In contrast to the oral mucosa, the epithelium of the forearm exhibited a muted response to NaCl and did not respond to changes in NaCl concentration. Thus, as assessed by the potentials evoked by changing NaCl concentrations, oral epithelial surfaces are more responsive than skin, and

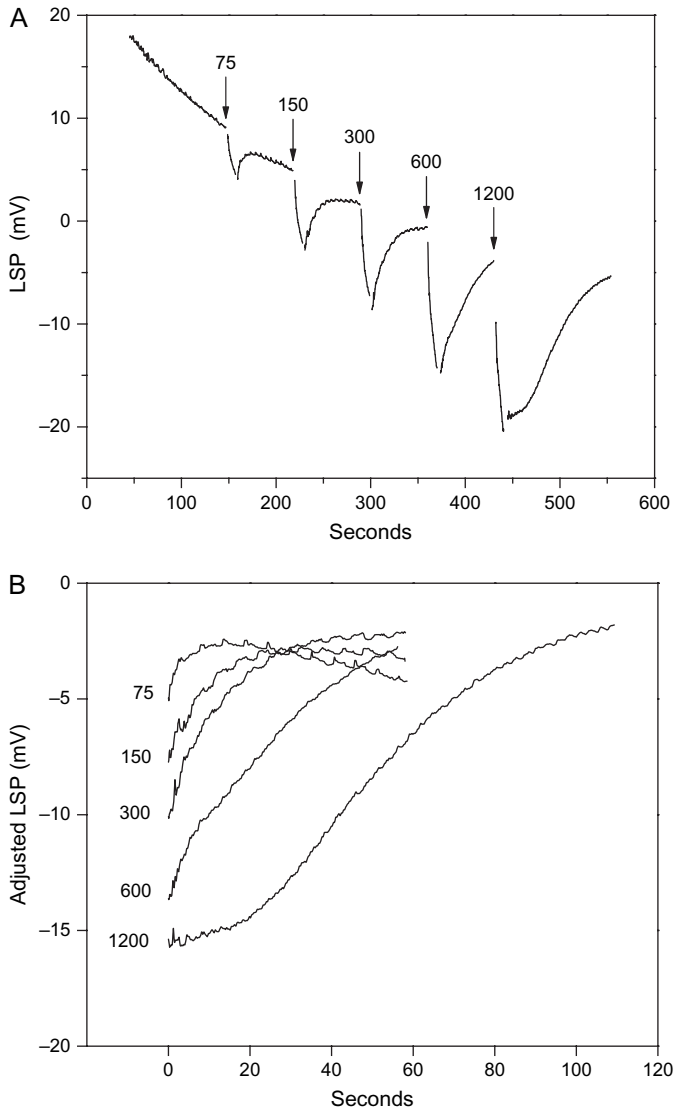

Figure 2 The LSP response to increasing salt concentration. In panel **(A)**, the lingual surface was superfused with 30 mM NaCl rinse, and at 60-s intervals, the lingual surface was superfused for 10 s with higher concentrations of NaCl as denoted. Increasing the salt concentration induced rapid electronegative excursions of the LSP, and the magnitude of the response increased with the NaCl concentration. Following return to rinse solution, the rate of recovery of the LSP appeared to be slower at higher NaCl concentration. Panel **(B)** illustrates the recovery of the LSP after exposure to differing concentrations of NaCl.

the lingual surface is sensitive to changes in NaCl concentration while the cheek mucosa and skin are not.

Figure 2B illustrates another characteristic of the lingual surface's response to salt. Following exposure to higher salt concentrations, the recovery time of the LSP increased. The recovery time also varies with the duration of exposure to higher salt concentrations with longer exposures prolonging the recovery (data not shown). For these reasons in the subsequent experiments described in the manuscript, changes in salt concentration were limited to 5 s.

Also illustrated in Figure 2A is the slow negative-going change in the baseline LSP, which appears to decay with continued exposure to the rinse solution. To assess the time course of the LSP response to a low concentration of salt, we exposed the lingual surface to 10 mM NaCl for approximately 6.5 min; 7 experiments were performed on 6 subjects; 1 subject was studied twice. During the initial 24 s of the protocol, the chamber was attached to the subject's tongue and the perfusion pumps were initialized. As shown in Figure 3 when rinse solution superfused the lingual surface, the LSP became electronegative rapidly and then the decline slowed. The average data, illustrated in Figure 3, fit a second-order exponential decay curve ($r^2 = 0.992$, $P < 0.001$), allowing the LSP response to salt to be partitioned into 2 time-dependent components. The fast time component had a $t_{½}$ of 3.9 ± 0.1 s, and the slower time component had a $t_{½}$ of 164 ± 2 s. As also shown by the SD band in Figure 3, the variability in the change in voltage among subjects increased over time. This variability was also reflected by the variability of the time constants for the individual experiments; the fast time component ranged from 1.8 to 15 s, whereas the slow constant ranged from 105 to 446 s.

