Brief Communication

Acid-Sensing Ion Channel-2 Is Not Necessary for Sour Taste in Mice

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The acid-sensitive cation channel acid-sensing ion channel-2 (ASIC2) is widely believed to be a receptor for acid (sour) taste in mammals on the basis of its physiological properties and expression in rat taste bud cells. Using reverse transcriptase-PCR, we detected expression of ASIC1 and ASIC3, but not ASIC4, in mouse and rat taste buds and nonsensory lingual epithelium. Surprisingly, we did not detect mRNA for ASIC2 in mouse taste buds, although we readily observed its expression in rat taste buds. Furthermore, in Ca^{2+} imaging experiments, ASIC2 knock-out mice exhibited normal physiological responses (increases in intracellular Ca^{2+} concentrations) to acid taste stimuli. Our results indicate that ASIC2 is not required for acid taste in mice, and that if a universal mammalian acid taste transduction mechanism exists, it likely uses other acid-sensitive receptors or ion channels.

Key words: **taste; sour; acid; receptor; ASIC; transduction**

Introduction

Much progress has been made in uncovering G-protein-coupled receptors that transduce sweet, bitter, and umami tastes (Margolskee, 2002; Gilbertson and Boughter, 2003), but identifying mechanisms for sour and salty has proved to be much more difficult. For instance, sour taste is elicited by acids, which penetrate the lingual epithelium, including taste buds, and stimulate a subset of taste receptor cells (Lyall et al., 2001; Richter et al., 2003). Protons are believed to act on acid-sensitive ion channels and depolarize taste receptor cells (Ugawa et al., 1998, 2003; Miyamoto et al., 2000; Stevens et al., 2001; Richter et al., 2003). Depolarization of acid-sensitive taste receptor cells produces an influx of extracellular Ca^{2+}, which, in turn, is presumed to lead to neurotransmitter release onto the synapses of gustatory sensory afferent fibers.

There are a number of candidate transducers for sour taste. In particular, several different acid-sensitive or proton-conducting ion channels have been identified in taste receptor cells and have been proposed to mediate acid taste in mammals. Such channels include epithelial sodium channels (Gilbertson et al., 1992; Gilbertson and Gilbertson, 1994; Ugawa et al., 1998; Liu and Simon, 2001; Lin et al., 2002), hyperpolarization-activated cyclic nucleotide-gated channels (Stevens et al., 2001), and unspecified cation and chloride channels (Miyamoto et al., 2000). However, the evidence is not compelling for any one of these channels as a definitive mechanism for sour taste transduction per se, because metabolically active cells often express a variety of pH-sensitive ion channels to cope with cytoplasmic acidification.

Acid-sensing ion channel-2 (ASIC2; also known as BNC1 and BNaC) is a member of a family of voltage-insensitive cation channels involved in mechanosensitivity and neuronal acid sensitivity (Waldmann and Lazdunski, 1998; Price et al., 2000; Sukharev and Corey, 2004). ASIC2 mRNA exists as two splice variants, ASIC2a and ASIC2b (Waldmann and Lazdunski, 1998). On the basis of studies in the rat, ASIC2 has recently been proposed as a mammalian sour taste receptor (Ugawa et al., 1998; Liu and Simon, 2001; Lin et al., 2002; Ugawa, 2003; Ugawa et al., 2003). First, reverse transcriptase (RT)-PCR, *in situ* hybridization, and immunostaining studies have shown that ASIC2 is expressed in rat taste receptor cells, and physiological responses in rat taste cells are consistent with ASIC-like channels (Ugawa et al., 1998, 2003; Liu and Simon, 2001; Lin et al., 2002). ASIC2 may also be expressed in human taste tissue (Huque et al., 2003). When expressed in oocytes, heteromers of ASIC2a and ASIC2b can be activated by acid taste stimuli (Ugawa et al., 2003). Nevertheless, there is no direct evidence for the involvement of ASIC2 as a generalized mammalian sour taste receptor. Indeed, in preliminary findings, behavioral responses to acid taste stimuli remained unaltered in mice lacking the ASIC2 gene (Kinnamon et al., 2000). Hence, we sought to clarify the role of ASIC2 as a candidate mammalian sour taste receptor. Surprisingly, we found that ASIC2 is not expressed in mouse taste buds, and that physiological responses to sour taste stimuli are robust and unaltered in ASIC2 knock-out (KO) mice.

