Role for epithelial Na^+ channels and putative Na^+/H^+ exchangers in salt taste transduction in rats

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Lundy, Robert F., Jr., David W. Pittman, and Robert J. Contreras. Role for epithelial Na^+ channels and putative Na^+/H^+ exchangers in salt taste transduction in rats. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R1923–R1931, 1997.—The effects of the epithelial Na^+ channel antagonists amiloride and benzamil and the Na^+/H^+ exchange antagonist 5-(*N*,*N*-dimethyl)-amiloride (DMA)-Cl on the integrated responses of the chorda tympani nerve to 30, 75, 150, 300, and 500 mM concentrations of NaCl, KCl, and NH_4Cl were assessed in male Sprague-Dawley rats. Based on evidence from other systems, 1 and 25 μM amiloride and benzamil were chosen to selectively inhibit epithelial Na^+ channels and 1 μM DMA was chosen to selectively inhibit Na^+/H^+ exchange. When added to stimulating salt solutions, amiloride, benzamil, and DMA were each effective in inhibiting responses to all three salts. The degree of inhibition varied with drug, salt, and salt concentration, but not drug dose. Amiloride suppressed NaCl responses to a greater degree than KCl and NH_4Cl responses, whereas DMA suppressed NH_4Cl responses to a greater degree than NaCl and KCl responses. In all but one case (25 μM amiloride added to KCl), drug suppression of taste nerve responses decreased with an increase in salt concentration. The present results suggest that *1*) epithelial Na^+ channels in rat taste receptor cells may play a role in KCl and NH_4Cl taste transduction; *2*) a Na^+/H^+ exchange protein may be present in taste receptor cells, representing a putative component, in addition to epithelial Na^+ channels, in salt taste transduction; and *3*) salt taste detection and transduction may depend on the utilization of a combination of common and distinct transcellular pathways.

amiloride analogs; chorda tympani nerve; amiloride hydrochloride; benzamil; 5-(*N*,*N*-dimethyl)-amiloride chloride; sodium chloride; potassium chloride; ammonium chloride

THE CHORDA TYMPANI NERVE, which innervates taste receptor cells found in fungiform papillae on the anterior two-thirds of the tongue, conveys peripheral taste information to the central nervous system. Taste solutions on the tongue come in contact with and stimulate taste receptor cells, thereby altering the activity of the chorda tympani nerve. Previous patch-clamp studies demonstrate that taste receptor cells express a variety of ion channels in their plasma membranes (1, 2, 13). It was shown that NaCl and KCl stimulate taste receptor cells in part by cations entering through specific ion channels (12, 31). In rats, amiloride-sensitive Na^+ channels have been shown to play a role in taste stimulation by sodium salts (3, 5, 7, 20, 26), whereas 4-aminopyridine-sensitive K^+ channels (4-AP K^+ channel) may play a role in taste stimulation by KCl (11, 26) and NH_4Cl (11). From the available evidence it appears that a component of taste receptor cell stimulation by sodium salts involves a pathway (epithelial Na^+ channels) not utilized by potassium and ammonium salts. Similarly, a component of taste receptor cell stimulation by potassium and ammonium salts involves a pathway (4-AP K^+ channels) not utilized by sodium salts. In contrast, other studies in rats provide evidence suggesting that NaCl and KCl may also stimulate taste receptor cells through the same mechanism involving amiloride-sensitive epithelial Na^+ channels (18, 19, 21).

A prior study by Minear et al. (19) demonstrated that when added to ongoing stimulation, amiloride suppressed chorda tympani nerve responses to KCl in rats. Amiloride concentrations of 0.3 and 1 μM suppressed taste nerve responses to 30 mM KCl but had little effect on responses to 100, 300, and 1,000 mM KCl. An increase in the amiloride dose to 10 and 100 μM suppressed all KCl responses by an average of 35%; the degree of amiloride suppression decreased as KCl concentration increased.

More recently, Lundy and Contreras (18) also showed that amiloride suppressed chorda tympani nerve responses to KCl. Furthermore, they found that NaCl responses, but not KCl responses, were temperature dependent. The absolute responses to NaCl were smaller at 25°C than at 35°C and amiloride (100 μM) suppression of NaCl responses were greater at 25°C than at 35°C. The evidence suggests that epithelial Na^+ channels are temperature dependent as a temperature decrease combined with amiloride suppressed NaCl responses to a greater degree than amiloride alone. The responses to KCl and amiloride suppression of KCl responses were unaffected by changes in temperature. Because of the sensitivity of epithelial Na^+ channels and the insensitivity of KCl stimulation to temperature shifts, Lundy and Contreras (18) concluded that amiloride suppression of KCl responses cannot be mediated by antagonism of KCl-stimulated entry of sodium ions into taste receptor cells. They concluded instead that amiloride suppressed KCl responses by direct antagonism of stimulating potassium ions into taste receptor cells.

Lundy and Contreras (18) also demonstrated that 100 μM benzamil, a more selective Na^+ channel antagonist than amiloride (15), was equally effective as 100 μM amiloride in suppressing chorda tympani responses to 500 mM NaCl. Furthermore, benzamil was found to be one-half as effective as amiloride in suppressing chorda tympani nerve responses to 500 mM KCl. Benzamil did not suppress chorda tympani nerve responses to 500 mM NH_4Cl. Lundy and Contreras (18) concluded that amiloride suppression of taste nerve responses to KCl likely involved epithelial Na^+ channels in addition to other membrane protein(s), whereas amiloride suppression of responses to NH_4Cl likely involved membrane protein(s) other than epithelial Na^+ channels. Despite this evidence, the extent to

which Na^+ channels may be involved in taste receptor cell stimulation by KCl remains unresolved because 100 μM concentrations of amiloride and benzamil are strong and unselective in their site of antagonism (see below). Furthermore, the mechanism of how amiloride is able to suppress NH_4Cl responses also remains unknown.

