Anion Modulation of Taste Responses in Sodium-sensitive Neurons of the Hamster Chorda Tympani Nerve

BRADLEY G. REHNBERG, BRUCE I. MACKINNON, THOMAS P. HETTINGER, and MARION E. FRANK

From the Department of BioStructure and Function and Center for Neurological Sciences, University of Connecticut Health Center, Farmington, Connecticut 06030

ABSTRACT Beidler's work in the 1950s showed that anions can strongly influence gustatory responses to sodium salts. We have demonstrated "anion inhibition" in the hamster by showing that the chorda tympani nerve responds more strongly to NaCl than to Na acetate over a wide range of concentrations. Iontophoretic presentation of Cl^- and acetate to the anterior tongue elicited no response in the chorda tympani, suggesting that these anions are not directly stimulatory. Drugs (0.01, 1.0, and 100 μM anthracene-9-carboxylate, diphenylamine-2-carboxylate, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonate, and furosemide) that interfere with movements of Cl^- across epithelial cells were ineffective in altering chorda tympani responses to 0.03 M of either NaCl or Na acetate. Anion inhibition related to movements of anions across epithelial membranes therefore seems unlikely. The chorda tympani contains a population of nerve fibers highly selective for Na^+ (N fibers) and another population sensitive to Na^+ as well as other salts and acids (H fibers). We found that N fibers respond similarly to NaCl and Na acetate, with spiking activity increasing with increasing stimulus concentration (0.01–1.0 M). H fibers, however, respond more strongly to NaCl than to Na acetate. Furthermore, H fibers increase spiking with increases in NaCl concentration, but generally decrease their responses to increasing concentrations of Na acetate. It appears that anion inhibition applies to taste cells innervated by H fibers but not by N fibers. Taste cells innervated by N fibers use an apical Na^+ channel, whereas those innervated by H fibers may use a paracellularly mediated, basolateral site of excitation.

INTRODUCTION

Questions concerning salt taste have generally focused on stimulatory aspects of cations. A role for anions in salt taste transduction was demonstrated when Beidler (1953) and later Beidler and Gross (1971) reported a wide range of responses by the rat chorda tympani nerve to equimolar concentrations of several sodium salts. Since then, several reports have considered the contributions of anions in making salts stimulatory (Formaker and Hill, 1988; Rehnberg, Hettinger, and Frank, 1989, 1992;

Address reprint requests to B. G. Rehnberg, Department of Biological Science, York College, Country Club Road, York, PA 17405-7199.

J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/93/03/0453/13 $2.00
Volume 101 March 1993 453–465

Elliott and Simon, 1990; Schiffman, Crumbliss, Warwick, and Graham, 1990; Ye, Heck, and DeSimone, 1991). Nevertheless, most questions about the role of anions in salt taste are unresolved: e.g., Which anions contribute to salt taste? Are some anions stimulatory and others inhibitory? Do anions only modify the stimulatory effects of cations or do they have their own separate actions? Do anions act via specific pathways (receptors or channels) or do they nonspecifically affect membrane excitability?

Harper (1987) has proposed a biophysical model of salt taste that includes contributions from anions. The stimulatory effectiveness of a variety of salts in integrated recordings from the chorda tympani nerve may be explainable on the basis of diffusion potentials generated at the interface between the stimulus solution and the tongue, probably in the region of the tight junctions. This model requires that the tight junctions be permeable to small stimulatory cations and anions, where both diffusion and sieving contribute to the differential effects.

The chorda tympani nerve of the hamster has two physiologically defined populations of fibers that respond to sodium salts (N and H fibers; Frank, Bieber, and Smith, 1988). N fibers innervate sodium-sensitive taste cells that have amiloride-blockable sodium channels (Hettinger and Frank, 1990). Excitation occurs when Na^+ in the stimulus passes through Na^+ channels in the apical membrane, thereby depolarizing the cell. Preincubating the hamster tongue with 10 μM amiloride completely and reversibly eliminated the transient and steady-state responses of N fibers to NaCl (Hettinger and Frank, 1990). In contrast, H fibers were relatively insensitive to amiloride, indicating that they innervate cells having a different sodium-sensing mechanism. Research thus far has primarily focused on how N and H fibers differentially respond to cations such as Na^+, K^+, NH_4^+, H^+, Mg^{2+}, and quinine$^+$ (Frank et al., 1988). If N and H fibers differ in their sensitivity to amiloride, it seems possible that they may differ in their susceptibility to inhibition by anions in salt stimuli.

The stimulatory effectiveness of NaCl and Na acetate are clearly different in whole-nerve recordings from the chorda tympani of the hamster (Rehnberg et al., 1989). A Na acetate concentration of 0.4 M was needed to produce a transient response equal to that produced by 0.1 M NaCl. Formaker and Hill (1988) have argued that, in rats, halide anions play more than just an indirect stimulatory role. Multi-unit responses of the rat chorda tympani to certain nonhalide sodium salts were completely eliminated by amiloride, whereas only partial blockage was seen for halide salts. Elliott and Simon (1990) made similar observations and have also presented data supporting the view that organic anions such as acetate inhibit the response of the rat chorda tympani to sodium salts. Our objective in this study is to use electrophysiological and pharmacological methods to investigate the role played by anions in salt taste in golden Syrian hamsters (*Mesocricetus auratus*). We address several specific questions: (*1*) Does the chorda tympani nerve respond differently to NaCl and Na acetate over a range of concentrations? (*2*) Do Cl^- and CH_3COO^- (acetate) play a direct role in stimulating taste cells? (*3*) Are anion-conducting pathways in taste cell membranes selective for Cl^- and CH_3COO^-? (*4*) Does the concept of anion inhibition apply to all sodium-sensitive taste cells innervated by chorda tympani fibers?

