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Acid-sensing ion channels in taste buds

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Summary. Taste receptor cells detect gustatory stimuli using a complex arrangement of ion channels, G protein-coupled receptors, and signaling cascades. Sour and salty tastes are detected by ion channels in the rat. Using a combination of homology screening and functional expression approaches, we screened a rat circumvallate papilla cDNA library and identified acid-sensing ion channel-2a (ASIC2a) and ASIC2b as candidates for the rat sour-sensing channels. *In situ* hybridization and reverse transcription-polymerase chain reaction experiments revealed that ASIC2a and ASIC2b transcripts were localized in taste bud cells. Immunohistochemistry and immunoprecipitation also revealed that both subunits were expressed in a subset of taste cells and that some of the cells expressed ASIC2a/ASIC2b heteromeric assemblies. Electrophysiological studies demonstrated that stimulation of acetic acid produced larger ASIC2 currents than did hydrochloric acid at the same pH. ASIC2a/ASIC2b channels generated maximal inward currents at pH ≤ 2.0, which agrees well with the *in vivo* pH-sensitivity of rat taste cells. The amiloride-sensitivity of ASIC2a/ASIC2b heteromer lessened with decreasing pH and almost completely disappeared at pH 2.0. These data suggest that ASIC2a and ASIC2b may play roles in sour taste transduction.

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Background

Sour taste is an indication of acidity and contributes to avoiding the ingestion of harmful and/or unpleasant substances. Spoiled foods, unripe fruits, and many naturally poisonous substances taste sour because of their high acidity. Protons are the causative agents of sourness, and proton-sensitive ion channels located in taste cells are believed to act as sour-taste receptors (Herness and Gilbertson, 1999; Gilbertson *et al.*, 2000; Miyamoto *et al.*, 2000; Lindemann, 2001).

Various mechanisms have been proposed to contribute to the detection of acids such as acid-sensing ion channels, 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB)-sensitive chloride channels, hyperpolarization-activated channels HCN1 and HCN4, potassium (K2P) channels, leakage-type K^+ channels, and transient receptor potential family members PKD1L3 and PKD2L1 (Ugawa *et al.*, 1998; Miyamoto *et al.*, 2000; Stevens *et al.*, 2001; Richter *et al.*, 2004b; Lin *et al.*, 2004; Liu *et al.*, 2005; Ishimaru *et al.*, 2006; Huang *et al.*, 2006).

Using a combination of molecular biological, pharmacological, and morphological approaches, we identified acid-sensing ion channels-2a (ASIC2a) and ASIC2b as candidates for sour taste sensors (Ugawa *et al.*, 1998; Ugawa *et al.*, 2003). The present review summarizes the role of acid-sensing ion channels in sour-taste transduction.

ASIC2a in rat taste bud cells

To isolate taste receptors, we initially constructed a rat circumvallate papilla cDNA library. Then we screened this using combined homology and functional expression approaches. Among 18 candidate clones by