Prior studies in kidney medulla microsomes (16), chick skeletal muscle cells (30), and hamster ovary cells transfected with Na^+/H^+ exchange proteins (23, 32) indicate that amiloride and benzamil significantly suppressed Na^+ influx through Na^+/H^+ exchange with apparent inhibitor constant (K_i) values in the range of 1.4–300 μM and 100–700 μM, respectively. The exact K_i values for amiloride and benzamil seemed to depend on which tissue and Na^+/H^+ exchange isoform was under investigation. The fact that 100 μM concentrations of amiloride and benzamil affect proteins other than epithelial Na^+ channels reduces the resolution concerning their site(s) of action on taste receptor cell stimulation by salts. The pharmacology of amiloride and benzamil antagonism of Na^+/H^+ exchange activity in taste receptor cells is unknown. Assuming that nontaste tissue and taste receptor cells have similar pharmacological properties, then the low concentrations of amiloride (1 μM) and benzamil (1 and 25 μM) used in the present study should exhibit selective antagonism of the epithelial Na^+ channel pathway. Only one study has examined the effects of various Na^+/H^+ exchange antagonists on chorda tympani nerve responses to NaCl in gerbils; the antagonists were found to be relatively ineffective agents (24).

Peripheral nerve recordings provide an indirect assessment of transcellular events occurring on the plasma membrane of taste receptor cells that can only be measured through other means. Thus our purpose is to use peripheral nerve recording to infer events that relate to taste receptor cell physiology. The present study used low amiloride and benzamil concentrations and various NaCl, KCl, and NH_4Cl concentrations to assess the extent to which epithelial Na^+ channels may be involved in receptor cell stimulation by these salts. The involvement of Na^+/H^+ exchangers was assessed by using low 5-(*N*, *N*-dimethyl)-amiloride (DMA)-Cl concentrations and the same salt stimuli. DMA is a more specific antagonist of Na^+/H^+ exchange than amiloride and benzamil (16, 23, 30, 32), showing very little if any inhibition of epithelial Na^+ channels at low micromolar concentrations (15, 17).

The present findings provide further evidence supporting the role of epithelial Na^+ channels in salt taste not only by sodium ions but also by potassium and bicarbonate ions. We provide additional evidence suggesting that inhibition of chorda tympani responses to KCl by high amiloride concentration (>25 μM) may be due in part to inhibition of protein(s) other than epithelial Na^+ channels (18). In addition, we provide evidence indicating that Na^+/H^+ exchangers may be present in the apical membrane of rat taste receptor cells. The activity of this putative protein may influence responses of the chorda tympani nerve to NaCl, KCl, and NH_4Cl salts.

METHODS

Subjects

Recordings were obtained from the whole chorda tympani nerve of 24 adult male rats weighing 250–450 g (Sprague-Dawley, Charles River Breeding Laboratories). Rats were housed in transparent plastic cages, a maximum of two per cage, in a temperature-controlled colony room on a 12:12-h light-dark cycle with lights on at 0500. All animals had free access to Purina Rat Chow 5001 and deionized-distilled water ad libitum.

Preparation

Rats were anesthetized with urethan (1.5 g/kg body wt) administered in two intraperitoneal injections spaced 15 min apart. Supplementary doses were administered as necessary to maintain a deep level of anesthesia. Rectal temperature was monitored throughout the experiment and maintained at 36–38°C with a heating pad. Before recording the tongue was kept moist with cotton soaked in physiological saline. The right chorda tympani nerve was located using a mandibular approach, and the sheath was removed. The whole nerve was placed on a nichrome wire electrode, and a similar indifferent electrode was placed on the underlying muscle tissue near the nerve for differential amplification (×10,000) of action potentials. The integrated signal (time constant 0.2 s) was recorded on a Graphtec Thermal Arraycorder for subsequent data analysis. The animal was grounded via the headholder.

The tongue was gently extended out from the oral cavity and fixed in place by attaching a small suture to the ventral surface of the tongue and securing the loose end of the suture to the table top with tape. For stimulus presentation we used a computer-controlled delivery system, designed and built at Florida State University. Our system provides precise control over flow rate and temperature with uninterrupted flow eliminating tactile and thermal transients from the recording. Stimuli were presented to the anterior portion of the tongue by computer-controlled stepping motors to maintain a constant flow rate of 50 μl/s. This flow rate approximates the fluid volume consumed by a normal rat licking from a drinking spout obtaining about 5–7 μl/lick at a rate of 6–7 licks/s (27). By way of four independently controlled input valves, the stimulus and rinse input lines were linked with a stimulus outflow tube directed over the anterior surface of the tongue. The four input valves were controlled by a custom Power Macintosh computer program that permitted rapid switching and/or mixing between two stimulus and two rinse channels while maintaining a continuous solution flow through the outflow tube. Therefore, a stock stimulus concentration could be diluted by defining a mixing ratio between a stimulus input line and a rinse line. The time required for a stimulus or water rinse to flow from an input valve to the tongue surface was about 2 s. The temperature of the rinse and stimulus solutions was controlled by a Peltier heat exchange device placed near the end of the stimulus outflow tube. A suction tube placed underneath the tip of the tongue of the rat removed solutions that flowed off the tongue.

Stimulation Protocols

During the recording session deionized-distilled water flowed continuously over the tongue at 35°C before and after stimulus presentation at the same temperature. Stimulating solutions were made from reagent grade chemicals dissolved in deionized-distilled water. The tongue was stimulated with 94, 85, 70, 40, and 0% dilutions of stock (500 mM) NaCl, KCl, and NH_4Cl solutions mixed with and without amiloride

(1 and 25 μM), benzamil (1 and 25 μM), and DMA (1 μM). These dilutions correspond to 30, 75, 150, 300, and 500 mM salt concentrations, respectively. The pH levels of the salt solutions alone or mixed with an antagonist varied little. For example, the pH levels for the five concentrations of NaCl, KCl, and NH_4Cl solutions ranged between 5.64 and 6.12, 6.09 and 6.48, and 5.55 and 6.01, respectively. The pH levels for the same concentrations of NaCl, KCl, and NH_4Cl solutions mixed with 1 μM amiloride ranged between 5.74 and 6.02, 5.96 and 6.29, and 5.45 and 5.76, respectively.