Materials and Methods

Animals. We used adult Sprague Dawley rats and adult C57/Bl mice (Jackson Laboratory, Bar Harbor, ME). Homozygous ASIC2 KO mice (Price et al., 2000) were generously supplied by Dr. M. Welsh (Howard Hughes Medical Institute, University of Iowa, Iowa City, IA). All procedures were performed under protocols approved by the National Insti-

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Table 1. PCR primers for ASIC1–4 from rat and mouse

Genes	Forward primer name, sequence	Reverse primer name, sequence	PCR product
rASIC1 (U94403), mASIC1 (XM_128133)	*ASIC1–1* 5′-GCCTATGAGATCGCAGGG-3′	*ASIC1–2* 5′-AAAGTCCTCAAACGTGCCTC-3′	305 bp
rASIC2 (U53211), mASIC2 (NM_007384)	*ASIC2–1* 5′-GAAGAGGAAGGGAGCCATGAT-3′	*ASIC2–2* 5′-GGCAGAAGTTCGCAATGTGT-3′	275 bp
	ASIC2–3 5′-CTCAGAGATGGGACTCGACTTC-3′	*ASIC2–4* 5′-TGGCTCCCTTCCTCTTCTTC-3′	551 bp
	ASIC2–3	*ASIC2–2*	807 bp
rASIC3 (NM_173135), mASIC3	*ASIC3–1* 5′-CCCAGCTCTGGACGCTATG-3′	*ASIC3–2* 5′-TCTTCCTGGAGCAGAGTGTTG-3′	414 bp
rASIC4 (NM_022234), mASIC4 (BC046481)	*ASIC4–1* 5′-GAATGTGCCGACCACACACT-3′	*ASIC4–2* 5′-GCAAGCAAAGTCTTCAAAGAGG-3′	563 bp

GenBank accession numbers for the cDNAs are in parentheses. r, Rat; m, mouse.

tutes of Health and University of Miami Institutional Animal Care and Use Committee.

RNA and RT-PCR. Taste epithelium was delaminated from underlying tissue by injecting collagenase and dispase (Gilbertson et al., 1993). Crypts of the circumvallate and foliate epithelium, highly enriched with taste buds, were then microdissected from surrounding nonsensory epithelium. Fungiform and palatal taste buds were individually aspirated from the serosal face of the epithelium. Total RNA was isolated from each tissue sample using the Absolutely RNA Nanoprep kit (Stratagene, La Jolla, CA). DNase-digested RNA was eluted, denatured, and reverse-transcribed in a 20 μl reaction with 200 U of SuperScript II (Invitrogen, Carlsbad, CA). PCR was performed in 25 μl using 1 μl of cDNA template.

We designed PCR primers using published cDNA sequences for rat and mouse ASICs (Table 1). All primers were located in isoform-specific regions that were identical between the two species. Hence, each primer pair efficiently amplified of both rat and mouse cDNA templates. Each pair of primers spanned at least one intron to distinguish between products amplified from cDNA and genomic DNA. To assure that we could reliably detect ASIC2 expression, we used two forward and two reverse primers that were used in three combinations (Table 1). Because no published cDNA were available for mouse ASIC3, we identified a unique sequence in the mouse genome that was >96% identical to the published rat cDNA sequence. These constituted the mouse orthologs as evidenced by identical intron locations and similar exon sizes. As controls, we used specific primers to amplify β-actin (5′-CACCCTGTGCTGCTCACC-3′; 5′-GCACGATTTCCCTCTCAG-3′, 328 bp product) and the taste-specific G-protein, α-gustducin (5′-GCAACCACCTCCATTGTTCT-3′; 5′-AGAAGAGCCCACAGTCTTTGAG-3′, 285 bp product). PCRs using positive control (brain), negative control (water in place of template), and the test cDNAs were run in parallel from master mixes. The identity of PCR products for all ASICs, amplified from taste tissue samples (ASIC1–3) or brain (ASIC4), was confirmed by DNA sequencing.