Next, we assessed the lingual response to repeated pulses of salt concentration. In 8 experiments (6 subjects, 2 studied twice), rinse solution superfused the lingual surface, and beginning at 90 s, a series of six 5-s pulses of 30 mM NaCl were introduced; 60 s of rinse separated the pulses of 30 mM NaCl. As shown by filled circles in Figure 4A, the pulses of 30 mM salt evoked an electronegative LSP; the error bars denote the SD. With repeated exposures to 30 mM salt, the amplitude of the evoked LSP was reduced [1-way repeated measures ANOVA, $\epsilon = 0.407$, $F(2.03,14.23) = 53.4$, $P < 0.001$]. Interestingly, the last 2 pulses in the series were not statistically different by the Tukey method, suggesting that the responsiveness of the evoked LSP achieves a plateau after about 5 min. These observations suggested that either prolonged exposure to 10 mM salt induces a form of habituation (or adaptation) or repeated exposures to 30 mM salt induce the habituation (or adaptation).

To distinguish between the above possibilities, the interval between 30 mM pulses was lengthened from 60 s to 4 min in 8 additional studies performed on 6 subjects. As shown by the open circles in Figure 4A, the evoked LSP was reduced by the same degree as in the previous protocol. This observation indicates that the prolonged exposure to rinse solution caused the reduction in amplitude of the LSP response to higher salt concentrations.

Using the above data, we explored the effect of normalizing the evoked LSP. Each experimental sequence of evoked potentials was divided by the first-evoked LSP. As shown in Figure 4B, the normalized data exhibited the same pattern of change over time as the raw data in Figure 4A, but normalizing reduced the SD. For visual comparison, the ordinate in Figure 4B spans the same range as the ordinate in Figure 4A. (In the 2-pulse protocol, the CV of the second-evoked LSP

Table 1 Effects of NaCl concentration on the surface potential at 3 locations on the tongue, the mucosal surface of the cheek, and the skin of forearm

	Tongue			Cheek	Forearm
	Tip	Mid	Rear		
60 mM NaCl	−23.2 ± 4.1	−20.5 ± 6.3	−22.8 ± 3.8	−23.7 ± 5.6	−6.3 ± 2.5*
300 mM NaCl	−31.2 ± 6.4	−30.2 ± 7.3	−32.5 ± 4.7	−28.4 ± 3.6	−6.9 ± 5.3*
P	<0.01	<0.01	<0.01	>0.05	>0.05

Values are means ± SD, $n = 5$. The surface potentials were measured at 3 midline locations on the dorsal tongue (at the tip, at midpoint which was 3–3.5 cm from the tip, and at the rear which was 6–6.5 cm from the tip), the mucosal surface of the cheek, and the volar surface of the forearm. Analysis by 2-way repeated measures ANOVA revealed significant effects of NaCl concentration [$\epsilon = 1$, $F(1,4) = 115.7$, $P < 0.001$] and location [$\epsilon = 1$, $F(4,16) = 20.9$, $P < 0.001$]. The effects of NaCl concentration at the different locations were assessed using the Tukey method, and the significance levels are indicated in the last row. *Forearm different from other locations at each salt concentration at $P < 0.01$.

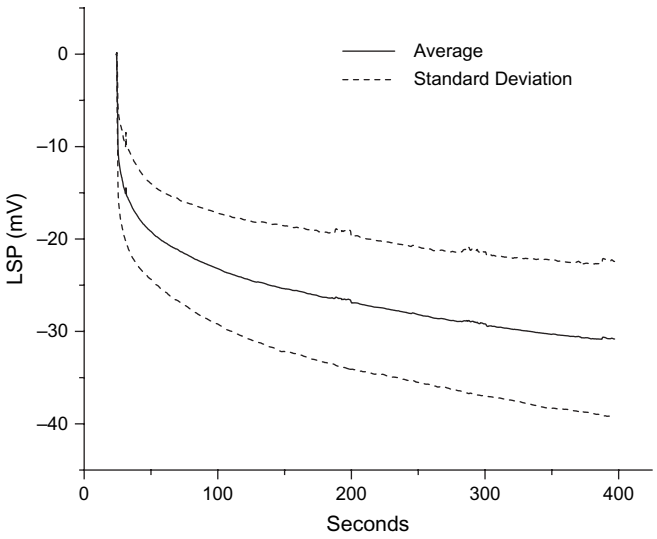

Figure 3 The time course of the LSP during exposure to 10 mM NaCl for 6.5 min. In 7 observations in 6 subjects, after initiation of flow, the LSP decreased rapidly but the rate slowed quickly. The average data fit a double exponential decay curve ($r^2 = 0.992$, $P < 0.001$), and the fast time component had a $t_{½}$ of 3.9 ± 0.1 s, whereas the slow component had a $t_{½}$ of 164 ± 2 s. The SD increased during the period of observation.

