Taste Receptor Cells Express pH-Sensitive Leak K⁺ Channels

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INTRODUCTION

Taste stimuli interact with receptors or ion channels of taste receptor cells (TRCs), which guides the acquisition of nutrients and avoidance of toxins. Acids elicit sour taste, with the degree of sourness a function of proton concentration. Mechanisms of sour transduction involve multiple ion channels (Gilbertson et al. 1992, 1993; Kinnamon and Roper 1988; Kinnamon et al. 1988; Lin et al. 2002a; Miyamoto et al. 1988, 1998; Stevens et al. 2001; Ugawa et al. 1998), membrane proteins (Bigiani and Roper 1994; Okada et al. 1987, 1993), and intracellular molecules (Liu and Simon 2001; Lyall et al. 2001; Richter et al. 2003; Stewart et al. 1998). Thus sour taste coding seems to integrate signals from multiple pathways.

TRCs are excitatory, generating spontaneous and evoked action potentials. The excitability is regulated by potassium channels that set resting membrane potentials (∆V_{rest}) and regulate action potential frequency (Hille 2001). In TRCs, control of ∆V_{rest} has been attributed to delayed rectifying K⁺ (K_{DR}) channels, leak K⁺ (∆K_leak) channels (Kolesnikov and Bobkov 2000; Miyamoto et al. 1991; Okada et al. 1986; Roper and McBride 1989), and inward rectifier K⁺ (K_{IR}) channels (Sun and Herness 1996). However, ∆V_{rest} in most TRCs is from −36 to −69 mV (Miyamoto et al. 2000), potentials where K_{DR} and K_{IR} conduct little current (Chen et al. 1996). Recently, a leak K⁺ channel (∆K_leak) was found in mouse taste buds cells, conducting time- and voltage-independent currents and contributing to setting ∆V_{rest} (Bigiani 2001). Its molecular identity has not been determined.

We previously reported that acids depolarize taste receptor cells by two different mechanisms: activation of an inward current, possibly mediated by acid-sensing ion channels (ASICs) (Lin et al. 2002a), and suppression of a steady-state leak conductance (Lin et al. 2002b). Preliminary studies revealed that the acid-suppressed conductance shared properties with the reported ∆K_leak in mouse (Bigiani 2001) and with the cloned two-pore domain K⁺ (K_{2p}) channels of the TASK family (TWIK-related acid-sensitive K⁺ channel) (Duprat et al. 1997; Girard et al. 2001; Kim et al. 1998, 2000; Leonoudakis et al. 1998, 2000; Rajan et al. 2000; Reyes et al. 1998), which are sensitive to extracellular pH (Millar et al. 2000) and contribute to the establishment of ∆V_{rest}. Recently, an additional member of the K_{2p} family, TALK-1 (TWIK-related alkaline pH activated K⁺ channel type 1) (Han et al. 2003), has been identified, whose properties closely match those of TASK-2. The voltage-independent activation and open-rectification of TASKs permit substantial current at both ∆V_{rest} and depolarized potentials. Thus TASKs could provide potential mechanisms for sour taste transduction and the control of ∆V_{rest} in TRCs. Since acid is present in foods commonly, and sour-sensitive TRCs respond broadly to stimuli with different modalities (Caicedo et al. 2002; Gilbertson et al. 2001; Sato and Beidler 1997), acid modification of ∆V_{rest} via TASKs may modulate other taste sensations.

Using H⁺ sensitivity as a reporter in whole cell recordings, we characterized TASK-like currents in TRCs. We show that some TRCs possess a highly H⁺-sensitive ∆K_leak conductance that controls ∆V_{rest}. Immunocytochemical and molecular biological approaches show the presence of TASK-like channels in rat taste buds, and TASK-2 is the most highly expressed of these channels. Together, we provide strong evidence for the presence of a subset of K_{2p} channels in TRCs and suggest...
potential roles in setting $V_{\text{rest}}$ and in sour taste transduction. Preliminary results have been published in abstract form (Burks et al. 2003; Lin et al. 2002a).