METHODS

Neural Recordings

Male hamsters (Charles River Laboratories, Wilmington, MA) weighing 100–150 g were deeply anesthetized with pentobarbital and the right chorda tympani nerve was exposed by the mandibular approach. Surgical procedures and recording technique have been previously described (Hyman and Frank, 1980). For whole-nerve recordings, the nerve was placed on a nichrome wire electrode and the differentially amplified responses were displayed on an oscilloscope. The amplified signal was also sent to a digital summator and strip chart recorder which printed summated whole-nerve responses using a bin size of 200 ms. Taste stimuli were presented using an overhead funnel (1.5 ml/s) and a glass flow chamber which limited stimulation to the anterior tongue. Whole-nerve recordings were made using NaCl and Na acetate at concentrations ranging from 0.003 to 1.0 M. Our response measure was the height of the peak transient phase above baseline activity.

For single-unit recordings, the chorda tympani was desheathed with forceps and divided into fine bundles of fibers. Responses were displayed on an oscilloscope and stored on magnetic tape. Criteria for identifying single-unit activity included low spontaneous rates, singular wave form, uniform spike height, and examination of latencies between contiguous spikes. Our response measure was the number of action potentials during the first 5 s of the response to the taste stimulus. "Search" stimuli used to categorize single-unit activity were 0.03 M NaCl, NH_4Cl, and sucrose (Sigma Chemical Co., St. Louis, MO). N fibers were those that responded relatively well to NaCl and weakly to sucrose and NH_4Cl, whereas H fibers responded well to both salts but weakly to sucrose (Frank et al., 1988). We measured the responses of both N and H fibers to NaCl and Na acetate at concentrations ranging from 0.003 to 1.0 M.

Single Fiber Cross-Adaptation

Adapting and test stimuli were presented using separate overhead funnels fitted with stopcocks. The adapting stimulus was continuously presented for 30 s and was immediately followed by the test stimulus for 15 s. After the test stimulus, the tongue was rinsed with distilled water for 60 s, which readied it for the next adapting stimulus. After using an adapting and test stimulus, the pair was immediately retested with the order of presentation reversed. Pilot recordings were done to determine moderate and equistimulatory concentrations of NaCl and Na acetate to be used for cross-adaptation. N fibers showed approximately equal transient and steady-state responses to 0.03 M NaCl and 0.03 M Na acetate. H fibers gave unpredictable and often weak responses to Na acetate and were therefore not amenable to the cross-adaptation approach.

Iontophoretic Presentation of Anionic Stimuli

Responses of the whole chorda tympani nerve to iontophoretically applied taste stimuli followed the protocol of Herness and Pfaffmann (1986). Current was delivered across a nichrome wire electrode positioned above but not in contact with the tongue inside the flow chamber and a stainless steel pin inserted into musculature of the rear leg. Constant cathodal current of 2–20 μA was delivered by a Cornerstone S-900 stimulator (Dagan Corp., Minneapolis, MN) through a Cornerstone S-910 isolation unit. Changes in electrode resistance due to polarization are effectively accommodated. Bridging solutions of 1 mM NaCl, Na acetate, and Na saccharin flowed through the tongue chamber at 1.5 ml/s, thereby simultaneously covering both the tongue and the wire electrode. The concentrations of the bridging solutions were near detection thresholds for the chorda tympani in the absence of current (Herness and Pfaffman, 1986). Cathodal current was expected to drive anions in the bridging solution (primarily Cl^-,

CH_3COO^-, or saccharin$^-$) toward the tongue and cations (primarily Na^+) away from the tongue.

The tongue was rinsed continuously between stimuli with distilled water at 1.5 ml/s for 60 s. To test a stimulus, a bridging solution flowed over the tongue for ~10 s before cathodal current was delivered for 10 s. A reference stimulus of 0.03 M NaCl was periodically presented without current to monitor nerve viability and the sensitivity of taste cells on the tongue.

Inhibitors of Anion-conducting Pathways

Four drugs that interfere with normal movements of Cl^- across a variety of epithelia were tested for efficacy in reducing chorda tympani responses to NaCl and Na acetate. Anthracene-9-carboxylate (A-9) and diphenylamine-2-carboxylate (DPC) are Cl^- channel blockers, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonate (SITS) is an inhibitor of anion exchange, and furosemide is an inhibitor of a Cl^- cotransporter. The drugs were tested at 0.01, 1.0, and 100 μM. Testing protocol followed that of Hettinger and Frank (1990). A 0.03-M taste stimulus was applied to the tongue and the response of the chorda tympani was recorded for ~20 s. Without rinsing the tongue, a mixture of 0.03 M stimulus and drug was presented and the response measured for an additional 20 s. The size of the steady-state responses during 5-s intervals before (R_c) and after (R_i) presentation of drug was used to quantify inhibition (Fig. 1). To allow for latencies of inhibition, R_i was not measured until 2 s after the drug made contact with the tongue. Inhibition was expressed as a percent of the control response: $100 \cdot R_i \cdot R_c^{-1}$. As a comparison, amiloride (a Na^+ channel blocker) was tested at 10 μM (Fig. 1), a concentration known to strongly reduce chorda tympani responses to NaCl (Hettinger and Frank, 1990).

