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Distribution of Amiloride-sensitive Sodium Channels in the Oral Cavity of the Hamster

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Abstract

The distribution of amiloride-sensitive sodium channels (ASSCs) in taste buds isolated from the oral cavity of hamsters was assessed by patch clamp recording. In contrast to the case for rats, taste cells from the fungiform, foliate and vallate papillae and from the soft palate all contain functional ASSCs. The differential distribution of ASSCs between the hamster and the rat may be important for understanding the physiology underlying the differing behavioral responses of these species to sodium salts.

Introduction

One of the most commonly described gustatory transduction mechanisms for sodium salts involves sodium ion permeation through apical amiloride-sensitive sodium channels (ASSCs) in taste cells (for review see Lindemann, 1996; Gilbertson and Kinnamon, 1996). A wealth of evidence implicates this mechanism in sodium salt transduction in a variety of species, but what is not clear is the distribution of ASSCs in taste buds in various structures of the oral cavity. Recent reports indicate that the sodium salts likely activate a number of transduction pathways in addition to sodium permeation through ASSCs. These pathways may include both undefined cellular mechanisms activated following the paracellular transport of sodium ions though the tight junctions between taste cells (Ye *et al.*, 1991; Simon *et al.*, 1993) and, possibly, the movement of sodium ions through other unidentified amiloride-insensitive transport pathways (Doolin and Gilbertson, 1996).

In rats, the relative contribution of amiloride-sensitive and -insensitive transduction mechanisms differs between the anterior and posterior tongue. Sodium salt responses from the anterior tongue (i.e. innervated by the chorda tympani) in the rat are amiloride-sensitive (Heck *et al.*, 1984; Brand *et al.*, 1985), while those in the posterior, or glossopharyngeal-innervated, tongue are not (Formaker and Hill, 1991). Consistent with these findings, we find that in rats functional ASSCs are present in ~2/3 of rat taste cells from the fungiform papillae and 1/3 of taste cells from the foliate papillae, but are absent from taste cells from the vallate papillae (Doolin and Gilbertson, 1996). The differential distribution of this transduction channel may be a factor contributing to the differing sensitivity to sodium salts between the anterior and posterior tongue (Spector and Grill, 1992). Unlike rats, hamsters find sodium salts aversive (Hettinger and Frank, 1990; Gilbertson and Gilbertson, 1994). Accordingly, the trasduction of sodium salts may be different in these two species. In the present study we have attempted to determine if the distribution of amiloride-sensitive sodium channels follows a similar pattern in the oral cavity of the hamster. Part of these results have appeared elsewhere in abstract form (Gilbertson *et al.*, 1997).

Methods, results and discussion

Taste buds were isolated from the fungiform, foliate and vallate papillae from male Golden Syrian hamsters (age 2–4 months) as previously described (Doolin and Gilbertson, 1996) using the method adapted from Béhé *et al.* (1990). For isolation of taste buds from the soft palate (hamsters and Sprague–Dawley rats, ages 2–4 months) and geschmacksstreifen (rats), the cheek and jaw were cut and an incision was made with a scalpel in the epithelium of the hard palate just anterior to the soft palate. The underside of the tongue was cut and the incision continued through the epiglottis and the muscular layer underlying the soft palate until the original incision in the hard palate was reached. At this point the tongue, a portion of the epiglottis and the soft palate were removed intact. The soft palate was injected between the muscle layer and epithelium with physiological saline containing 5 mg/ml dispase, 0.5 mg/ml collagenase A

(Boehringer Mannheim, Indianapolis, IN) and 1 mg/ml trypsin inhibitor (Doolin and Gilbertson, 1996). The tissue was incubated in Ca^{2+}–Mg^{2+}-free saline containing 2 mM BAPTA for ~35 min until the palatine epithelium could be peeled free and the taste buds removed. Amiloride (10 μM) was added to all dissociation solutions to help protect against enzymatic degradation of ASSCs (Garty and Edelman, 1983; Gilbertson *et al.*, 1993). Taste buds from the soft palate and geschmacksstreifen in male 2- to 4-month-old Sprague–Dawley rats, which were not examined in our earlier study (Doolin and Gilbertson, 1996), were included to facilitate comparisons with the hamster.