**METHODS**

**Electrophysiological recordings**

Adult Sprague-Dawley male rats were used in this study. Vallate papillae taste bud isolation, whole cell patch-clamp recordings, and data acquisition were as described previously (Lin et al. 2002a). The bath solution (Tyrode’s) was comprised of (in mM) 140 NaCl, 5 KCl, 1 MgCl$_2$, 2 CaCl$_2$, 10 HEPES, 10 glucose, and 10 sodium pyruvate (pH 7.4 with NaOH). Acidic solutions were obtained by adding 1 M citric acid or HCl to the bath solution to obtain the desired pH. K$^+$ channels were kept (pH 7.4 with NaOH). Acidic solutions were obtained by adding 1 M citric acid or HCl to the bath solution to obtain the desired pH. K$^+$ channel inhibitors BaCl$_2$ (5 mM) and 10 mM tetraethylammonium (TEA; Sigma Chemical, St. Louis, MO) were added to Tyrode’s and bath-applied to taste cells. The intracellular pipette solution contained (in mM) 140 KC1, 2 MgCl$_2$, 10 HEPES, 11 EGTA, 1 ATP, and 0.4 GTP (pH 7.2 with KOH). To ensure that recordings were obtained from TRCs, we applied depolarizing voltage steps to induce voltage-gated K$^+$ and/or Na$^+$ current, since nonsensory epithelial cells and some glia-like taste cells (Akabas et al. 1990; Bigiani 2001) do not possess these currents. For steady-state measurements, holding current was recorded at various holding potentials and 10- or 20-mV hyperpolarizing voltage pulses were used to monitor membrane conductance. Statistical analyses and curve fittings were conducted using Origin 6.1.

**Immunocytochemistry**

Rats were anesthetized with sodium pentobarbital (40 mg/kg) or ketamine–xylazine (100-20 mg/kg) and perfused transcardially with 0.1 M PBS followed by buffered 4% paraformaldehyde. The tongue and positive control tissues of brain and kidney were removed and postfixed for 2 h before being transferred into PBS with 25% sucrose overnight. The tissues were frozen and cut with a cryostat into free-floating 30-µm-thick sections. Sections containing taste buds of foliate and vallate papilla were selected, rinsed, and incubated in blocking solution containing 2% normal goat or donkey serum, 0.3% Triton X-100, and 1% bovine serum albumin in PBS for 1.5 h. The sections were incubated with polyclonal antibodies against TASK-1, TASK-2 (Alomone Labs), or TASK-3 (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:50 to 1:50 dilutions in the blocking solution overnight at 4°C, followed by rinsing and incubation with the FITC-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA) overnight at 4°C. Sections were incubated with polyclonal antibodies against TASK-1, TASK-2 (Alomone Labs) overnight at 4°C, washed to establish procedures (Gilbertson and Fontenot 1998), washed to remove nonadherent cells, and immediately placed into 1.5-mL microfuge tubes with 200 µL RNA later (Ambion, Austin, TX). The taste buds were centrifuged at 6,000 rpm for 7 min. The resulting pellet was resuspended in lysis buffer from the RNeasy Mini Kit from Qiagen (Valencia, CA), and RNA was extracted according to manufacturer’s instructions, including DNase I treatment. For positive or negative controls, RNA was extracted from ~100 mg of brain tissue (for TASK-3), kidney (for TASK-1, TASK-2, and TALK-1), pancreas (TALK-1), and liver (TALK-1) using Tri Reagent (MRC, Cincinnati, OH) according to the manufacturer’s instructions.