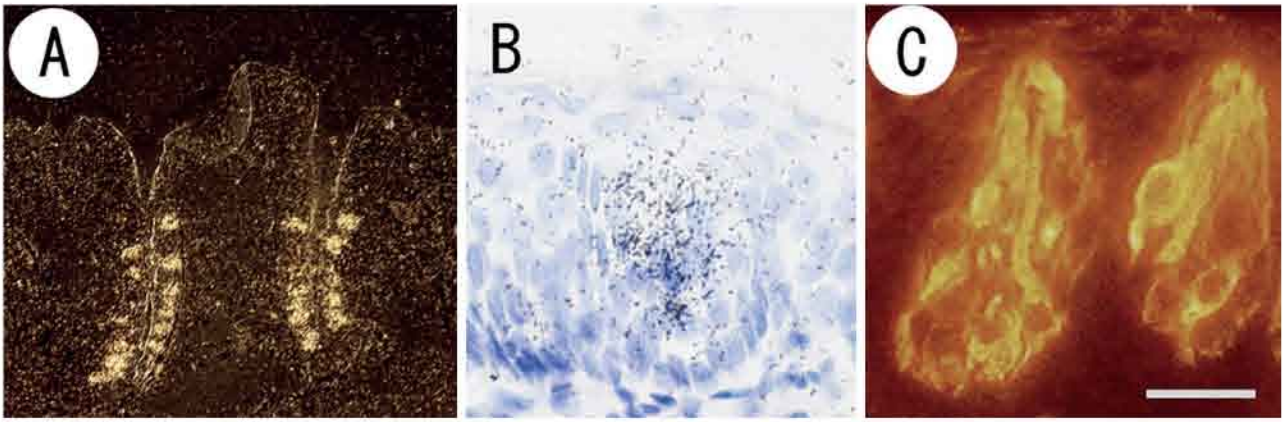

Fig. 1. Localization of acid-sensing ion channel-2a (ASIC2a). **A**,**B:** Expression of ASIC2a mRNA in the rat circumvallate papilla by *in situ* hybridization. **A:** The circumvallate papilla, stained with an antisense probe, in a dark-field photomicrograph. **B:** The dense hybridization signals for ASIC2a mRNA accumulate only in the central portion of the taste buds under bright-field illumination. **C:** Immunohistochemical localization of ASIC2a in the circumvallate taste buds. Intense ASIC2a immunoreactivity can be seen in a subset of taste cells (approximately 20-30%) in the taste buds. Bar: 50 μm

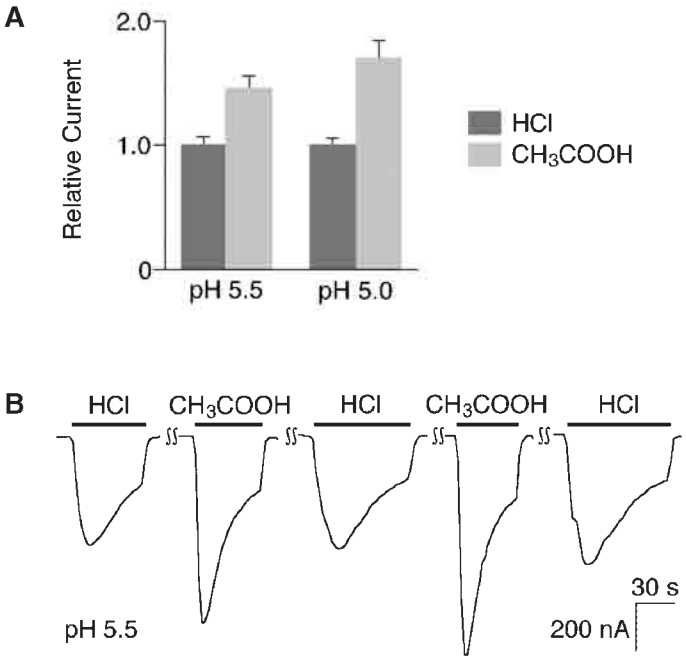

Fig. 2. Acetic acid enhances acid-sensing ion channel-2a (ASIC2a) channel activity. **A:** Whole-cell currents of ASIC2a-expressing oocytes at -70 mV. Data are plotted relative to the current elicited by acidic recording solutions (ND140) adjusted with HCl. Values are the mean ± SEM from seven different oocytes. **B:** Representative current trace of an oocyte injected with cRNA encoding ASIC2a. ND140 solution (containing (in mmol/L): NaCl 140; KCl 2; $CaCl_2$ 1.8; $MgCl_2$ 1; 2-(4-morpholino)-ethanesulfonic acid (MES) 5, pH 5.5) adjusted with either HCl or CH_3COOH was applied alternately.

the first screening, we found that one clone produced a large inward current in response to the rapid pH drop. Sequence analysis revealed that this clone was identical to mammalian degenerin-1 (MDEG1) although minor nucleotide differences were found in non-coding regions. Mammalian degenerin-1 is now called acid-sensing ion channel-2a (ASIC2a) (Waldmann and Lazdunski, 1998; Price *et al.*, 2000; Duggan *et al.*, 2002).

In situ hybridization revealed that intense hybridization signals for ASIC2a mRNA were found in most taste buds on both sides of deep clefts in the rat circumvallate papillae but not in any surrounding tissues (Fig. 1A). Under higher magnification, the dense hybridization signals were seen to accumulate in the central portion of the taste buds, suggesting that the mRNA were not localized in the basal cells (Fig. 1B). A ribonuclease protection assay also showed the expression of ASIC2a transcripts in the papillae (data not shown). Thus, ASIC2a mRNA was exclusively expressed in the taste bud cells in the rat circumvallate papillae.