Whole-cell patch clamp recordings were made from individual taste receptor cells maintained in isolated taste buds as previously described (Gilbertson, 1995; Doolin and Gilbertson, 1996). We used our standard criteria to identify cells as taste receptor cells and not epithelial cells (Gilbertson *et al.*, 1993). That is, cells must have an elongate morphology, evidence of voltage-activated ion channels and recordings that were stable for >5 min. The taste buds were plated into a 0.75 ml chamber consisting of a slide coated with Cell-Tak (Bollaborative Biomedical Prod., Bedford, MA) and a Sylgard O-ring and checked for voltage-activated ion channel activity in normal physiological saline (Tyrode; Gilbertson *et al.*, 1993). The extracellular solution then was changed to a K^+-free saline consisting of (in mM): NaCl, 140; tetraethylammonium bromide, 5; $CaCl_2$, 1; $MgCl_2$, 1; HEPES, 10; glucose, 10; Na pyruvate, 10; tetrodotoxin, 0.0005, pH 7.4. The pipette solution contained (in mM): CsCl, 150 mM; $CaCl_2$, 1 (free Ca^{2+} ~10^{-8} M); $MgCl_2$, 2; HEPES, 10; EGTA, 11; Na_2ATP, 2; NaGTP, 0.4, pH 7.2 with Tris-OH. The use of K^+-free solutions eliminated the possibility of contamination of the records by K^+ currents. To test for the presence of ASSC activity, amiloride (30 μM) or benzamil (10 μM), the amiloride analogue specific for ASSCs (Kleyman and Cragoe, 1988), was added to the K^+-free saline and changes in the whole-cell Na^+ current were monitored (cf. Figure 1). Electrophysiological recording conditions were identical to those previously described (Doolin and Gilbertson, 1996). All chemicals except those listed otherwise were obtained from Sigma Chemical Co. (St Louis, MO).

In the present study we recorded from >135 taste cells in order to determine the distribution of ASSCs in the oral cavity of the hamster. In addition, because our earlier characterization of ASSCs in rat (Doolin and Gilbertson, 1996) did not include taste cells from the soft palate or geschmacksstreifen, we looked for ASSC activity in these cells as well. We did not, however, include taste cells from the nasoincisor duct for technical reasons. Conservatively, we classified as amiloride-sensitive only those cells that showed a >15% decrease in current measured at –80 mV, which was reversible upon return to amiloride-free (or benzamil-free) solution. In most cases, we recorded whole-cell currents in response to a command voltage ramp from –80 to +80 mV in the absence and presence of either amiloride (30 μM) or benzamil (10 μM). Amiloride sensitivity was evident as a decrease in the slope of the whole cell current presumably caused by an inhibition of ASSCs (Figure 1). The total amiloride-sensitive current was estimated by subtracting the current in the presence of the inhibitor from the control current. There were no significant differences between the effects of amiloride and benzamil in the present study, so the results from these two compounds were pooled. Cells responding to either compound are referred to as amiloride-sensitive.

In the hamster, over half of the cells from each of the four taste bud types examined showed evidence of ASSC activity (Figure 1). There was no significant difference among the taste bud types in terms of both magnitude of amiloride-sensitive (A-S) currents and the proportion of A-S current relative to the total current. In cells classified as amiloride-sensitive, the total A-S current (at –80 mV) accounted for 15 to >95% of the total current under the conditions of these experiments, and in most cells accounted for ~40–50% (mean = 48.3 ± 12.0%; median = 42.5% of total current; n = 72 cells). The mean reversal potential of