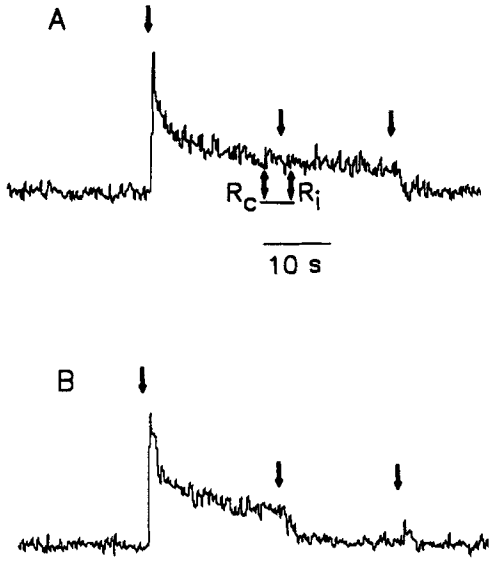

FIGURE 1. Responses of the whole chorda tympani nerve to 0.03 M NaCl and the effects of (*A*) ineffectual and (*B*) strong inhibitors. The first arrow marks the onset of NaCl; the second arrow shows the onset of either (*A*) a mixture of 0.03 M NaCl and 1.0 μM A-9 or (*B*) 0.03 M NaCl and 10 μM amiloride; and the third arrow indicates a water rinse. The measures used to calculate percent inhibition (R_c and R_i; see Methods) are shown in *A*.

RESULTS

NaCl is a more potent stimulus than Na acetate when recording from the whole chorda tympani nerve (Fig. 2). Up to 0.3 M, the concentration–response functions

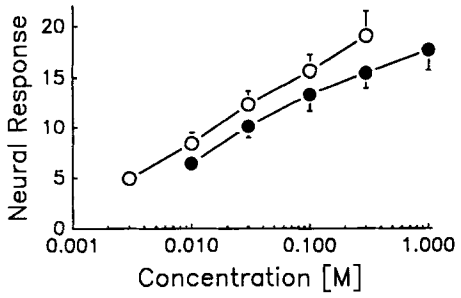

FIGURE 2. Summated responses of the whole chorda tympani nerve to NaCl (○) and Na acetate (●). Responses are in strip-chart units. Means and standard errors were calculated using data from six preparations.

for these stimuli were parallel, with NaCl eliciting greater neural activity at each test concentration.

The two populations of sodium-sensitive fibers in the chorda tympani nerve show different patterns of response to NaCl and Na acetate. N fibers responded strongly and, in general, similarly to equimolar concentrations (Fig. 3). Responses by 13 N fibers to 0.03 M NaCl ($\bar{X}$ = 136.6 spikes/5 s) and 0.03 M Na acetate ($\bar{X}$ = 116 spikes/5 s) were not significantly different (paired t test, $P = 0.156$). In contrast, H fibers reacted differently to the two salts (Fig. 4). Increased firing to NaCl was observed for increased concentrations between 0.03 and 0.3 M. Responses to Na acetate leveled off or decreased at concentrations above 0.1 M and were generally less than for NaCl at the same concentrations. Responses by 8 H fibers to 0.3 M NaCl ($\bar{X}$ = 89.4 spikes/5 s) were significantly greater than the responses to 0.3 M Na acetate (32.9 spikes/5 s; paired t test, $P < 0.01$). Overall trends can be seen in a composite graph based on Figs. 3 and 4, as well as recordings from other fibers (Fig. 5). A two-way ANOVA using the responses of N fibers to NaCl and Na acetate between 0.01 and 1.0 M showed significant effects due to stimulus concentration ($F = 10.6$, $P < 0.01$) but not due to stimulus type ($F = 0.18$, $P = 0.58$). A similar

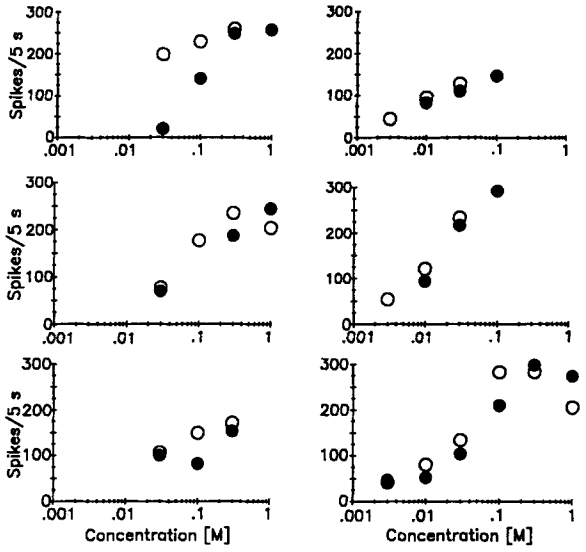

FIGURE 3. Representative responses of N fibers to NaCl (○) and Na acetate (●). Each figure represents one N fiber.