Amiloride concentrations >100 μM have been shown to inhibit various cellular proteins such as Na^+/H^+ exchangers and Na^+/Ca^{2+} exchangers and to suppress protein synthesis and enzyme function (15). Additionally, lengthy and repeated amiloride pretreatment has been found to reduce baseline chorda tympani nerve activity (3, 5) and to require prolonged interstimulus intervals of 10–20 min to completely reverse the suppressive effect of the drug (3, 5). Consequently, amiloride concentrations above 100 μM and lengthy pretreatment should be avoided. Recent studies have adopted a procedure involving the addition of amiloride to NaCl during sustained neural activity (8, 18, 19). When added to the stimulus, amiloride was present for a short duration (10–20 s), yet was more effective in inhibiting NaCl responses without comprising spontaneous nerve activity (8, 18, 19). Furthermore, the suppressive effect of amiloride was reversed within seconds (8, 18, 19). The present stimulation protocol was adopted to avoid drug concentrations and durations that may disrupt membrane components other than the ones targeted by the drug that can reversed relatively quickly without compromising spontaneous neural activity.

Each salt was presented in an ascending concentration series. Within a concentration series each concentration was presented twice first following *protocol A* and then *protocol B*. Both protocols consisted of a 30-s stimulation period followed by a 1.5- to 2-min water rinse. In *protocol A* the stimulus was presented continuously for 30 s. In *protocol B* stimulation consisted of three consecutive segments with the salt concentration presented alone for the first 10 s, the salt mixed with a drug presented for the second 10 s, and the salt concentration presented alone for the last 10 s with uninterrupted stimulus flow over the entire 30-s stimulation period. An example of the stimulation paradigm is depicted in Fig. 1. The completion of an ascending salt concentration series was followed by a 5-min water rinse, after which a different salt series was presented. The order of salt series presentation was random (e.g., NaCl-KCl-NH_4Cl, KCl-NH_4Cl-NaCl, and so forth). In all cases the order of salt presentation did not affect the responses; the absolute responses and the degree of drug-induced suppression were the same regardless of whether a given salt appeared early or late in the overall sequence (all P values > 0.68). Occasionally, a portion of the stimulation series was repeated to replace recordings confounded by noise artifacts or by a temporary reduction in nerve sensitivity due either to excessive moisture accumulation on the electrode or to a change in the position of the nerve on the electrode. For each rat all three salts and a single concentration of an antagonist were tested.

Data Reduction and Analysis

All initial measures were recorded from a Graphtec Thermal Arraycorder in arbitrary chart units. As depicted in Fig. 1, response magnitudes were measured during *seconds 6–10* and *14–20* after stimulus on set. For each measurement period the average response magnitude was measured directly from the chart recording and used for data analysis. The stability of a recording was evidenced by an invariable level of baseline activity throughout the recording session for all experiments. As shown in Fig. 2, concentration response functions similar to those observed previously in our own laboratory (19) and other laboratories (10) were obtained. Figure 2 shows the relative response magnitudes of the chorda tympani nerve to NaCl, KCl, and NH_4Cl solutions mixed with and without 1 μM amiloride, benzamil, and DMA. Subsequent figures, presented as percent control response, were derived from these data and the corresponding data for 25 μM amiloride and benzamil (not shown). The percent control response was calculated as follows: [(B20/B10)/(A20/A10)] × 100. The notations A10 and A20 represent average response magnitudes to each salt presented alone during *seconds 6–10* and *14–20*, respectively (*protocol A*). The notation B10 represents the average response magnitude, measured during *seconds 6–10*, to each salt presented alone; B20 represents the average response magnitude, measured during *seconds 14–20*, to each salt mixed with an antagonist (*protocol B*). Thus each concentration of each salt had its own control response. For example, the control response for 30 mM NaCl mixed with a drug was the preceding stimulation with 30 mM NaCl by itself, that for 75 mM NaCl with a drug was the preceding stimulation with 75 mM NaCl by itself, and so on for other salts and salt concentrations. This type of analysis takes into account any changes in response magnitude that may occur during continuous salt stimulation in the absence of an antagonist.

As can be seen in Fig. 1, *A*-*C*, response magnitudes during continuous salt stimulation depend on not only the particular salt but also on the concentration. Response magnitude increases progressively during stimulation with 500 mM NH_4Cl but not with the two lower concentrations (Fig. 1*C*). In contrast, response magnitudes to 500 mM NaCl and KCl peak early during stimulation and decline gradually. This type of analysis removed the variable adaptation rates across stimuli, and to a lesser extent across animals, as a possible confound. Subsequent figures and statistical analysis therefore are representative only of the effects of an antagonist on response magnitudes.

To assess the effects of an antagonist, one-factor analysis of variance tests (ANOVA) were conducted with drug (amiloride, benzamil, and DMA) or drug concentration (1 and 25 μM) as the between-group factor and repeated measures over salt concentration as the within-group factor. In some instances, post hoc contrast analyses were used to determine the source of group differences. Data from animals with any missing values were eliminated automatically from the ANOVA program. All data analyses were done using SYSTAT; P values < 0.05 were considered statistically significant. All values are presented as the means ± SE.

RESULTS

Comparison of 1 μM Drug Suppression Within Salts

NaCl. Figure 3*A* shows the effect of 1 μM amiloride ($n = 6$), benzamil ($n = 4$), and DMA ($n = 4$) on the chorda tympani nerve responses to NaCl plotted as a percent control response. The percent control response corresponds to the fraction of the nerve response unaffected by drug application (drug insensitive). A significant between-subjects drug main effect was evident ($F_{2,11} = 8.43$, $P < 0.01$). A post hoc contrast analysis revealed that amiloride suppression was greater than DMA suppression for every NaCl concentration ($F_{1,11} \geq 6.7$, $P \leq 0.025$) except 150 mM NaCl ($F_{1,11} = 2.48$, $P = 0.14$). Benzamil suppression was also greater than