was 30%, and after normalization, the CV was reduced to 9.3%. In the 6-pulse protocol, the CVs of the subsequent evoked LSPs were 18.3%, 19%, 19.7%, 20.0%, and 20.1%, sequentially, and normalizing reduced the respective CVs to 6.5%, 7.6%, 9.7%, 10.5%, and 11.3%.) These data indicate that the normalized evoked LSP is more consistent from session to session than is the raw LSP. Furthermore, these data indicate that the "normalized" lingual response appears to be reasonably consistent among subjects.

In rats, NaCl, LiCl, and KCl stimulate the activity of the CT nerve, but the stimulated activity is not uniformly inhibited by amiloride. Amiloride inhibited the CT activity stimulated by NaCl and LiCl but had no effect on the CT activity stimulated by KCl (DeSimone et al. 1984; Ye et al. 1994). In order to assess whether the LSP responses in humans to these salts are similar to the CT activity responses in rats, we examined subjects whose NaCl-induced LSP had exhibited at least minimal amiloride sensitivity (A-S, $n = 4$) and subjects whose LSP had exhibited amiloride insensitivity (A-I, $n = 5$). The salts at 150 mM sequentially superfused the lingual surfaces in the absence of amiloride and then in the presence of 100 μM amiloride. The 4 A-S subjects exhibited similar LSP responses, and these responses differed from the responses of the 5 A-I subjects. Typical LSP responses are illustrated in Figure 5. In A-S subjects, NaCl and LiCl affected the LSP similarly in the absence of amiloride, whereas KCl caused the LSP to become more electropositive. The KCl effect on the LSP was reversed by returning to NaCl. Adding amiloride to NaCl caused the LSP to become more electropositive, and the directionality continued when the salt was changed to LiCl. In the presence of amiloride, KCl had little effect on the LSP. In A-I subjects, changing from NaCl to LiCl or to KCl had little effect on the LSP whether or not amiloride was in solution. Therefore, the amiloride-sensitive component of the LSP exhibits cation selectivity similar to that exhibited by the CT activity in rats.

To assess the relationship between the evoked LSP and the salt taste intensity, we utilized a protocol that allowed for data normalization. Accordingly, the reference solution or modulus (100 mM NaCl) was presented prior to each presentation of test solution (50, 100, 300, and 1000 mM NaCl). Seven subjects were instructed to compare the salt intensity of the test solution to the reference solution, which was assigned an intensity value of 100. As shown in Figure 6A, the evoked LSPs and the reported intensity scores increased with the NaCl concentration, and their patterns of increase had similar appearances. When regressed against one another, the evoked LSPs and the intensity scores correlated well ($r^2 = 0.992$, $P < 0.01$).

Data were transformed by converting the raw intensity scores to a logarithmic scale and by normalizing the evoked LSPs to the reference pulse preceding each test pulse. The CV for the raw intensity scores averaged 40.9%, which was reduced to 9.4% by converting to the logarithmic scale. The CV of evoked raw LSPs averaged 27.9%, which was reduced to 12.0% by normalizing. As shown in Figure 6B, the transformed intensity scores correlated well with the normalized