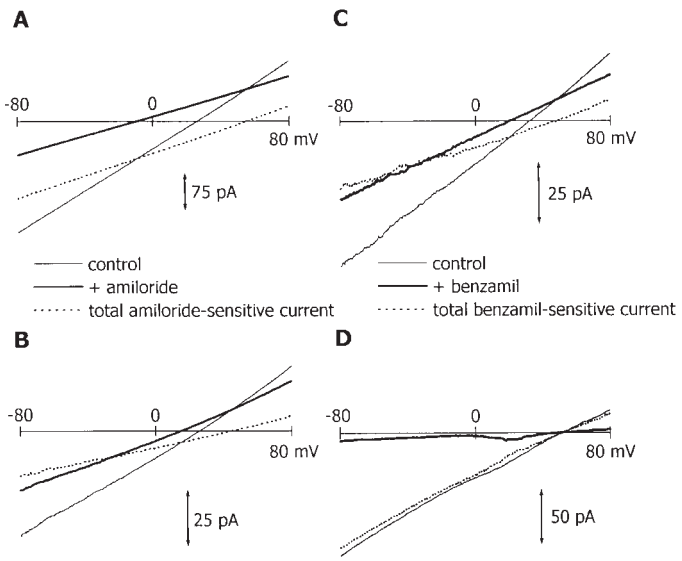

Figure 1 Whole-cell currents in hamster taste cells display amiloride or benzamil sensitivity, consistent with presence of ASSCs. Currents were elicited by 450 ms voltage ramps from –80 to +80 mV with a holding potential of –80 mV and were recorded in nominally K^+-free solutions (see text). The fungiform **(A)** and vallate **(B)** taste cells shown were treated with amiloride (30 μM), while the foliate **(C)** and palate **(D)** taste cells were treated with benzamil (10 μM). The inhibition of these currents by these compounds was voltage independent. The relative magnitude of the amiloride-sensitive currents, determined by subtraction of the current in the presence of the inhibitor from the control current, shown here represents the normal variation both within and across taste bud types. Reversal potentials for the amiloride- or benzamil-sensitive currents for A–D were +55.6, +47.0, +50.4 and +52.3 mV respectively, which is close to the predicted equilibrium potential for Na^+ based upon the Nernst equation (+65.8 mV).

the amiloride-sensitive current in all hamster taste buds was +49.2 ± 7.3 mV, which is close to the predicted value for the Na^+ equilibrium potential of +65.8 mV based upon applying the Nernst equation (Hille, 1992) to the sodium concentrations in our extracellular and pipette solutions. The deviation may reflect contributions from the permeability of other ions through ASSCs or the inability of the inhibitor concentrations used to completely block all ASSC activity.

As shown in Table 1, the greatest proportion of amiloride-sensitive cells in the hamster was found in the soft palate, though there was no statistically significant difference between the various taste buds in terms of percentage of cells containing ASSCs. However, the distribution of ASSCs in the hamster appears to be very different from that found in the rat (Table 1) in experiments performed under similar conditions (Doolin and Gilbertson, 1996). While there was no significant difference between rats and hamsters in the percentage of responsive cells in fungiform ($P = 0.672$) or foliate taste buds ($P = 0.064$), hamsters had a significantly greater proportion of amiloride-sensitive cells in the vallate ($P < 0.001$) and palatine taste buds ($P = 0.007$) than did rats (Pearson chi-square; Table 1). Interestingly, within the palate of the rat there was also a greater proportion of cells with ASSCs in the geschmacksstreifen than in the more posterior soft palate, though this difference was not statistically significant. Thus, hamsters appear to have a relatively uniform distribution of ASSCs in the oral cavity, while rats have a more discrete distribution of ASSCs that, on the surface at least, seems to correlate with innervation by branches of the VIIth nerve (chorda tympani or greater superficial petrosal). Whatever the reason, it is clear from this and our previous study (Doolin and Gilbertson, 1996) that the distribution of functional ASSCs is different in these two species.

The distribution of ASSCs in isolated taste buds found in the present study parallels our recent work on the identification of amiloride-sensitive Na^+ transport pathways in gustatory epithelia from the hamster and rat (Gilbertson and Zhang, 1998). That is, amiloride applied to the apical surface inhibited ouabain-sensitive Na^+ transport (mucosal to serosal) in epithelia containing fungiform, foliate, vallate and palatine taste buds in the hamster. In the rat, however, amiloride-sensitive Na^+ transport is present in the fungiform-, foliate- and palatine-containing epithelia but is absent from the vallate-containing epithelia. In all cases, amiloride was only effective on the mucosal surface suggesting that the ASSCs seen in the present study were likely apically restricted. Thus, there seems to be a good correlation between the presence of amiloride-sensitive sodium transport measured using Ussing chambers and the presence of functional ASSCs determined by patch clamp recording. The widespread distribution of ASSCs in the oral cavity of the hamster also argues against there being a requirement for innervation of the VIIth nerve in expression of functional ASSCs. This case is less clear for the rat, however, and will require direct testing of the link between specific innervation and ASSC expression through cross-innervation studies of the glossopharyngeal and chorda tympani nerves in this species.