Using a rabbit anti-ASIC2a antibody, we examined the cellular distribution of ASIC2a proteins in the rat taste buds. We found that intense ASIC2a immunoreactivity was localized in a subset of taste bud cells (approximately 20-30%) in the circumvallate papillae, not in surrounding tissues (Fig. 1C). Further immunoelectron microscopic analyses revealed that some of the ASIC2 positive cells had the characteristics of taste-receptor cells (type III cells; Murray *et al.*, 1969), which make synaptic contacts with gustatory afferent fibers (data not shown).

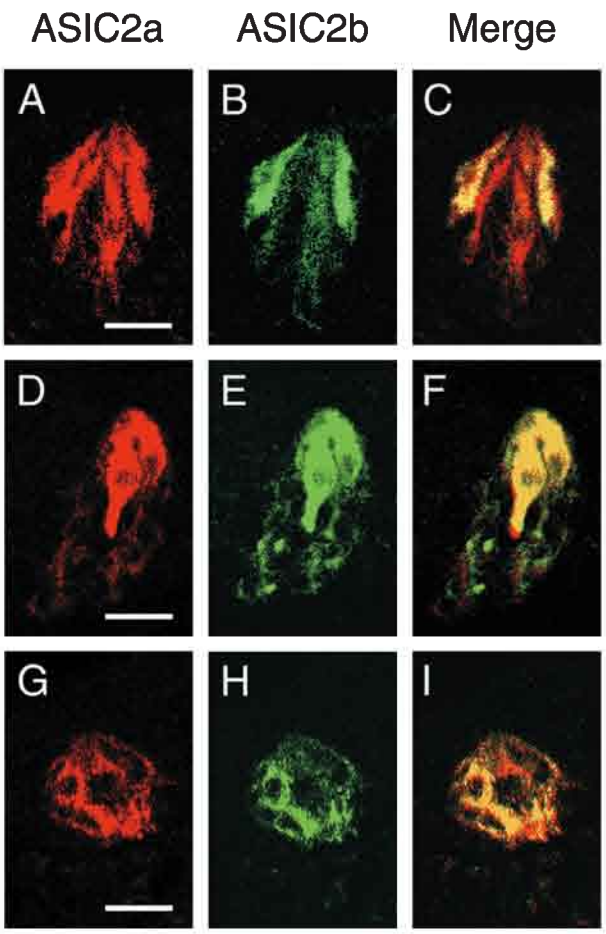

Fig. 3. Colocalization of ASIC2a and ASIC2b within single taste cells. Immunohistochemical analyses with anti-ASIC2a and anti-ASIC2b antibodies were performed on contiguous semithin sections (2 μm). ASIC2a-staining in rat circumvallate, foliate, and fungiform papillae (**A**, **D**, and **G**, respectively). ASIC2b-staining in the papillae (**B**, **E**, **H**). Regions of overlap (**C**, **F**, **I**). Both ASIC2a and ASIC2b proteins were, at least in part, colocalized in a subpopulation of taste cells in the circumvallate (*upper row*), foliate (*middle row*), and fungiform (*lower row*) papillae. Scale bars: 25 μm

Acetic acid enhances ASIC2a currents

Protons cause sourness, but sour taste varies depending on whether the acid is organic or inorganic. From ancient times, vinegar has been used as a seasoning for sour taste, and it has long been known that acetic acid, the main ingredient of vinegar, is a more sour than hydrochloric acid at the same pH level. Similarly, lactic acid and citric acid exhibit more sour taste than hydrochloric acid at the same proton concentration (Lindemann, 1996).

To investigate this phenomenon, we examined acid-activated currents in *Xenopus* oocytes expressing ASIC2a by the application of acidic solutions adjusted to pH 5.5 or 4.5 with acetic acid or hydrochloric acid. Stimulation by acetic acid generated larger inward currents compared with currents induced by hydrochloric acid at an equal pH (Fig. 2A,B). These response patterns of ASIC2a are similar to the characteristics of the *in vivo* sour-taste perception of the two acids. These data suggest that extracellular protons can directly activate the ASIC2a channel and, simultaneously, the organic anions are able to enhance the ASIC2a channel activity.