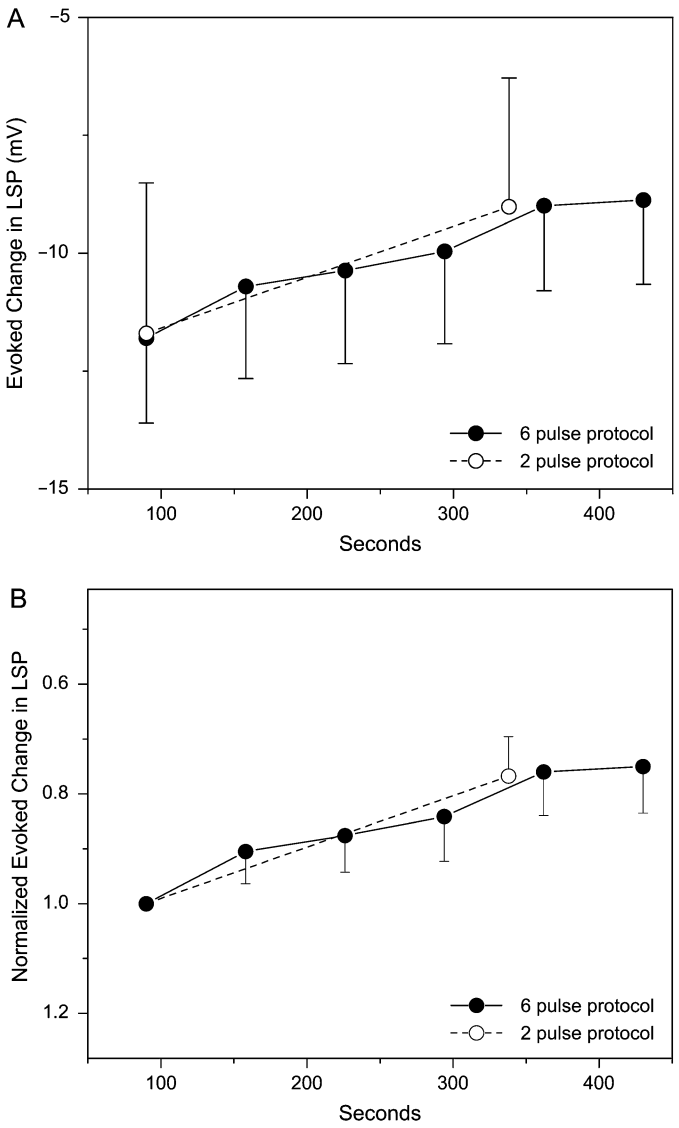

Figure 4 The LSP response to repeated exposures of 30 mM NaCl. In panel **(A)**, 8 observations were obtained in 6 subjects. Thirty millimolar NaCl was introduced for 5 s 6 times (filled circles); 60 s of rinse separated each exposure. The magnitude of the evoked response (change in LSP) to 30 mM NaCl decreased with time [1-way repeated measures ANOVA, $\epsilon = 0.407$, $F(2.03,14.23) = 53.4$, $P < 0.001$]. To assess whether the reduced response was an effect of time or an effect of the repeated exposures to 30 mM NaCl, the response to 30 mM NaCl was evaluated after 4 min (open circles) in 6 subjects (8 observations). Because the reductions in the evoked responses in the 2 protocols were similar over time, it appears that the prolonged exposure to rinse solution decreases the response to 30 mM NaCl. In panel **(B)**, the data in panel (A) were reanalyzed by normalizing. Prior to averaging, each experimental sequence was divided by the first-evoked LSP. For comparison, the ordinate spans the same range as the ordinate in panel (A). As can be seen, this method of normalizing reduces the SD of the evoked LSP.

evoked LSP ($r^2 = 0.978$, $P < 0.02$). Three of the subjects participated in repeat experiments with equally good results. In addition, a nearly identical protocol was conducted with 8 subjects; the difference in protocol was that the modulus

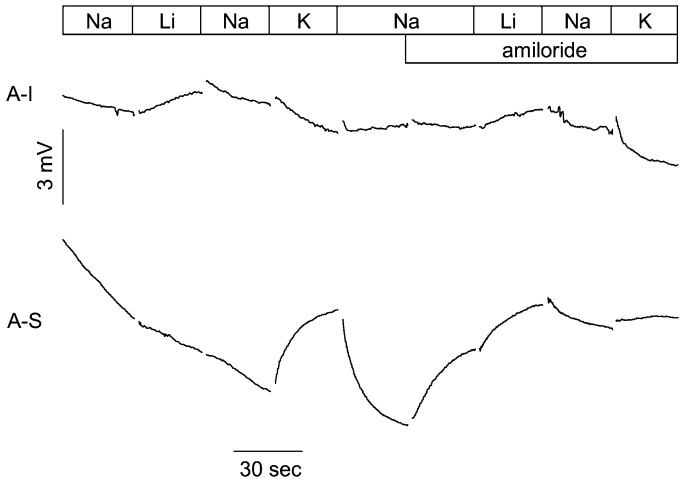

Figure 5 Effects of NaCl, LiCl, KCl, and amiloride on the LSP. Representative experiments from 2 subjects, one whose LSP response was sensitive to amiloride (A-S) and another whose LSP response was insensitive to amiloride (A-I). The concentration of the salt solutions superfusing the lingual surface was 150 mM. The initial sequence of solutions had no amiloride, and 100 μM amiloride was added to the subsequent sequence. In the A-S subject, NaCl and LiCl affected the LSP similarly in the absence of amiloride, whereas KCl caused the LSP to become more electropositive. The electropositive effect of KCl was reversed by return of NaCl. Adding amiloride to NaCl caused the LSP to become more electropositive and that directionality continued when LiCl replaced NaCl. In the presence of amiloride, KCl had little effect on the LSP. In the A-I subject, changing from NaCl to LiCl or to KCl had little effect on the LSP whether or not amiloride was present in the solutions.