Table 1 Relative proportions of taste cells containing functional ASSCs in the hamster and rat

Taste bud type	Hamster	Rat	*P*
Fungiform	60.0% [30] (52.5%[a])	65%[b] [51]	0.672
Foliate	63.6% [22]	37%[b] [27]	0.064
Vallate	68.4% [19]	0%[b] [31]	<0.001
Soft palate	84.2% [19]	38.5% [13]	0.007
Geschmacksstreifen	N/A[c]	66.7% [12]	N/A

Data shown reflect percentage of cells containing functional ASSCs determined by the decrease in current seen during application of either amiloride (30 μM) or benzamil (10 μM) as shown in Figure 1. Numbers in square brackets refers to the number of cells tested.

[a]Reported in Gilbertson *et al.* (1993) for 118 cells.

[b]Data taken from Doolin and Gilbertson (1996).

[c]Hamsters, unlike rats, do not contain a discernible geschmacksstreifen. Significance values (*P*) for the comparison of the percentage of cells containing ASSCs between the rat and the hamster were determined using the Pearson chi-square test. χ^2 values for fungiform, foliate, vallate and palate were 0.179, 4.432, 28.663 and 7.161 respectively, with one degree of freedom.

It has been reported that, in addition to Na^+ permeation through ASSCs, sodium salts may be transduced in mammalian taste cells via paracellular pathways leading to taste cell activation (Ye *et al.*, 1991; Simon *et al.*, 1993) or via undefined amiloride-insensitive Na^+ transport pathways (Doolin and Gilbertson, 1996; Gilbertson and Zhang, 1998). Because in the present study we have focused exclusively on ASSCs, it is unclear what this differential distribution of ASSCs between the rat and the hamster may mean in terms of overall sodium salt responsiveness. Nonetheless, it is intriguing that these two species, one of which (rat) finds low to isotonic concentrations of NaCl acceptable (Breslin *et al.*, 1993) while the other (hamster) finds most NaCl concentrations aversive (Hettinger and Frank, 1990; Gilbertson and Gilbertson, 1994), exhibit such contrasting distributions of this sodium salt transduction channel. The presence of ASSCs in hamster vallate taste buds and their absence in rat vallate taste buds may be linked to these underlying salt preferences since, broadly speaking, the glossopharyngeal nerve is believed to be more responsive to aversive stimuli than the chorda tympani (Harada and Smith, 1992; Ninomiya *et al.*, 1994).

These experiments also represent the first report of electrophysiological (patch clamp) recording from isolated taste buds other than those found in the tongue. Though there are roughly equal numbers of taste buds in the

fungiform papillae and soft palate in the hamster [18.0 versus 13.8% of total taste buds respectively (Miller and Smith, 1985)], there has been little or no direct information about the physiology of these cells. Recordings from the greater superficial petrosal (GSP) nerve, which innervates taste buds in the palate, have demonstrated that there is significant sodium salt responsiveness in this area (Harada *et al.*, 1991; Harada and Smith, 1992; Harada, 1994), consistent with our findings in the present study and in the isolated lingual epithelium (Gilbertson and Zhang, 1998). Moreover, it may be these palatine taste buds that contribute to the continued responsiveness to sodium salts even following bilateral transection of the chorda tympani and glossopharyngeal nerve (Sollars and Bernstein, 1994).

Our finding of functional ASSCs in palatine taste buds is consistent with recent behavioral data suggesting that the sodium responsiveness in the palate is mediated, at least in part, via amiloride-sensitive mechanisms. Amiloride-sensitive transduction mechanisms are generally considered to be responsible for the narrowly tuned sodium responses in the oral cavity, permitting the discrimination of NaCl from KCl (Spector *et al.*, 1996). Spector and colleagues speculated on the presence of palatine ASSCs since their results showed that amiloride was much more effective at compromising NaCl versus KCl discrimination (Spector *et al.*, 1996) than was chorda tympani transection (St John *et al.*, 1997). Thus, this implies that amiloride-sensitive mechanisms exist elsewhere than the anterior tongue. Recent reports of the amiloride sensitivity of NaCl responses recorded in the GSP are conflicting. While Harada *et al.* (1997) failed to demonstrate significant effects of amiloride on NaCl responses, Sollars and Hill (1997) showed that amiloride could significantly inhibit responses to sodium salts. Our present results in taste cells and in palatine epithelia (Gilbertson and Zhang, 1998) are more consistent with the latter finding. Clearly, elucidating the physiological properties and chemosensitivity of the taste cells from the soft palate will be important to the overall understanding of gustatory processing in the oral cavity.

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