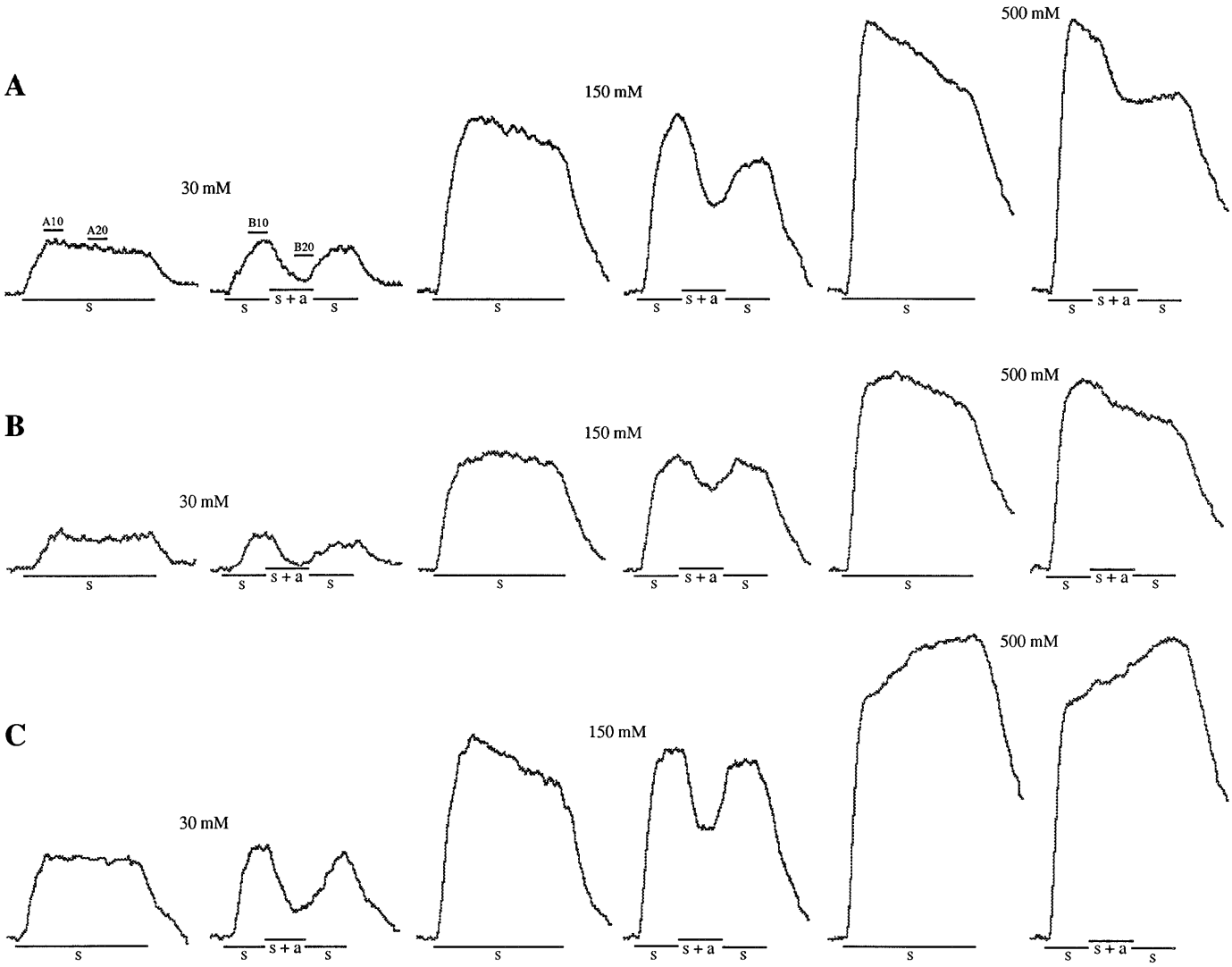

Fig. 1. Integrated responses of chorda tympani nerve to 2 presentations of 30, 150, and 500 mM concentrations of NaCl (*A*), KCl (*B*), and NH_4Cl (*C*). Responses on *left* for each salt concentration were to the stimulus presented alone without an antagonist; responses on the *right* were to the salt mixed with 1 μM amiloride. Horizontal bar (s) under each response reveals period of stimulation of the salt alone (s) or the salt mixed with amiloride (s + a). Longer bar on *left* of each salt concentration represents 30 s of salt stimulation in absence of amiloride. Three shorter horizontal black bars under responses on *right* of each salt concentration represent 3 consecutive 10-s periods of uninterrupted stimulus flow. With 30 mM NaCl as an example, the first shorter black bar (s) represents a 10-s presentation of salt alone, second shorter black bar (s + a) represents a 10-s presentation of salt mixed with amiloride, and third shorter black bar represents a 10-s presentation of salt alone again.

DMA suppression but only for the lowest NaCl concentration ($F_{1,11} = 9.52$, $P = 0.01$). Amiloride and benzamil suppression of NaCl responses was statistically similar. In addition, there was a significant within-subjects salt concentration main effect ($F_{4,44} = 31.6$, $P < 0.01$). This indicates that the fraction of the nerve response inhibited by amiloride, benzamil, and DMA decreased with an increase in NaCl concentration (i.e., drug effectiveness decreased). In general, 1 μM amiloride and benzamil tended to suppress NaCl responses more than did 1 μM DMA. This was most apparent with weak NaCl concentrations.

KCl. Figure 3*B* shows the effect of 1 μM amiloride ($n = 6$), benzamil ($n = 4$), and DMA ($n = 4$) on the chorda tympani nerve responses to KCl plotted as a percent control response. There was a significant within-subjects salt concentration main effect ($F_{4,36} = 15.3$, $P < 0.01$). This is indicative of the decrease in the relative effectiveness of all three drugs as KCl concentration increased. A between-subjects drug main effect was not significant ($F_{2,9} = 1.95$, $P = 0.19$). This suggests that 1 μM concentrations of amiloride, benzamil, and DMA suppressed KCl responses to the same degree.