was assigned an intensity value of 10 instead of 100. The second set of experiments also demonstrated that the log intensity score correlated with the normalized LSP ($r^2 = 0.935$, $P < 0.04$). The correlation between the LSP and the salt taste intensity suggests that the LSP is a component of the signal transduction system involved in salt taste.

Because in earlier experiments (Figure 4) the amplitude of the LSP evoked by repeated pulses of 30 mM NaCl changed with time and eventually appeared to achieve a plateau, we analyzed the time course of the LSPs evoked by the 100 mM NaCl modulus in these experiments. We again observed that the amplitude of the evoked LSP decreased with time and appeared to achieve a plateau after 5 min (data not shown).

Next, we assessed the simultaneous effects of amiloride on salt taste intensity and on the LSP. However, the variability of reported salt intensities using our chamber, which exposed a small area (55.4 mm^2) of the lingual surface to test solutions, was large as indicated by SDs in the intensity scores in Figure 6A. Hence, we selected subjects who could reliably taste salt when using our chamber. Of 14 subjects, 8 correctly reported the salt intensities on both sides of the tongue during their first test. Seven of these subjects were retested, and 3 correctly identified the difference between salt solutions on both sides of the tongue. These 3 subjects correctly identified salt concentrations on repeated testing were classified as "salt tasters" and were selected for further testing.

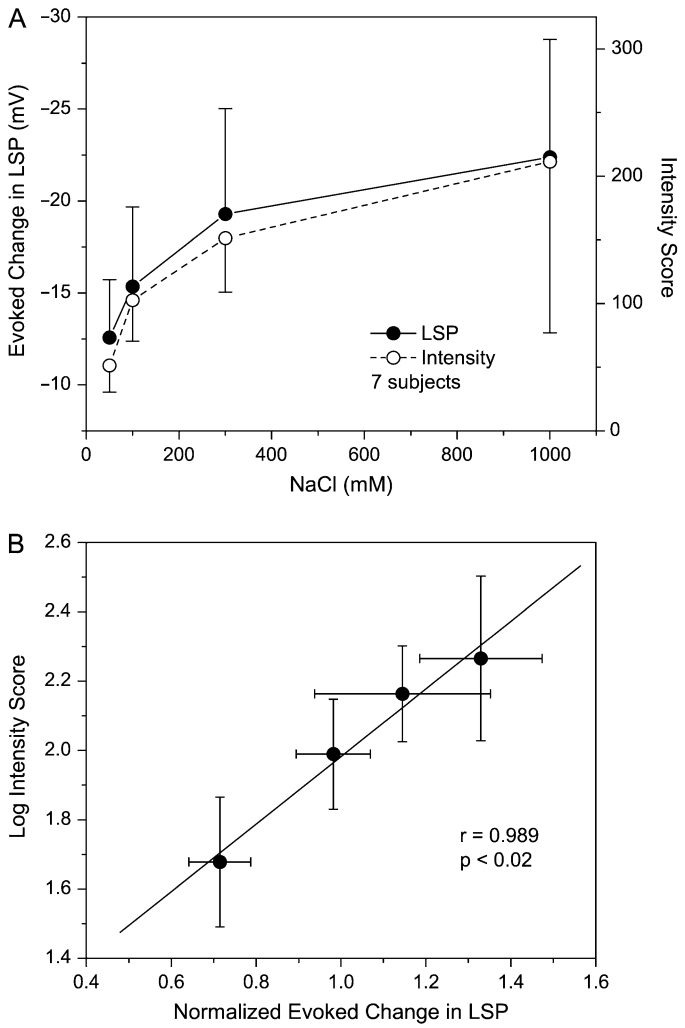

Figure 6 The LSP and salt taste vary with the concentration of salt. In panel **(A)**, while monitoring the LSP, subjects were instructed to compare the salt intensity of test solutions to the reference solution (100 mM NaCl), which was assigned an intensity value of 100. The evoked unnormalized evoked change in the LSP and the reported intensity scores exhibited similar patterns. In panel **(B)**, the LSP data were normalized to the reference pulse preceding each test pulse, and the raw intensity scores were transformed to a logarithmic scale. As shown, the simultaneously measured LSP and intensity scores correlated linearly.