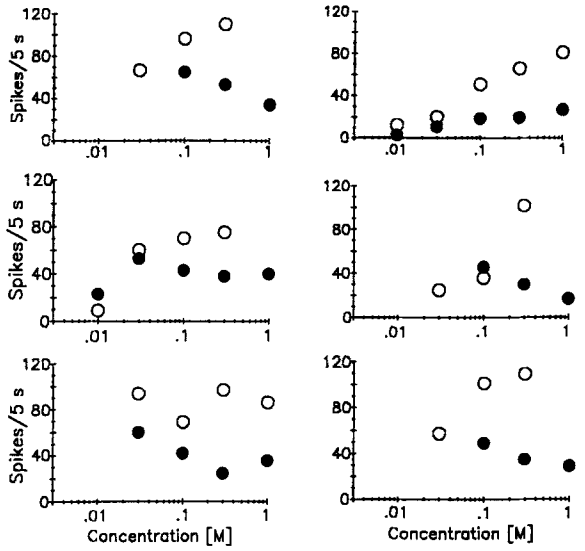

FIGURE 4. Representative responses of H fibers to NaCl (○) and Na acetate (●). Each figure represents one H fiber.

ANOVA for H fibers showed significant effects due to both stimulus concentration ($F = 6.74$, $P < 0.01$) and stimulus type ($F = 27.56$, $P < 0.01$).

Cross-adaptation data indicate that Cl^- and CH_3COO^- differentially affect the taste cells innervated by N fibers. Patterns of self-adaptation were similar for NaCl and Na acetate (Fig. 6, *A* and *B*). Both stimuli elicited clear transient responses that gradually decayed toward lower steady-state levels. Presentation of the "test" stimuli produced no new transient responses or consistent increases in steady-state neural activity. One unit showed a strong "off" response when the tongue was rinsed with water (Fig. 6 *B*). Patterns of cross-adaptation for NaCl adapt/Na acetate test resembled the data for self-adaptation (Fig. 6 *C*). After N fiber responses to NaCl had decayed toward steady-state levels, presentation of Na acetate elicited no increase in firing rate. For each fiber tested, spike activity was lower during the first 5 s after presentation of Na acetate compared with the 5 s immediately before Na acetate. The reciprocal cross, however, produced a different pattern of cross-adaptation (Fig. 6 *D*). After adapting to Na acetate, the number of spikes in the 5 s after the NaCl test presentation was significantly greater than in the 5 s before ($P < 0.05$, two-tailed

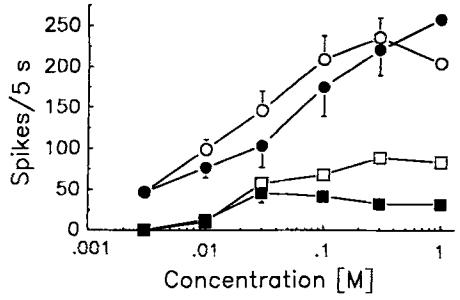

FIGURE 5. Summary of neural responses to NaCl (*open symbols*) and Na acetate (*filled symbols*) by N fibers (*circles*) and H fibers (*squares*). Means and standard errors were calculated using data from fibers that include those shown in Figs. 3 and 4.

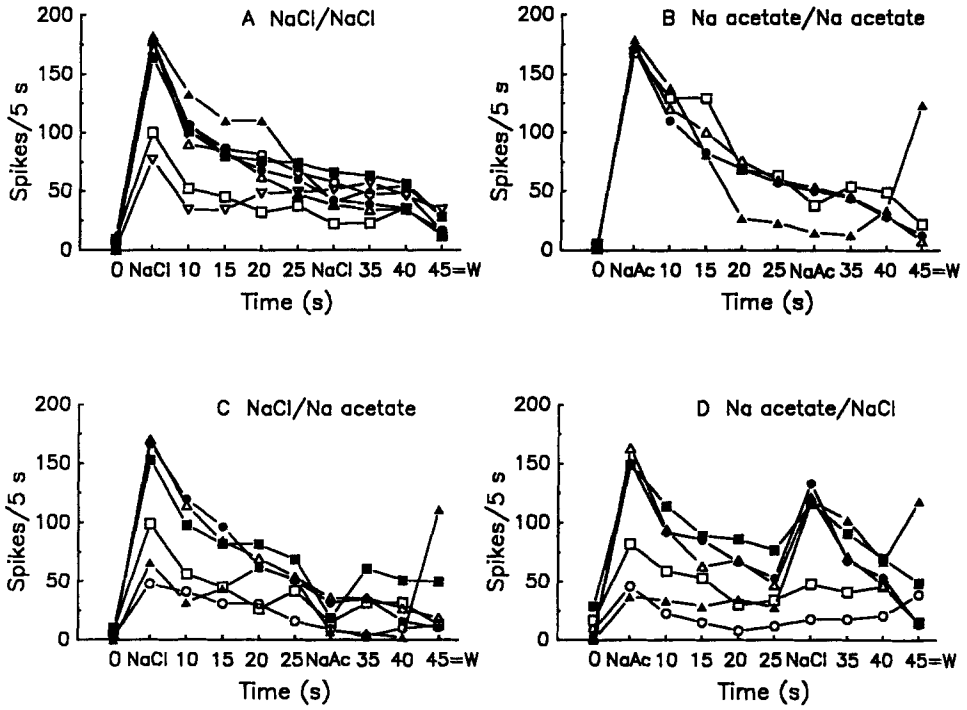

FIGURE 6. Self- and cross-adaptation of N fibers in the chorda tympani nerve. Each line represents spiking activity for one N fiber. The adapting stimulus was presented at 5 s and the test stimulus at 30 s. Water rinse is indicated by W. (*A*) Self-adaptation to 0.03 M NaCl. (*B*) Self-adaptation to 0.03 M Na acetate. (*C*) Response to 0.03 M NaCl followed by 0.03 M Na acetate. (*D*) Response to 0.03 M Na acetate followed by 0.03 M NaCl.