NH_4Cl. Figure 3*C* shows the effect of 1 μM amiloride ($n = 6$), benzamil ($n = 3$), and DMA ($n = 4$) on the chorda tympani nerve responses to NH_4Cl, plotted as a percent control response. There was a significant within-subjects salt concentration main effect ($F_{4,40} = 45$, $P < 0.01$). As found for both NaCl and KCl, the fraction of the nerve response inhibited by amiloride, benzamil,

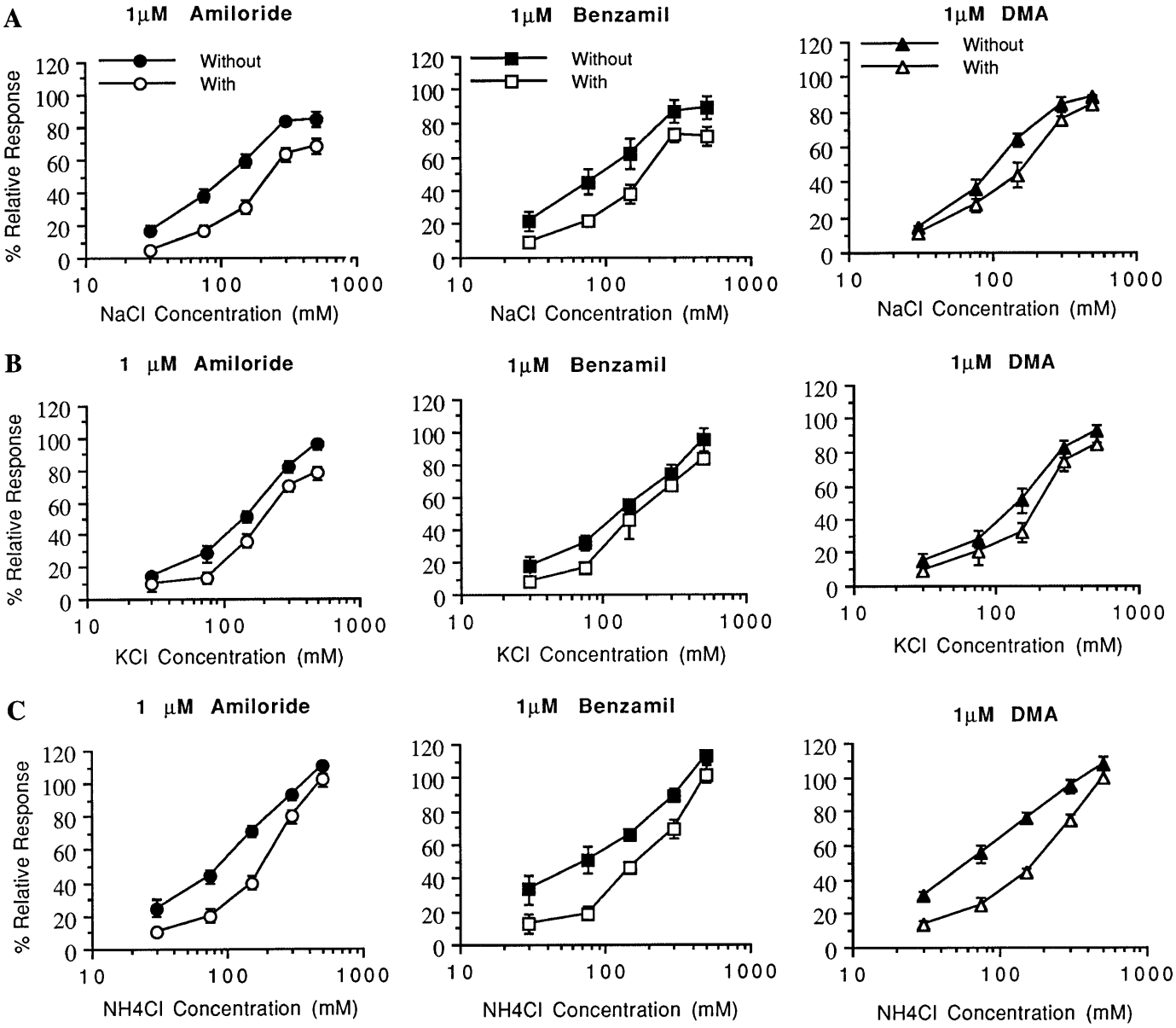

Fig. 2. Relative responses of integrated chorda tympani nerve to ascending concentration series of NaCl (*A*), KCl (*B*), and NH_4Cl (*C*) solutions mixed with (open symbols) and without (solid symbols) 1 μM concentrations of amiloride, benzamil, and 5-(*N*,*N*-dimethyl)-amiloride (DMA). Salt concentrations were 30, 75, 150, 300, and 500 mM.

and DMA decreased with an increase in NH_4Cl concentration. A between-subjects drug main effect was not significant ($F_{2,10} = 0.13$, $P = 0.87$). Therefore, the inhibitory effect of 1 μM concentrations of amiloride, benzamil, and DMA was the same across all NH_4Cl concentrations.

Comparison of 25 μM Drug Suppression Within Salts

NaCl. The percent control responses of the chorda tympani nerve to NaCl solutions in the presence of 25 μM amiloride ($n = 5$) and benzamil ($n = 4$) are depicted in Fig. 4*A*. There was a significant within-subjects salt concentration main effect ($F_{4,28} = 11.3$, $P < 0.01$), indicating that amiloride and benzamil suppression decreased with an increase in NaCl concentration. A between-subjects drug main effect was not significant ($F_{1,7} = 0.03$, $P = 0.86$). Thus as seen with 1 μM concentrations, 25 μM amiloride and benzamil suppressed NaCl solutions to a similar degree.