To assess the effects of amiloride on salt taste and on the LSP, the screening protocol was altered. On one side of the tongue, 10 μM amiloride was added to rinse and subsequent test solutions following the first pair of test solutions. On the other side of the tongue, amiloride sensitivity of the LSP was assessed (to assess the reproducibility of the effect of amiloride on the Δ slope, the left and right sides of the tongue were studied in random order in 10 subjects. We utilized 100 μM amiloride to maximize the inhibitory effect. In 12 pairs of observations [2 subjects were studied twice], amiloride induced statistically similar changes in slope. The differences between the paired sets of measurements averaged 0.020 mV/s and did not approach statistical significance. These data indicate that on a given day, the Δ slope measurement is reproducible. An additional subject was found to have an amiloride response on one side of the tongue only; no data from that subject were utilized in this report) after the last test pulse. Typical results for the salt tasters are shown in Figure 7. Amiloride lowered the intensity scores for 100 mM NaCl from 98.7 ± 8.2 to 76.7 ± 15.3 (paired t-test, $P < 0.05$) and for 300 mM NaCl from 113.2 ± 3.3 to 71.7 ± 10.4 (paired t-test, $P < 0.03$). Amiloride also eliminated the subjects' ability to discriminate salt concentrations as assessed by the difference between the intensity scores for the 2 salt concentrations: 21.3 ± 6.3 in the absence of amiloride versus –5.0 ± 8.7 (paired t-test, $P < 0.03$) in the presence of amiloride. Amiloride also affected the LSP, increasing the average Δ slopes by 0.072 ± 0.020 (paired t-test, $P < 0.03$). These results were replicated in these 3 salt tasters and indicate that amiloride inhibits the ability to taste salt and the LSP simultaneously in some people.

Discussion

These experiments demonstrated that the LSP is affected by the application of NaCl. The changes in the LSP varied with time and with the NaCl concentration. The NaCl-evoked LSP correlated with the human ability to discriminate salt concentrations applied to the lingual surface. However, subjects exhibited diversity in their ability to taste salt. In a small group of subjects who proved to be adept at discriminating salt concentrations, amiloride impaired the ability to taste salt and affected the LSP.

Imposition of the Na^+ and Cl^- gradients across epithelia generates evoked potentials, and based on the design of these experiments, the negative-going evoked potential indicates that the Na^+ gradient is the dominant contributor. In comparison to the skin of the forearm, the lingual epithelium responded more briskly to NaCl, and in comparison to the cheek mucosa and the forearm skin, the lingual epithelium responded to changing concentrations of NaCl. Thus, the lingual epithelium exhibits unique electrical properties in response to NaCl.

In response to application of various NaCl concentrations, the evoked lingual epithelium exhibits rapid changes in the LSP. With more prolonged application of salt, the LSP continues to decline slowly. If the LSPs were simply due to ion flux through a fixed conductive pathway or interaction with a fixed Na^+-selective structure similar to an ion-specific electrode, the anticipated electrical response to the imposed salt concentration would attain a plateau rapidly. The negative-going drift in the LSP may well represent a continued response of the lingual epithelium to sodium. The prolonged recovery of the LSP upon reapplication of rinse solution after exposure to high salt concentrations also suggests an active response of the lingual epithelium. These electrical responses are likely to involve the generation and dissipation of ion concentrations on the serosal aspect (blood side) of the