Wilcoxon's matched-pairs signed-rank test). The strongest test responses to NaCl were seen in the more vigorously responding fibers.

Whole-nerve responses to iontophoretically delivered anionic stimuli suggest that neither Cl^- nor CH_3COO^- directly contributes to the stimulatory effectiveness of NaCl and Na acetate. Responses shown in Fig. 7 are representative of four

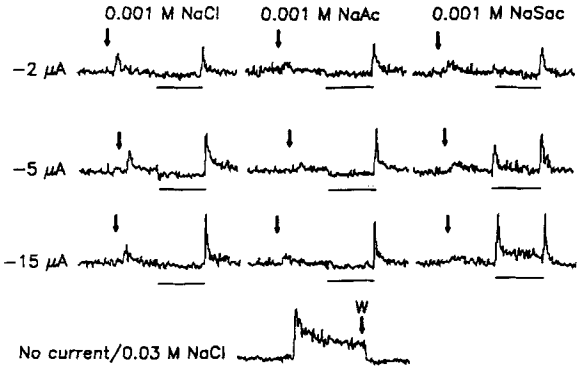

FIGURE 7. Responses of the whole chorda tympani nerve to 0.001 M NaCl, Na acetate, and Na saccharin in the presence and absence of 2, 5, and 15 μA of cathodal current. Arrows indicate presentation of the chemical stimuli and the horizontal bars show the 10-s periods during which current was delivered. Also shown is a response to 0.03 M NaCl in the absence of current.

preparations using the iontophoretic protocol. In all cases, the chorda tympani showed either no response or a slightly decreased baseline response to 1 mM NaCl or Na acetate over a range of cathodal currents. Iontophoretic presentation of the saccharin$^-$ anion, however, produced a typical chorda tympani response consisting of both transient and steady-state phases. In addition, responses to saccharin$^-$ increased with increasing current and reached magnitudes comparable to the response to chemically applied 0.03 M NaCl (Fig. 7). Breaking the current produced a characteristic off response for all stimuli (Herness and Pfaffmann, 1986).

Furosemide, SITS, DPC, and A-9 had no clear effect on whole-nerve responses to 0.03 M NaCl (Fig. 8 *A*). Neural responses in the presence of a wide range of drug concentrations were similar to control responses. A two-way ANOVA showed no

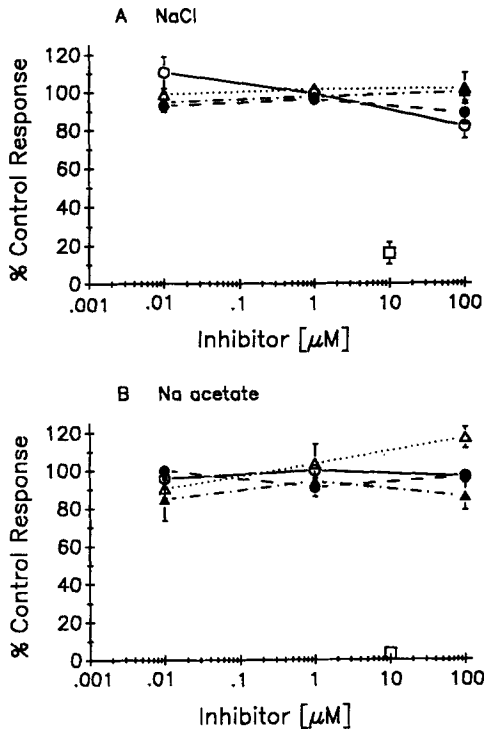

FIGURE 8. Whole chorda tympani responses to (*A*) 0.03 M NaCl and (*B*) 0.03 M Na acetate in the presence of furosemide (○), A-9 (●), SITS (△), DPC (▲), and amiloride (□). The neural response in the presence of the inhibitor is expressed as a percentage of the response in the absence of the inhibitor. Means and standard errors were calculated using data from three to five preparations.

significant effects due to either inhibitor type ($F = 1.01$, $P = 0.40$) or inhibitor concentration ($F = 1.57$, $P = 0.22$). A similar ANOVA using responses to Na acetate (Fig. 8 *B*) showed a significant effect due to inhibitor type ($F = 3.38$, $P = 0.03$) but not to inhibitor concentration ($F = 1.21$, $P = 0.32$). In contrast to the negligible effects of the above inhibitors, 10 μM amiloride reduced responses of the chorda tympani to near baseline levels for both NaCl and Na acetate (Figs. 1 and 8).

DISCUSSION

Each fungiform papilla in the hamster has a taste bud that contains ~50 cells. The neural output from electrolyte-sensitive taste cells travels centrally in N and H fibers

in the chorda tympani nerve. Our recordings therefore reflect stimulus interactions at the apical membrane of taste cells as well as those intracellular events that bear on the transduction of salt taste. Details such as stimulus specificities of taste cells within the buds and the degree of convergence of separate taste buds onto the same neural fiber are unresolved matters. Nevertheless, recordings from single N and H fibers reflect transduction events occurring in a relatively small number of salt-sensitive taste cells. Our findings suggest that there are two kinds of salt sensitivities in fungiform taste buds and two corresponding ways of reacting to salt anions.