KCl. Figure 4*B* illustrates the percent control responses of the chorda tympani nerve to KCl solutions in the presence of 25 μM amiloride ($n = 5$) and benzamil ($n = 4$). A between-subjects drug main effect was not significant ($F_{1,6} = 0$, $P = 0.98$). However, there was a significant within-subjects salt concentration main effect ($F_{4,24} = 8.7$, $P < 0.01$) and a drug times salt concentration interaction ($F_{4,24} = 4.1$, $P = 0.01$). The significant interaction indicates that the suppression functions differed between the two drugs across KCl concentration. Amiloride suppression of KCl responses

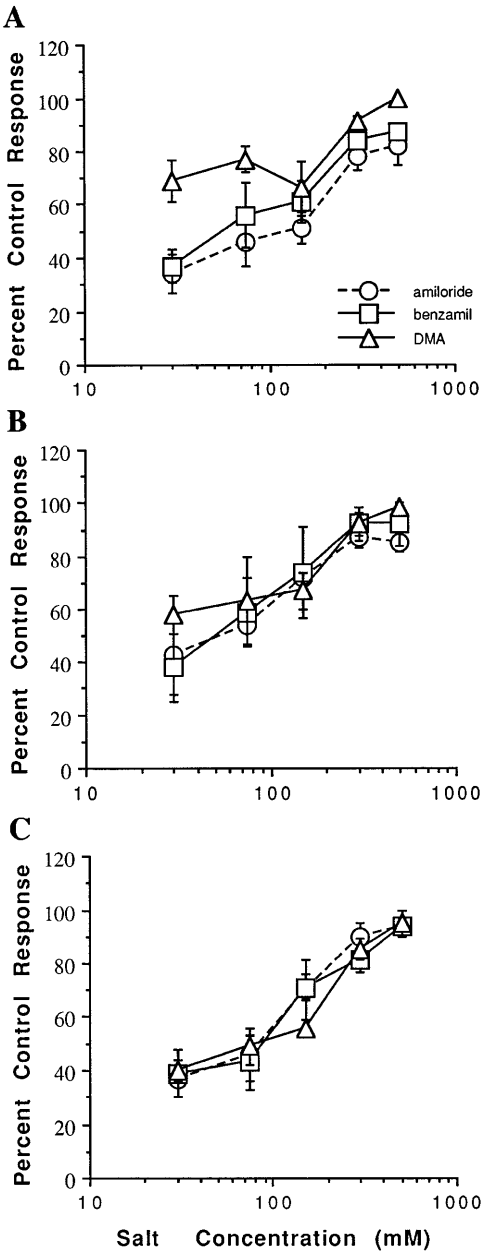

Fig. 3. Percent control responses of integrated chorda tympani nerve to ascending concentration series of NaCl (*A*), KCl (*B*), or NH_4Cl (*C*) solutions mixed with 1 μM amiloride, benzamil, and DMA. Salt concentrations were 30, 75, 150, 300, and 500 mM.

did not vary much, averaging about 40% across KCl concentration. However, benzamil suppression decreased progressively with increasing KCl concentration.

NH_4Cl. In Fig. 4*C* the percent control responses of the chorda tympani nerve to NH_4Cl solutions in the presence of 25 μM amiloride ($n = 5$) and benzamil ($n = 4$) are compared. A significant within-subjects salt concentration main effect ($F_{4,28} = 28.2$, $P < 0.01$) indicated that amiloride and benzamil suppression decreased with an increase in NH_4Cl concentration. A between-subjects drug main effect was not significant ($F_{1,7} = 0.68$, $P = 0.43$). Thus, as seen with 1 μM concentrations of amiloride and benzamil, 25 μM amiloride and benzamil suppressed NH_4Cl responses to a similar degree.

Comparison of Drug Suppression Between Salts

Figure 5 shows the percent suppression of the chorda tympani response to NaCl, KCl, and NH_4Cl collapsed across concentration and plotted as a function of drug (1 μM). A two-way ANOVA revealed a significant drug main effect ($F_{2,32} = 5.1$, $P = 0.01$) and a significant salt times drug interaction ($F_{4,32} = 3.4$, $P = 0.02$). A salt main effect was not significant ($F_{2,32} = 1.9$, $P = 0.16$). A post hoc analysis on the drug main effect revealed that

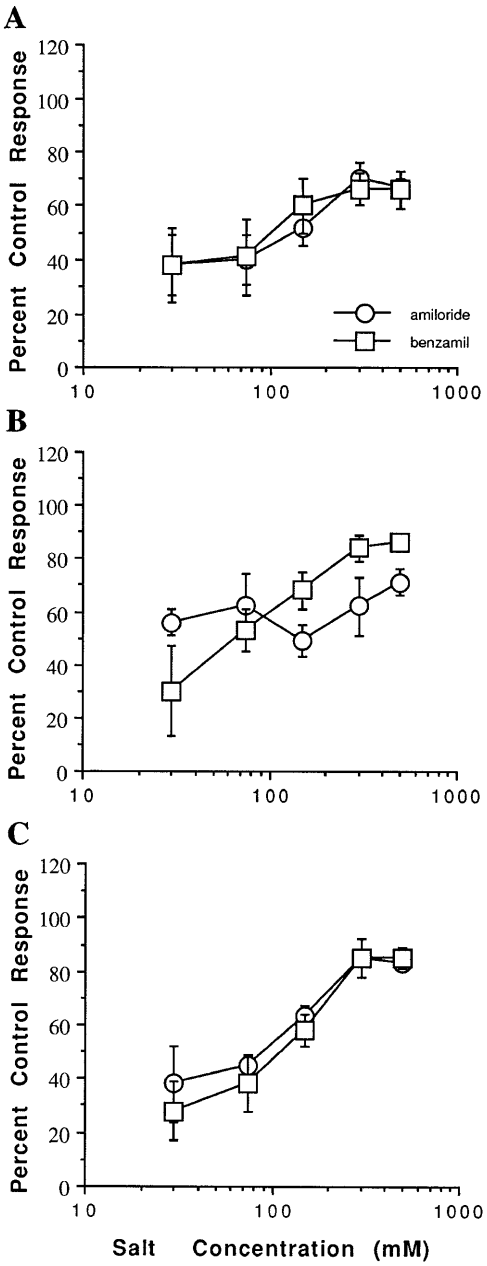

Fig. 4. Percent control responses of integrated chorda tympani nerve to ascending concentration series of NaCl (*A*), KCl (*B*), or NH_4Cl (*C*) solutions mixed with 25 μM amiloride and benzamil. Salt concentrations were 30, 75, 150, 300, and 500 mM.