**RT-PCR**

First-strand cDNA was synthesized using the OmniScript RT Kit (Qiagen). The maximum volume of taste RNA or 50 ng of brain RNA was used for the reaction, with the total volume being 20 µL. Reactions were also set up in which the reverse transcriptase enzyme was omitted as a control to detect genomic DNA contamination. After first-strand synthesis, 2 µL of cDNA was added to a PCR reaction mix [final concentration: 500 mM KCl, 100 mM Tris-HCl (pH 8.3), 2.0 mM Mg$^{2+}$, 1:1 TaqMaster PCR enhancer (Eppendorf, Westbury, NY), 200 µM dNTPs, 500 nM forward and reverse primers, and 1.25 U Taq polymerase]. The following primer sequences were used for the three TASK channels and TALK-1 in the RT-PCR assays: TASK-1 (accession no. AF031384; rat), 5'-TTGTTTTTGTTTGGTTCTCGT-3' (sense, nucleotides 1728–1747), 5'-GTGACCTGGACAAGAGACCC-3' (antisense, 1688–1887); TASK-2 (accession no. AF319542; mouse), 5'-CAACCACCTTCATGCTGTTG-3' (sense, 557–575), 5'-ACTTCCAGCATCTGTAGGG-3' (antisense, 896–915); TASK-3 (accession no. AF192366; rat), 5'-CCGATGACACCTCTGCTG-3' (sense, 481–498), 5'-GGACAACACCCCCGCTCTTGT-3' (antisense, 989–907); and TALK-1 (accession no. AY404471; mouse), 5'-AAGGGAACCTCACCACATTC-3' (sense, 251–270), 5'-AGAAGCCTCTCAGGAAAC-3' (antisense, 593–610). Amplification by regular PCR included an initial 5-min denaturation step followed by 40 cycles of a three-step PCR: 30-s denaturation at 95°C, 30-s annealing at a predetermined optimal temperature (62°C for TASK-1 and TALK-1, 57°C for TASK-2, 59°C for TALK-3), and 45-s extension at 72°C, and concluding with a 7-min final extension step. Amplified sequences were visualized by electrophoresis in 2% agarose gels poured using 1× TAE buffer (40 mM Tris-Acetate, 1 mM EDTA) or by real-time technology. cDNA to be sequenced was either purified directly after PCR using the QiAquick PCR purification kit (Qiagen) or extracted from agarose gels using the QiAquick gel extraction kit. Sequences were determined by the dye-terminator method using an ABI (Foster City, CA) Model 3100 Automatic Sequencer.
qPCR

To quantify TASK-1, TASK-2, TASK-3, and TALK-1 mRNA levels among the different taste epithelia, we used a two-tube RT-PCR assay with the PCR step conducted in a real-time thermal cycler (SmartCycler, Cepheid, Sunnyvale, CA). The procedures for first-strand synthesis are the same as described earlier, except the reaction was scaled to 100 µl. Two microliters of cDNA was used for each qPCR reaction. The HotMaster Taq DNA polymerase kit (Eppendorf) was used, with the following final concentration: 1X reaction buffer, 3.5 mM MgCl₂, 200 µM dNTPs, 300–900 nM sense and antisense primers, 300–900 nM fluorescent probes, and 1.25 U HotMaster Taq.

Two-step PCR protocols were used to amplify TASK1 and TASK3 (15-s denaturation at 95°C and 60-s annealing and extension at 60°C) and TALK-1 (15-s denaturation at 95°C and 60-s annealing and extension at 62°C), while a three-step PCR protocol (15-s denaturation at 95°C, 30-s annealing at 57°C, and 30-s extension at 72°C) was used to amplify TASK2. Primers and probes were designed for the three TASK channels, TALK-1, and the housekeeping gene, GAPDH, using Oligo 6.0 Primer Analysis Software (Molecular Biology Insights, Cascade, CO). Primer and probe sequences for the qPCR assays are listed in Table 1. We used a TaqMan (ABI) detection system in which the primer pairs for channel-specific sequences were multiplexed with the primer pairs for GAPDH for comparison of expression levels in the three types of taste buds (Bustin 2000).

Channel-specific probes were labeled at the 5' end with carboxyfluorescein fluorescent dye (FAM) as the reporter fluorophore and Black Hole quencher-1 (BHQ-1) at the 3' end. The GAPDH probe was labeled with carboxy-X-rhodamine fluorescent dye (ROX) as the reporter fluorophore and Black Hole quencher-2 (BHQ-2) as the quencher. All probes were obtained from Integrated DNA Technologies (Coralville, IA). All qPCR assays were carried out in triplicate, and a minimum of three independent experiments was conducted.