Recently, Ye et al. (1991) provided the first direct experimental evidence that anions of salt stimuli gain access to basolateral regions of taste cells and thereby influence excitability. A small chamber was attached to the anterior tongue of rats to present taste stimuli and to voltage- and current-clamp the enclosed epithelial patch. Simultaneously recording from the chorda tympani nerve permitted correlations of transepithelial potentials and the neural taste responses to NaCl, Na acetate, and Na gluconate. They discovered an inverse relationship between the magnitudes of neural response and corresponding transepithelial potential response. Presenting the three taste stimuli while clamping the voltage to −90 mV (inside electronegative) produced steady-state responses of equal magnitude. Their interpretation is that voltage-clamping the field potentials removes the effects of diffusing Cl^-, CH_3COO^-, or gluconate$^-$, and that the observed neural responses result only from the sodium cation. According to this hypothesis, the degree of anion inhibition corresponds to the field potential surrounding taste cells, which in turn is determined by the paracellular shunting of stimulus anions. This interpretation is similar to Harper's model (1987) in which apical receptor stimulation by cations is modulated by diffusion potentials.

The paracellular shunt model of salt stimulation has appeal and is supported by the data of Ye et al. (1991), but it is based on taste responses from the whole nerve. The chorda tympani nerve in both the rat (Frank, Contreras, and Hettinger, 1983) and hamster (Frank et al., 1988) has two populations of nerve fibers (N and H) that respond strongly to sodium salts. In its simplest formulation, the paracellular shunt model should affect all sodium-sensitive fibers similarly. Our data indicate that the concept of anion inhibition may have relevance only to the H population. H fibers generally respond more vigorously to NaCl than to Na acetate and the slope of the concentration–response curve is greater for NaCl. N fibers are more likely to respond equally to NaCl and Na acetate. Consequently, the single-fiber perspective posits a salt sensitivity that is not simultaneously anion dependent and amiloride sensitive. Instead, these two defining characteristics of salt taste appear to be segregated by population. The population of N fibers is amiloride sensitive and anion independent, whereas H fibers are amiloride insensitive and anion dependent. We believe that there are two independent and fundamentally different mechanisms for detecting sodium salts in the hamster. That is not to say that anions are without influence on the excitability of N fibers. Our cross-adaptation data for N fibers, for example, show that NaCl and Na acetate do not reciprocally cross-adapt. Clearly, there are still many unanswered questions about basic transduction mechanisms in salt taste.

Although not made explicit, the notion of "anion inhibition" has existed since Beidler (1953, 1954) used a rat chorda tympani preparation to show that anions

modify the efficacies of sodium salts. Our understanding of the meaning of anion inhibition is that the cationic component of a salt is responsible for stimulating salt-sensitive cells and the associated anions in some way reduce the cation's capacity to stimulate. If NaCl is the most potent of all sodium salts, Cl^- would be the weakest inhibitor among all anions. This is still an unsettled matter since two recent studies of responses by the rat chorda tympani came to different conclusions about the functional role of CH_3COO^-. Formaker and Hill (1988) argued that CH_3COO^- was not inhibitory, whereas Elliott and Simon (1990) concluded that CH_3COO^- did inhibit the response to Na^+. If CH_3COO^- inhibits responses to Na^+ in hamsters, the affected taste cells would be those innervated by H fibers. As the concentration of Na^+ and CH_3COO^- increases, the activity of these fibers stays the same or even decreases.

Differential responses of taste systems to the monovalent NaCl and Na acetate cannot be explained by differences in species dissociation or in activity coefficients. Such physiochemical variables would be expected to have more substantial effects for divalent ions at high concentrations (Sostman and Simon, 1991). NaCl and Na acetate are expected to fully dissociate. At 0.1 M, the activity coefficients of NaCl and Na acetate are nearly identical (0.78 and 0.79, respectively; Handbook of Chemistry and Physics, 71st ed.). At 1.0 M, they are 0.66 and 0.76, respectively. If results were plotted in terms of activity, the differences would increase slightly.

Driving anions toward the tongue with current while simultaneously withdrawing cations suggests that neither Cl^- nor CH_3COO^- directly excites taste cells on the anterior tongue. Even relatively large cathodal currents produced neither transient nor steady-state responses. Iontophoretic presentation of the saccharin anion, however, showed that specific classes of anions can be highly stimulatory using this protocol. Although the exact mechanism of iontophoretic stimulation is not known, Herness and Pfaffmann (1986) found that several organic anions having either sweet or bitter qualities to hamsters were good iontophoretic stimuli. Recording from the chorda tympani and using cathodal current, they found the following efficacy series: Na saccharin > Na *m*-nitrobenzene sulfonate > Na picrate > Na *m*-nitrobenzoate > Na cholate > NaCl. In our study, switching to anodal current with either NaCl, Na acetate, or Na saccharin in the flow chamber caused very large neural responses, presumably due to Na^+ moving toward the tongue (Pfaffmann and Pritchard, 1980). Thus, it appears that Cl^- and CH_3COO^- have the capacity to modulate the stimulatory effectiveness of Na^+ without being taste stimuli themselves. If Cl^- and CH_3COO^- are only stimulus modulators and Na acetate is a less potent taste stimulus than NaCl, then CH_3COO^- could be viewed as a stronger inhibitor of the response to Na^+. These data therefore provide support for the concept of anion inhibition.