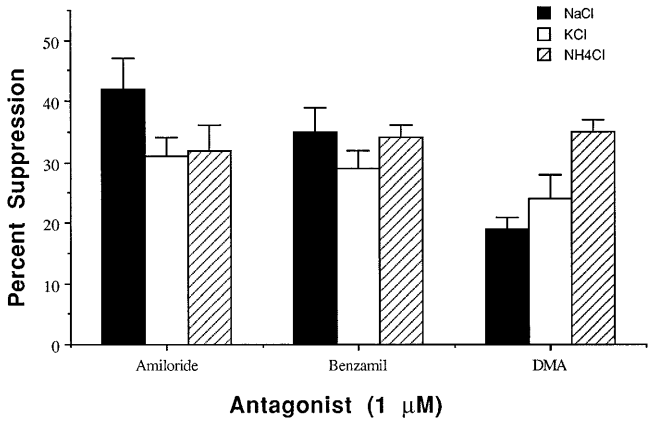

Fig. 5. Percent suppression of integrated chorda tympani nerve response to NaCl (solid bars), KCl (open bars), and NH_4Cl (hatched bars) collapsed across concentrations and plotted as function of antagonist. Antagonists were 1 μM concentrations of amiloride, benzamil, and DMA.

amiloride and benzamil suppression was similar but greater than DMA suppression. A similar analysis on the salt times drug interaction showed that amiloride suppression of NaCl responses was greater than the suppression of KCl and NH_4Cl responses. In contrast, DMA suppression of NH_4Cl responses was greater than the suppression of KCl and NaCl responses. Benzamil suppression was similar between salts.

DISCUSSION

The present study provides further evidence supporting the role of epithelial Na^+ channels in not only NaCl taste transduction, but also in KCl and NH_4Cl taste transduction. Amiloride and benzamil (1 and 25 μM) significantly inhibited chorda tympani nerve responses to the above salts at concentrations from 30 to 500 mM. Amiloride suppressed NaCl responses to a greater degree than KCl and NH_4Cl responses. Moreover, the present results also indicate that a Na^+/H^+ exchanger may be present in rat taste receptor cells. The addition of 1 μM DMA to stimulating solutions of NaCl, KCl, and NH_4Cl produced substantial inhibition of taste nerve responses. DMA suppressed NH_4Cl responses to a greater degree than NaCl and KCl responses. The present findings are consistent with the notion that both epithelial Na^+ channels and Na^+/H^+ exchangers may be important components of the process of salt taste transduction.

NaCl Nerve Responses

Prior studies have demonstrated unequivocally that amiloride, the epithelial Na^+ channel antagonist, inhibits the integrated responses of the whole chorda tympani nerve to NaCl in rats (5, 19, 20, 31). The results from the present study are in agreement with findings from prior studies and extend those findings to include benzamil, a more selective Na^+ channel antagonist than is amiloride (15). Amiloride and benzamil suppressions of NaCl responses were found to be similar. Given that benzamil is a more selective antagonist, it is likely that amiloride and benzamil suppression of NaCl responses was mediated through antagonism of the epithelial Na^+ channel pathway.

Nerve responses to NaCl were also inhibited by 1 μM DMA, a more selective Na^+/H^+ exchange antagonist than are amiloride and benzamil (15). DMA (1 μM) was previously shown to decrease by 4% the short-circuit current in isolated frog skin (17). In this type of preparation, the short-circuit current represents transcellular Na^+ flux of which a large degree can be accounted for by ion conductance through epithelial Na^+ channels. The possibility exists in the present study that DMA inhibited NaCl responses through antagonism of the epithelial Na^+ channel pathway. However, this seems unlikely for two reasons. First, DMA inhibited NaCl (30–150 mM) responses by 30%. Second, the effect of DMA between salts (NH_4Cl > KCl = NaCl) was different from the effect of amiloride between salts (NaCl > KCl = NH_4Cl). It appears that the effect of DMA on NaCl responses was more likely mediated through antagonism of Na^+/H^+ exchangers. Given the 1:1 stoichiometry of Na^+/H^+ antiporters (25), DMA inhibition of NaCl responses may have been indirect, through changes in intracellular pH. If so, this may be indicative of the sensitivity of peripheral NaCl taste processing to intracellular pH. Taken altogether, it appears that epithelial Na^+ channels and Na^+/H^+ exchangers may be important membrane components in NaCl taste.

The present finding implicating the involvement of a Na^+/H^+ exchange pathway in NaCl transduction is inconsistent with a prior study investigating the role of Na^+/H^+ exchange in NaCl taste. Schiffman et al. (24) examined the effect of selective Na^+/H^+ exchange antagonists on taste nerve responses to NaCl and concluded that a Na^+/H^+ exchange pathway was not involved in NaCl transduction in gerbils. There are methodological differences between the present and prior studies. Besides the species difference, Schiffman and colleagues (24) applied the Na^+/H^+ exchange antagonists after a pretreatment regimen, whereas the present study added the antagonist to NaCl during sustained neural activity. Although the Na^+/H^+ exchanger is a ubiquitous protein occurring in multiple isoforms (23, 32), our results are merely suggestive and the presence of a Na^+/H^+ exchange protein in taste cell membranes must be verified using other techniques such as patch clamp or immunocytochemistry.

KCl Nerve Responses

Prior studies showed that amiloride inhibited the integrated responses of the whole chorda tympani nerve (18, 19) as well as single fiber responses of chorda tympani N units to KCl in rats (21). The present findings add to the mounting evidence indicating amiloride to be an effective antagonist of chorda tympani nerve responses to not only sodium salts but also to nonsodium salt solutions in rats and extend those findings to include the inhibitory action of benzamil. The addition of low amiloride (1 μM) and benzamil (1 and 25 μM) concentrations to ongoing stimulation with KCl solutions inhibited taste nerve responses to a

similar degree. Based on the known pharmacology of these antagonists in other systems (14, 15, 23, 32), it appears that the effects of amiloride and benzamil on KCl responses were mediated through antagonism of the epithelial Na^+ channel pathway. On the basis of findings from a prior study (18), it is unlikely that amiloride acts by inhibiting KCl-stimulated entry of sodium ions.