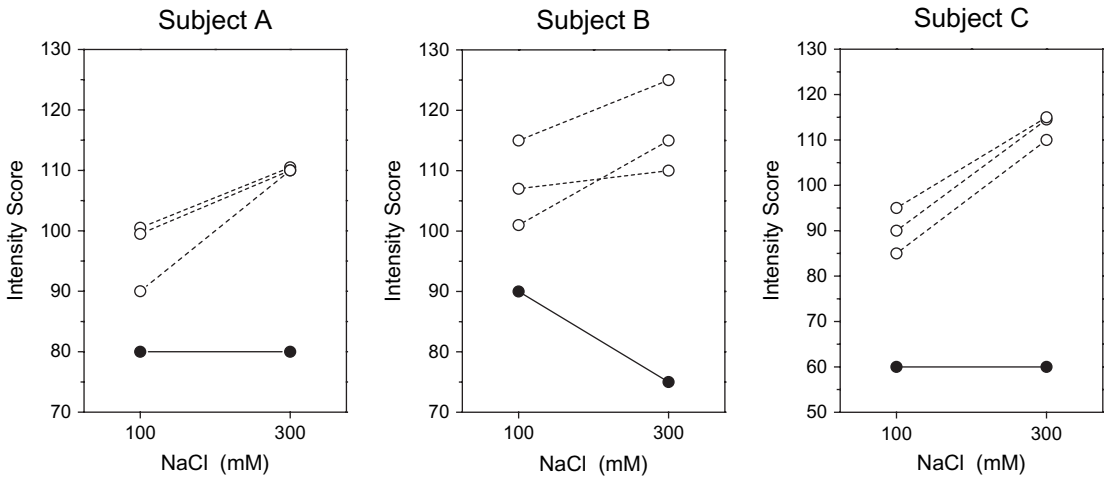

Figure 7 Salt taste is inhibited by amiloride. Experiments from 3 subjects who were capable of tasting the difference between 100 and 300 mM NaCl using the chamber. Salt intensity scores in the absence of amiloride are denoted by open circles, whereas the filled circles present the intensity scores in the presence of 10 μM amiloride.

lingual epithelium as well as the lingual epithelial cellular responses to sodium.

The present studies in humans complement earlier work performed in animals by DeSimone, Heck, and collaborators (DeSimone et al. 1981; Heck et al. 1984; DeSimone and Ferrell 1985; Ye et al. 1994; Stewart et al. 1996; Kloub et al. 1998). The human LSP and the animal open circuit voltage vary with the concentration of sodium applied to the lingual surface and the taste intensity of sodium or the CT activity. Amiloride also affects the LSP and salt taste in some humans in the same way it affects the CT activity in rats. Theoretically, the electrical measurements are identical, but in practice, they differ. In the present work, the influence of the junction potential, generated by the varying salt concentration at the interface of the solution and the electrode (or bridge), was taken into account, whereas in the earlier animal studies, the influence of the junction potential was not considered. If we had not accounted for the junction potential, an offset in the LSP would have resulted, altering the relationship between the LSP and the intensity of salt taste. Interestingly, such an offset was observed between the open circuit voltage and CT activity in the animal studies, which is consistent with the effect of the junction potential (Heck et al. 1989). Also, the present study utilized continuously flowing fluid through the chamber to minimize the unstirred microclimate at the lingual surface, whereas in the animal studies, fluid was injected into the chamber only when the solution was changed, allowing the development of standing ion gradients in the region near the lingual surface or an unstirred microclimate. Additionally, the present study was performed on conscious volunteers, whereas the earlier electrophysiological studies were performed on anesthetized animals that had undergone significant surgery in order to record CT nerve activity. Anesthesia and the stress of surgery affects hormone secretion, can affect cardiac output and organ perfusion, and may have influenced the lingual epithelium and/or the CT nerve. Some of these technical differences may have contributed to the slow negative-going drift of the LSP in response to prolonged exposure of the human lingual epithelium to sodium, a finding which contrasts to the stability of the open circuit voltage and short circuit current when the animal tongue was exposed to sodium for a prolonged period. Despite these differences, our findings corroborate the essential notion that cellular sodium transport is involved in salt taste in human beings.

Not all subjects exhibited an LSP that was sensitive to the ENaC inhibitor, amiloride. In those subjects who did exhibit amiloride sensitivity, the LSP response to NaCl, LiCl, and KCl showed selectivity. Specifically, NaCl and LiCl supported electronegative polarization, whereas KCl allowed depolarization, and amiloride blocked the electronegative polarization induced by Na^+ and Li^+. Amiloride-insensitive subjects did not exhibit cation selectivity. The pattern of cation selectivity observed in amiloride-sensitive subjects is similar to the cation selectivity observed in the CT activity in rats (DeSimone et al. 1984; Ye et al. 1994). These data support the notion that ENaC is active in the lingual surface of some individuals. However, we must be cautious as ENaC subunits may exist in nongustatory epithelial cells on the tongue surface as well as in TRCs (Lin et al. 1999; Lu et al. 2008), and our study did not identify the lingual location of ENaC responding to amiloride.

Although we demonstrated that the ability to taste salt correlated well with LSP, there was considerable variability in the subjects' abilities to distinguish salt concentrations reliably. When we searched for subjects who could reliably distinguish 300 mM NaCl from 100 mM using our chamber, 21% of the subjects were capable of fulfilling the task. Our chamber with its limited area of exposure of the lingual surface to solutions probably contributes to the low number of salt tasters and is in keeping with the direct relationship

between the number of fungiform papillae being stimulated and the sensitivity to NaCl (Doty et al. 2001). Thus, we speculate that the identified salt tasters had a higher density of fungiform papillae on the apical portions of their tongues accounting for their greater ability to distinguish between salt concentrations. However, that only a fraction of the population can have their salt taste assessed reliably with this chamber means that these data reflect a subset of the population. Hence, these data must be interpreted with some caution as they might not reflect the entire population.