Our work emphasizes that N fibers respond similarly to NaCl and Na acetate, whereas H fibers do not. This appears to be generally true, but cross-adaptation data show that N fibers can also distinguish between NaCl and Na acetate. Although it seemed that Na acetate inhibited neural responses after adaptation to NaCl, a simpler interpretation is more likely. The self-adaptation data show that test stimuli were applied to the tongue before the neural response had reached a steady-state level. After adapting to NaCl for 30 s, neural responses continued to decline when the test stimulus was either NaCl or Na acetate. In addition, the appearance of

inhibition by Na acetate was enhanced because N fibers seem to respond to NaCl slightly stronger than to Na acetate.

After adapting to Na acetate, N fibers significantly increased spiking rates to NaCl. This strongly suggests that chloride and acetate play different roles in the tonic excitability of N fibers. Taste cells adapted to Na acetate seem to be momentarily released from adaptation by Cl^-, which permits another transient response to Na^+. Explaining why N fibers respond to NaCl after adapting to Na acetate is made difficult by our lack of understanding of chemosensory adaptation. It is not known if Na adaptation results when Na^+ ions traverse apical Na channels at a progressively decreasing rate or when intracellular processes become progressively less responsive to constant levels of incoming Na^+. In either case, the ability of Cl^- to facilitate the Na response after adaptation to Na acetate is noteworthy since Cl^- itself is not a gustatory stimulus.

If gustatory adaptation involves a decreasing sodium conductance, the process may be analogous to sodium self-inhibition, a mechanism by which sodium is thought to block its own channel from the mucosal side (Lewis, Hanrahan, and Van Driessche, 1984; Garty and Benos, 1988). Sodium permeability across the stratum granulosum of frog skin increases sharply but transiently to a step increase in outside sodium (Fuchs, Larsen, and Lindemann, 1977). The transient peak in sodium permeability decays to a lower steady-state level with a time constant measured in seconds. To apply the self-inhibition model to our data in Fig. 6 *D*, the chloride anion would in some way release sodium from its inhibitory binding site and thereby transiently increase channel permeability to sodium. The resulting transient response to NaCl would once again decay as sodium self-inhibition reestablishes itself, resulting in decreased channel permeability. Drugs such as benzimidazolyl-guanidinium (BIG) and benzthiazolyl-guanidinium (BTG) are thought to increase sodium permeability through sodium channels by lowering the apparent affinity of Na^+ for the self-inhibition site (Fuchs et al., 1977).

Sodium transport can be influenced by a wide variety of chemical interactions at epithelial barriers. The relative effectiveness of mucosal anions in supporting sodium transport across toad urinary bladder was $SCN^- > I^- > NO_3^- > Br^- > Cl^- >$ propionate$^-$ > CH_3COO^- > tartrate$^-$ > citrate$^-$ > $SO_4^= > HPO_4^= > F^- > N_3^-$ (Singer and Civan, 1971). This ordering could not be explained by either anion size or electrophoretic mobility in aqueous media. Similarly, Mierson, Heck, DeSimone, Biber, and DeSimone (1985) concluded that anions at the apical surface of canine lingual epithelium influence the sodium transport step at the apical border. Aldosterone and the antidiuretic hormones vasopressin and oxytocin increase sodium conductance in epithelia by activating quiescent (nonconducting) sodium channels already present in apical membranes (Lewis et al., 1984; Garty and Benos, 1988). Activation of sodium channels by hormones, however, develops much more slowly (minutes to hours) than gustatory cross-adaptation. Finally, mucosally applied pharmacological agents such as BIG and BTG increase sodium transport and epithelial conductance across frog skin, suggesting that the sodium channel is associated with apical regulatory sites (Fuchs et al., 1977). We conclude from these studies that the flux of sodium across epithelia can be strongly influenced by chemical interactions with anions, hormones, and drugs.

The conductance of extracellular ions through the apical membrane is a mechanism that could contribute to the depolarization of taste cells during excitation. The existence of amiloride-sensitive Na^+ channels in taste cells innervated by specific populations of chorda tympani fibers in rats (Ninomiya and Funakoshi, 1988) and hamsters (Hettinger and Frank, 1990) is strong support for this hypothesis. Although Na^+ appears to passively diffuse through ungated channels in the apical membrane, the fate of the associated anion is unknown. Our data show that neither Cl^- nor CH_3COO^- pass through anion-conducting pathways that are blockable by furosemide, SITS, DPC, or A-9. Pharmacological research has previously established that these drugs can block Cl^- movements across epithelia, although we have not directly verified it for lingual epithelium. Elliott and Simon (1990) tested several inhibitors of anion exchangers, Cl^- cotransporters, and Cl^- channels on the response of the rat chorda tympani nerve to NaCl. None of the drugs depressed either the total response or the amiloride-insensitive portion, which led them to suggest the existence of anion-permeable tight junctions. Anion-specific permeability of tight junctions surrounding taste cells may play a role in determining the overall stimulatory effectiveness of a sodium salt. Large or multivalent anions would not traverse this paracellular pathway as easily as small monovalent anions and their salts would be less stimulatory (Elliott and Simon, 1990). The permeability of tight junctions to ions is variable in the vicinity of taste buds. Holland, Zampighi, and Simon (1991) showed that tight junctions around lingual taste cells in the dog were permeable to cationic lanthanum (used as $LaCl_3$), whereas surrounding nonsensory epithelium was impermeable.