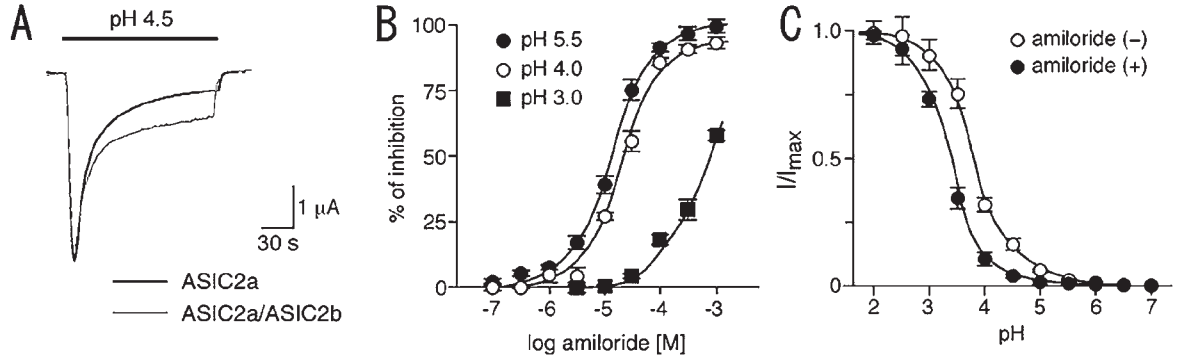

Fig. 4. Functional expression of acid-sensing ion channel (ASIC)-2a and ASIC2b. **A:** Representative whole-cell current traces of ASIC2a homomeric and ASIC2a/ASIC2b heteromeric channels expressed in *Xenopus* oocytes. Acidic pH solutions (pH 4.5) were present in the bath during the times indicated by a bar. **B:** X-and Y-axes represent concentrations of amiloride and % of blocked currents (compared to the maximal induced current at each pH value), respectively. **C:** The pH-dependency of the heteromer was shifted to more acidic pH values in the presence of 100 μM amiloride The values in **B** and **C** represent mean ± SEM of at least 5 experiments.

ASIC2b in rat taste bud cells

According to the present findings, mucosal acids can depolarize taste-receptor cells through ASIC2a channel activation, but there are some discrepancies to be addressed. First, acidic stimuli elicit action potentials from rat taste cells in a pH-dependent manner, with maximal responses at pH < 2.0 (Pfaffmann *et al.*, 1967) — although the ASIC2a generates its maximal currents at approximately pH 3.0 (Ugawa *et al.*, 2001). Second, the acid-evoked responses recorded in acid-selective nerve fibers (H-best fibers) that primarily carry information for sour taste are barely inhibited by amiloride in the rat (Ninomiya and Funakoshi, 1988), whereas the ASIC2a current is amiloride blockable.

To solve those problems, we further screened the rat circumvallate papilla cDNA library with a partial ASIC2a cDNA probe at low stringency and obtained several independent clones. The longest cDNA clone showed a high homology with ASIC2a and turned out to be identical to ASIC2b, an N-terminal splice variant of ASIC2a. Reverse transcription-polymerase chain reaction (RT-PCR) analysis also confirmed the expression of ASIC2b mRNA in rat taste cells (data not shown). Immunohistochemistry of consecutive thin sections with antibodies against ASIC2a and ASIC2b revealed that ASIC2a and ASIC2b immunoreactivities coexisted in single taste bud cells in the rat circumvallate, foliate, and fungiform papillae (Fig. 3). As described elsewhere (Lingueglia *et al.*, 1997; Ugawa *et al.*, 2001), ASIC2b interacts with ASIC2a to attenuate ASIC2a channel desensitization (Fig. 4A). More importantly, ASIC2b lowers the pH sensitivity of the ASIC2a channel: the ASIC2a/ASIC2b heteromer generates its maximal currents at pH < 2.0. These data are in good accordance with previous *in vivo* recordings of acid-evoked responses in rat taste cells. According to our data (Ugawa *et al.*, 2003), the amiloride sensitivity of the heteromer decreases with decreasing extracellular pH and is almost completely abolished at pH 2.0 (Fig. 4B, C). These pharmacological properties provide persuasive explanations for the amiloride insensitivity of rat sour-taste transduction because most previous electrophysiological experiments have used strongly acidic conditions (pH ≤ 3.0) as the sour-taste stimuli.

Both ASIC2a and ASIC2b subunits belong to the ASIC family of cation channels. Members of this family form homomeric and heteromeric proton-activated channels in the mammalian central and peripheral nervous systems (Waldmann and Lazdunski, 1998). Although ASIC2 is abundantly expressed in the rat circumvallate and fungiform papillae, ASIC2 expression is not detected in these tissues of the mouse. Here instead of ASIC2, ASIC1 and ASIC3 are expressed in the circumvallate and fungiform papillae (Richter *et al.*, 2004a). The expression pattern for each ASIC subunit differs by the species. Thus, the physiological role of each ASIC subunit in taste transduction may vary widely among species.

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