Ca^{2+} imaging. Ca^{2+} transients in taste receptor cells were recorded in slices of circumvallate papillae containing intact taste buds using laser scanning confocal microscopy as described previously (Caicedo et al., 2000; Caicedo and Roper, 2001; Richter et al., 2003). We only included data from taste cells that exhibited Ca^{2+} responses ($\Delta F/F > 0.1$) to stimulation with 100 mM citric acid. The response amplitude was defined as the peak $\Delta F/F$ elicited by focal application of citric acid to the taste pore (Caicedo et al., 2002; Richter et al., 2003). To compare results across preparations, the number of cells responding to citric acid was normalized to the number of cells responding to KCl depolarization (50 mM) in the same imaged field. Citric acid solutions were prepared fresh before each experiment. Lingual slices were continually bathed in Tyrode's buffer containing the following (in mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 $NaHCO_3$, 10 HEPES, 10 glucose, and 10 sodium pyruvate, pH 7.4.

Results

Approximately 25% of taste cells respond to acidic taste stimuli (Richter et al., 2003). If ASIC2 is a key sour tranduction channel in mice, its mRNA should be readily detected in taste buds using RT-PCR. Hence, we investigated whether ASIC2, a candidate mammalian sour taste receptor, is expressed in taste buds from rat and mouse. We designed PCR primers in fully conserved regions of the cDNA to ensure that ASIC2 sequences from both species would be efficiently amplified. All primers were located in regions common to the splice variants ASIC2a and ASIC2b and would amplify both isoforms. As expected, brain cDNA template from both rat and mouse yielded strong amplification products for all ASICs tested. We were surprised to find that ASIC2 was not evident in mouse taste buds from the circumvallate, fungiform, foliate papillae, and the palate (Fig. 1*a*,*b*). Because this finding was unexpected, we repeated PCR reactions and obtained the same result with at least 10 independent RNA preparations from mouse taste buds. The lack of ASIC2 expression was confirmed with three different combinations of validated primers located in three different exons (Fig. 1*b*, Table 1). Taste cDNA samples that failed to amplify ASIC2 sequences nevertheless were positive in control PCRs for β-actin and α-gustducin (a taste-specific G-protein). We consistently detected robust ASIC2 expression in mouse brain, rat brain, and rat taste buds using the same PCR primers. Because mice respond to acid taste stimuli in behavioral and Ca^{2+}-imaging experiments (Bachmanov et al., 1996; Richter et al., 2003), our findings imply that an acid-responsive mechanism(s) other than ASIC2 must transduce acid taste perception in this species.

For comparison, we assessed whether other ASICs are expressed in rat and mouse taste buds. Using RT-PCR, we observed that ASIC1 and ASIC3 are each expressed in taste buds from both species, whereas ASIC4 was not detectable in the taste buds of either species (Fig. 1). In mouse nontaste epithelium samples, ASIC1 expression was either lacking or only a faint PCR product was observed. We note that a similar inconsistent distribution of ASIC1 expression in taste versus nonsensory epithelium was also observed in human lingual tissue (Huque et al., 2003).

As an additional test of ASIC2 as a sour taste receptor, we recorded functional responses to acid taste stimuli in taste buds from mice in which both splice variants of ASIC2 were knocked out (Price et al., 2000). We have shown previously using confocal imaging that focal application of citric acid (a common sour taste stimulus) to the taste pore of rat and mouse taste buds elicits a robust Ca^{2+} influx in ~25% of taste cells (Caicedo et al., 2002; Richter et al., 2003). Taste cells that respond to focally applied citric acid represent a subset of the cells (approximately half) that respond to KCl depolarization, suggesting that acid taste stimuli elicit Ca^{2+} influx via voltage-gated Ca^{2+} channels (Richter et al., 2003). We found that citric acid-evoked Ca^{2+} responses in taste cells from ASIC2 KO mice were indistinguishable from those in