In conclusion, our data show that the taste system of the hamster reacts differently to NaCl and Na acetate. The Cl^- and CH_3COO^- ions do not enter taste cells through anion-selective pathways, nor do they directly stimulate taste cells. The concept of anion inhibition is supported by our data, but only in reference to one of two populations of salt-sensing neurons in the chorda tympani nerve. Furthermore, our data together with those of Hettinger and Frank (1990) show that neurons responsive to salts are not simultaneously anion and amiloride sensitive. Amiloride-insensitive H fibers were found to be sensitive to anions, whereas responses by N fibers are blocked by amiloride but are relatively anion insensitive.

We thank M. Barry, B. Formaker, and J. London for critical readings of earlier versions of this report.

Our research was supported by NSF grant BNS-8519638 and by NIH grants NS-16993 and DC-00058. B. G. Rehnberg was partially supported by NIH training grant 000225.

Original version received 2 March 1992 and accepted version received 29 October 1992.

REFERENCES

Beidler, L. M. 1953. Properties of chemoreceptors of tongue of rat. *Journal of Neurophysiology.* 16:595–607.

Beidler, L. M. 1954. A theory of taste stimulation. *Journal of General Physiology.* 38:133–139.

Beidler, L. M., and G. W. Gross. 1971. The nature of taste receptor sites. *In* Contributions to Sensory Physiology. W. D. Neff, editor. Academic Press, NY. 97–127.

Elliott, E. J., and S. A. Simon. 1990. The anion in salt taste: a possible role for paracellular pathways. *Brain Research.* 535:9–17.

Formaker, B. K., and D. L. Hill. 1988. An analysis of residual NaCl taste response after amiloride. *American Journal of Physiology.* 255:R1002–1007.

Frank, M. E., S. L. Bieber, and D. V. Smith. 1988. The organization of taste sensibilities in hamster chorda tympani nerve fibers. *Journal of General Physiology.* 91:861–896.

Frank, M. E., R. J. Contreras, and T. P. Hettinger. 1983. Nerve fibers sensitive to ionic taste stimuli in chorda tympani of the rat. *Journal of Neurophysiology.* 50:941–960.

Fuchs, W., E. H. Larsen, and B. Lindemann. 1977. Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *Journal of Physiology.* 267:137–166.

Garty, H., and D. J. Benos. 1988. Characteristics and regulatory mechanisms of the amiloride-blockable Na^+ channel. *Physiological Reviews.* 68:309–373.

Handbook of Chemistry and Physics. 71st ed. 1991. David R. Lide, editor. CRC Press, Inc., Boca Raton, FL.

Harper, H. W. 1987. A diffusion potential model of salt taste receptors. *Annals of the New York Academy of Sciences.* 510:349–351.

Herness, M. S., and C. Pfaffmann. 1986. Iontophoretic application of bitter taste stimuli in hamsters. *Chemical Senses.* 11:203–211.

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

Holland, V. F., G. A. Zampighi, and S. A. Simon. 1991. Tight junctions in taste buds: possible role in perception of intravascular gustatory stimuli. *Chemical Senses.* 16:69–79.

Hyman, A. M., and M. E. Frank. 1980. Sensitivities of single nerve fibers in the hamster chorda tympani nerve to mixtures of taste stimuli. *Journal of General Physiology.* 76:143–173.

Lewis, S. A., J. W. Hanrahan, and W. Van Driessche. 1984. Channels across epithelial cell layers. *Current Topics in Membranes and Transport.* 21:253–293.

Mierson, S., G. L. Heck, S. K. DeSimone, T. U. L. Biber, and J. A. DeSimone. 1985. The identity of the current carriers in canine lingual epithelium in vitro. *Biochimica et Biophysica Acta.* 816:283–293.

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

Pfaffmann, C., and T. Pritchard. 1980. Ion specificity of "electric taste." *In* Olfaction and Taste. Vol. 17. H. van der Starre, editor. IRL Press, London. 175–178.

Rehnberg, B. G., T. P. Hettinger, and M. E. Frank. 1989. Asymmetrical neural cross-adaptation of hamster chorda tympani responses to sodium and chloride salts. *Chemical Senses.* 14:663–672.

Rehnberg, B. G., T. P. Hettinger, and M. E. Frank. 1992. Salivary ions and neural taste responses in the hamster. *Chemical Senses.* 17:179–190.

Schiffman, S. S., A. L. Crumbliss, Z. S. Warwick, and B. G. Graham. 1990. Thresholds for sodium salts in young and elderly human subjects: correlation with molar conductivity of anion. *Chemical Senses.* 15:671–678.

Singer, I., and M. M. Civan. 1971. Effects of anions on sodium transport in toad urinary bladder. *American Journal of Physiology.* 221:1019–1026.

Sostman, A. L., and S. A. Simon. 1991. Trigeminal nerve responses in the rat elicited by chemical stimulation of the tongue. *Archives of Oral Biology.* 36:95–102.

Ye, Q., G. L. Heck, and J. A. DeSimone. 1991. The anion paradox in sodium taste reception: resolution by voltage-clamp studies. *Science.* 254:724–726.