Although the number of salt tasters who were identified is small, they appear to exhibit an association between their ability to taste salt and amiloride's ability to block salt taste and to affect the LSP. A mechanistic relationship between salt taste and the likely target of amiloride, ENaC, exists in many animal species, but the relationship has not been established in humans. Following the initial study led by Schiffman et al. (1983) indicating a significant effect of amiloride in human salt taste, the studies have been impressive in their inability to document a strong relationship between salt taste and an amiloride-sensitive pathway (Halpern et al. 1992; McCutcheon 1992; Tennissen 1992; Ossebaard and Smith 1995, 1996; Smith and Ossebaard 1995; Tennissen and McCutcheon 1996; Anand and Zuniga 1997; Ossebaard et al. 1997; Halpern 1998; Halpern and Darlington 1998). However, Ossebaard and coworkers did observe that amiloride altered how subjects described the taste qualities of salt, blocking the minor sour quality of NaCl without affecting the saltiness of NaCl (Ossebaard and Smith 1995, 1996; Smith and Ossebaard 1995; Ossebaard et al. 1997). Although we did not assess the taste qualities of salt, we observed that amiloride affected both salt taste and the LSP in the subjects who were most adept at tasting salt. Interestingly, we also found an individual who exhibited amiloride-sensitive LSP on only one side of the tongue, indicating that electrically active ENaC need not be distributed uniformly over the lingual surface. Nevertheless, in salt tasters, a parallel appears to exist between the ability of amiloride to blunt salt taste and to induce a change in the LSP, consistent with a common mechanism involving ENaC.

In order to identify salt tasters, we screened subjects for the ability to discriminate salt concentrations reliably, a procedure that differed from earlier studies. Because most subjects failed the screening, the variability due to poor subject performance was minimized. With less stringent discrimination criterion, more subjects would likely be included. It would be interesting in further study to see whether the ability to discriminate salt intensity was linked to variability in amiloride sensitivity. Anand and Zuniga observed that amiloride increased the variance of responses of subjects to sodium and lithium, but not to potassium (Anand and Zuniga 1997). Such an observation is consistent with amiloride having a profound effect on some individuals, and they noted that amiloride affected 90% of the subjects to varying degrees and had no effect on 10% of subjects. Others have also noted that amiloride applied to the anterior tongue does not blunt the ability of some subjects to detect salt (Halpern et al. 1992; McCutcheon 1992; Tennissen 1992; Tennissen and McCutcheon 1996).

Why there should be such heterogeneity in salt taste is unknown, but genetic and environmental factors may have roles. Shigemura et al. have demonstrated that genetic variation in alpha ENaC accounts for variation in the amiloride sensitivity of taste cells of mice when monitored by CT activity and discussed the possibility that altered regulation and trafficking of ENaC expression could also affect taste (Shigemura et al. 2008). In this regard, humans appear to exhibit considerable variability in the subunits of ENaC expressed in taste cells, including alpha ENaC (Huque et al. 2002). We also speculate that the human ability to discriminate salt concentrations may not be essential for survival in modern society because salt is ever present. However, in the wild, salt is not always available and animals unable to detect salt are at survival disadvantage (Jacobs 1978; Denton et al. 1985). Presumably, a similar disadvantage would occur to humans who are not adept at tasting salt and who exist in a setting in which salt is not freely available. Because of our environment, the physiological factors and triggers that enhance the ability to taste and/or detect salt may have little impact as they may be suppressed. However, the ability to taste and/or detect salt may participate in diseases in which normal physiology is altered. Candidate diseases in which salt taste may have a role are those in which the body's salt content is an acknowledged factor, for example, hypertension and congestive heart failure.

In summary, we have demonstrated that the Na-evoked changes in the LSP correlate with the human ability to taste salt. We observed that there is considerable variability in subject ability to taste salt. In a small number of subjects who were adept at tasting salt, amiloride blocked the ability to taste salt and affected the LSP. These findings may be consistent with a role of ENaC in salt taste in some humans.

Funding

Department of Veterans Affairs in the form of a Merit Review Grant to G.M.F.

Acknowledgements

The authors thank Victoria A. Bickel for manufacturing the resin chambers.

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Accepted February 15, 2009