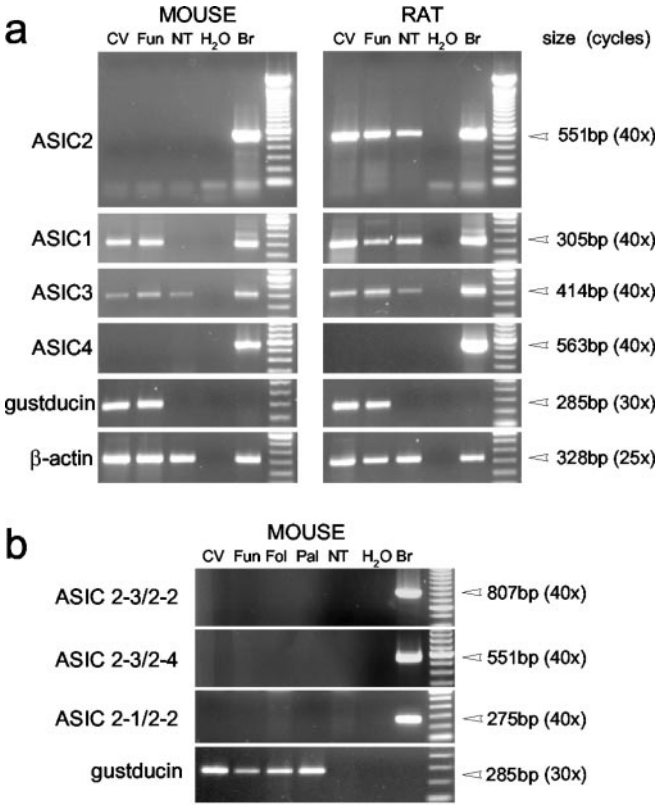

Figure 1. RT-PCR shows that ASIC2 expression is lacking in mouse taste buds. *a*, RT-PCRs for ASIC1–4 performed in parallel on RNA isolated from taste buds and nontaste epithelium from rat and mouse. For ASIC2, amplification using the ASIC2–3/ASIC2–4 combination of primers (Table 1) is shown. Each vertical lane represents a single cDNA preparation tested for expression of all four ASICs and two control genes. Ten independent mRNA preparations from mouse taste buds were tested (circumvallate, foliate, fungiform, and palatal taste buds); two are shown here. Gustducin (a taste-specific G-protein) and β-actin control PCRs validated the quality of each sample. Amplification for the ASICs was performed to 40 cycles to reveal even low-level expression. *b*, RT-PCR using three combinations of primers to test expression of ASIC2 in taste buds isolated from four different taste fields. CV, Circumvallate; Fun, fungiform; Fol, foliate; Pal, palate; NT, nontaste lingual epithelium; Br, brain.