Interestingly, the present results indicate that KCl responses were also inhibited by 1 μM DMA. Based on the high selectivity of DMA at low concentrations (14, 15, 17, 23, 32), it appears that DMA inhibition of KCl responses could be mediated through antagonism of the Na^+/H^+ exchange pathway. Similar to the effect of DMA on NaCl responses, it may be that DMA inhibition of KCl responses was indirect, through a reduction in intracellular pH.

The Na^+/H^+ exchange system is frequently found in parallel with a K^+-conductive pathway in epithelial tissue. In frog kidney for example, inhibition of Na^+/H^+ exchange activity by amiloride reduces the net transepithelial K^+ secretion through a Ba^{2+}-sensitive K^+-conductive pathway (outward current) (22). The same effect on K^+ secretion is mimicked by manipulations that decrease intracellular pH, such as removal of extracellular Na^+. In the taste system, if one assumes that the entry of stimulating potassium ions is decreased by a reduction in the exit of potassium ions, it becomes apparent that DMA inhibition of Na^+/H^+ exchange might affect the magnitude of nerve responses to KCl stimulation. Future studies will be required to determine the sequence of events leading to DMA inhibition of KCl taste responses. Taken altogether, it appears that epithelial Na^+ channels, Na^+/H^+ exchangers, and 4-AP K^+ channels (11, 26) may be important membrane components in KCl taste.

In the present study, 25 μM amiloride suppressed KCl responses (≥150 mM) to a greater degree than did 1 μM amiloride and 1 and 25 μM benzamil. This is consistent with a prior study showing that 100 μM amiloride suppressed KCl (500 mM) responses more than did 100 μM benzamil (18). The greater suppression by 25 μM amiloride than 1 and 25 μM benzamil indicates that amiloride is having some undisclosed membrane effect beyond Na^+ channel antagonism during KCl simulation. Thus, in addition to antagonizing the epithelial Na^+ channel pathway, strong amiloride doses may antagonize some other unidentified membrane protein(s), resulting in additional inhibition of nerve responses to strong KCl solutions. Although highly speculative, it may be that the additional effect of amiloride during KCl simulation involves antagonism of Na^+/H^+ exchangers.

NH_4Cl Nerve Responses

The present findings demonstrated that amiloride and benzamil also antagonize chorda tympani nerve responses to a wide range of NH_4Cl concentrations. Based on the known pharmacology of these antagonists in other systems (14, 15, 23, 32), it appears that the effects of amiloride and benzamil on NH_4Cl responses were mediated through antagonism of the epithelial Na^+ channel pathway. It is unlikely that amiloride acts by inhibiting NH_4Cl-stimulated entry of sodium ions. For instance, the addition of 1 μM amiloride and benzamil resulted in more inhibition of NaCl responses than addition of 1 μM DMA (Fig. 2). If NH_4Cl stimulated the entry of sodium ions then 1 μM DMA should result in less inhibition than the inhibition from 1 μM amiloride and benzamil. However, amiloride, benzamil, and DMA inhibition of NH_4Cl responses were of similar magnitude.

In a recent study, Lundy and Contreras (18) suggested that 100 μM amiloride suppression of taste nerve responses to NH_4Cl likely involved membrane protein(s) other than amiloride-sensitive Na^+ channels. Results from the present study support such a hypothesis insofar as 1 μM DMA significantly suppressed chorda tympani nerve responses to NH_4Cl. Based on the high selectivity of DMA at low concentrations (14, 15, 17, 23, 32), it appears that DMA inhibition of NH_4Cl responses was likely mediated through antagonism of the Na^+/H^+ exchange pathway. Again, this possibility may be indicative of the sensitivity of peripheral NH_4Cl taste processing to intracellular pH. Based on evidence cited previously, epithelial Na^+ channels, Na^+/H^+ exchangers, and 4-AP K^+ channels (11, 26) may be important membrane components in NH_4Cl taste.

In summary, the present findings provide further evidence in support of the notion that taste receptor cells utilize epithelial Na^+ channels to detect NaCl, KCl, and NH_4Cl solutions and activate chorda tympani neurons. In addition, the present findings provide the first evidence implicating the involvement of Na^+/H^+ exchange in salt transduction. A role for the putative Na^+/H^+ exchange pathway in receptor cell stimulation by NaCl, KCl, and NH_4Cl awaits a more direct examination.

Perspectives

The chorda tympani nerve has been shown to be critical for discrimination among NaCl, KCl, and NH_4Cl tastants, using a variety of behavioral measures (4, 9, 28, 29). For example, amiloride (29) or bilateral transection of the chorda tympani nerve (28) disrupts the response patterns of rats trained to respond differently, depending on whether the stimulus is NaCl or KCl. These findings suggest that the input from the narrowly tuned N best chorda tympani fibers (6) are critical for discriminating NaCl from KCl (29). However, if epithelial Na^+ channels were the critical pathway, then one might expect these channels to be involved exclusively in sodium salt stimulation.

Our results combined with findings from other studies (11, 26) suggest that the ability to discriminate sodium, potassium, and ammonium salts may require the utilization of a combination of transcellular mechanisms. For example, the available evidence indicates that epithelial Na^+ channels and Na^+/H^+ exchangers may be important components of NaCl transduction. In the case of KCl and NH_4Cl, it appears that 4-AP K^+ channels (11, 26), epithelial Na^+ channels, and Na^+/H^+

exchangers may be components of the transduction process.

If the above schema holds true, then in future studies it would be of great interest to know whether individual taste receptor cells are capable of using one or more of these mechanisms for salt detection. For example, do individual salt-sensitive taste cells have epithelial Na^+ channels, 4-AP K^+ channels, and Na^+/H^+ exchangers all on their receptive apical membrane or just a subset of the three membrane proteins? It would also be of interest to know the distribution and relative location of different types of salt-sensitive taste cells within taste buds and across taste papilla. Furthermore, some of these salt-sensitive taste cells may be innervated by N units and/or H units, or some other physiological group of taste neurons. Previous single chorda tympani fiber studies in rats (21) and hamsters (8) indicate that epithelial Na^+ channels are present exclusively in taste receptor cells innervated by N units.

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