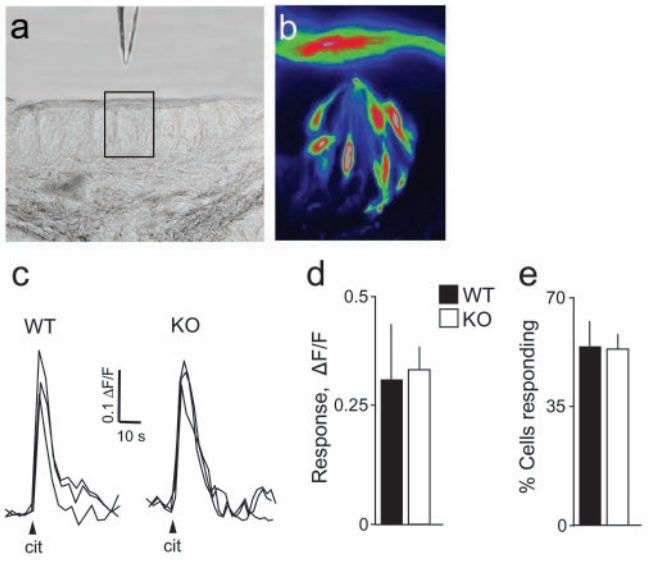

Figure 2. Ca^{2+} imaging shows that acid taste responses are unaltered in ASIC2 knock-out (KO) mice. *a*, Differential interference contrast micrograph of a ~100 μm thick slice of mouse circumvallate papilla containing numerous taste buds. A puffer pipette is visible above the tissue and was used to apply citric acid directly to the pores of taste buds. *b*, Pseudocolor confocal image (488 nm) of a circumvallate taste bud (box in *a*) in which individual taste receptor cells have been loaded with the fluorescent Ca^{2+} indicator Ca-Green Dextran (CGD) for functional Ca^{2+} imaging. The mucosal surface of the epithelium shows a layer of adsorbed CGD. The intensity of this fluorescence did not change during stimulus application and did not affect $\Delta F/F$ in taste cells. *c*, Superimposed responses (CGD fluorescence, $\Delta F/F$) from three taste receptor cells to focal stimulation with 100 mM citric acid (cit; arrowhead). Representative traces from a WT and an ASIC2 KO mouse are shown. *d*, Mean amplitude $\pm$ SEM of citric acid-induced Ca^{2+} responses in WT mice ($n = 31$ cells; 5 animals; closed bar) and KO mice (16 cells; 2 animals; open bar). *e*, Percentage (mean $\pm$ SEM) of taste cells responsive to citric acid relative to the number of KCl-responsive cells in WT (closed bars) and KO (open bars) mice. The sample population is the same as in *d*. The incidence of acid responsive cells is presented as normalized to KCl-responsive cells to control for variability in dye loading across preparations.

wild-type mice (WT) (Fig. 2). Specifically, the magnitude and time course of the Ca^{2+} response, as well as the incidence of acid-responsive taste cells, were almost identical between KO and wild-type mice (Fig. 2*c–e*).

Discussion

Our study shows that ASIC2 is not expressed in mouse taste cells, and that taste cells from ASIC2 KO mice respond normally to acid stimuli. These results are consistent with a preliminary report that in behavioral studies, ASIC2 KO mice responded normally to acid taste stimuli (Kinnamon et al., 2000). Collectively, these findings indicate that ASIC2 is not an acid taste receptor in mice. Although acid transduction in rats is consistent with the properties of ASIC2 (for review, see Ugawa, 2003), our observations indicate that this channel is not a universal mammalian sour receptor. Our study cautions against extrapolating taste transduction mechanisms from one animal model (in this case, rat) to other species.

We also found that other ASIC genes, specifically ASIC1 and ASIC3 but not ASIC4, are expressed in taste tissues from rats and mice. In rats, ASIC1 was expressed in taste and nonsensory epithelium alike. In contrast, in mice, ASIC1 was preferentially expressed in taste buds relative to nonsensory epithelium, as also reported in human lingual tissues (Huque et al., 2003). ASIC3 channels also appear to be expressed in taste and nonsensory epithelia in both species. The presence of ASIC2 in mouse taste tissue was suggested in a preliminary report (Buffington et al., 2002). We also observed that RNA extracted from circumvallate papillae from mouse (including connective tissue, muscle, nerve, and other cell types) occasionally yielded very low levels of RT-PCR product for ASIC2. It is possible that in circumvallate papillae, a small number of cells other than taste receptor cells may express ASIC2. A thorough understanding of sour taste transduction remains to be uncovered, particularly a mechanism that is common to all mammalian taste buds, if this exists. The ability of cells to cope with an excess of protons and to regulate their intracellular pH is crucial. There are numerous mechanisms, including channels, pumps, and transporters that serve this purpose (Reeh and Kress, 2001; Waldmann, 2001; Alper, 2002). Hence, one might expect that taste cells express one or more of these channels, pumps, or transporters. It would be instructive to ascertain which, if any, of these proteins are unique to those taste receptor cells that respond to acid stimulation.

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