For quantitative analysis, fluorescent signals of the samples were plotted against the respective qPCR cycle number. The cycle at which the growth curve crossed 30 fluorescent units was defined as the cycle threshold (C_T). This user-defined threshold was selected to occur during the log-linear phase of the growth curve, which is inversely proportional to the starting amount of target in the sample. Exact cycle thresholds were measured for the three TASK channels and TALK-1 as well as for the housekeeping gene, GAPDH. \( \Delta C_T \) was calculated by subtracting the GAPDH \( C_T \) from the individual K2P channel \( C_T \). Comparing \( \Delta C_T \) values allowed for detection of relative transcript abundance between different sets of pooled taste bud types by normalizing TASK channel expression to a constitutively expressed gene. Therefore, the smaller the \( \Delta C_T \), the higher that the K2P channel is expressed in the particular taste bud type. For relative quantitation of our samples, the arithmetic formula \( 2^{-\Delta C_T} \) was used and takes into account the amount of target, normalized to an endogenous reference and relative to a calibrator.

The K2P channel with the highest expression (or the lowest \( \Delta C_T \)) for each set of pooled taste receptor cells was defined as the calibrator for that set. The calculation of \( \Delta C_T \) involved subtraction of the \( \Delta C_T \) for each channel from the \( \Delta C_T \) calibrator value. The relative amount of target expression was determined according to the following relation (Applied Biosystems 1997)

\[
\Delta C_T = C_T^{\text{target}} - C_T^{\text{GAPDH}}
\]

\[
\Delta C_T = C_T^{\text{target}} - C_T^{\text{GAPDH}}
\]

where, \( C_T \) is the cycle threshold for the K2P channels or GAPDH determined empirically, and \( C_T^{\text{GAPDH}} \) is the cycle threshold for the calibrator, the most highly expressed channel in each assay. Mean relative expression values and SD were calculated from the three individual sets of pooled taste bud types. To determine if there were significant differences among the expression of K2P channels in the three taste bud types, multiple pairwise comparisons were made using a one-way ANOVA followed by Bonferroni's posthoc test for significance (SPSS 10.0, SPSS, Chicago, IL).

To determine if the efficiencies of the target and reference (GAPDH) amplification were consistent across template dilutions, we evaluated the \( \Delta C_T \) values for each set of K2P primers and GAPDH in three separate multiplexed reactions. For each of the PCR reactions, the absolute value of the slope of the log input versus \( \Delta C_T \) was < 0.1, showing equal amplification efficiencies for the different starting template concentrations (cf. Fig. 6, inset). There was no effect on \( C_T \) values when the GAPDH primers were either limited or not limited in the reactions.

RESULTS

Patch-clamp recording

Whole cell patch-clamp recordings were performed on freshly isolated vallate TRCs, and acidic stimuli were bath-applied. From a total 168 TRCs recorded, 19 cells responded to a pH drop from pH 7.4 to 5 (acidified with citric acid or HCl),

![Figure](http://example.com/figure.png)

**Table 1. Nucleotide sequences for the primers and probes used in the qPCR assays**

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<th>Target</th>
<th>GenBank Accession No.</th>
<th>Species</th>
<th>Sense Primer/Antisense Primer/Probe</th>
<th>Corresponding Nucleotide Sequence</th>
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<td>AF031384</td>
<td>Rat</td>
<td>5'-AACAGGAGCTCGTACGTACGT-3'</td>
<td>747–766</td>
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<td>5'-CTCTGATCACGCTCTCTGCTG-3'</td>
<td>861–880</td>
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<td></td>
<td>5'-TCTGATACGCGGACCCAGAGGGTT-3'</td>
<td>821–845</td>
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<td>Mouse</td>
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<td></td>
<td></td>
<td>5'-ACTTCCAGCCATCTGTAGGG-3'</td>
<td>592–636</td>
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<td>TASK-3</td>
<td>AF192366</td>
<td>Rat</td>
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<td>5'-ATCCGCGCAGAGGTTTTGAGGAGGG-3'</td>
<td>595–618</td>
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**Note:** TASK and TALK channel probes were labeled with the fluorescent reporter carboxyfluorescein fluorescent dye (FAM) on the 5' end and the quencher Black Hole quencher-1 (BHQ-1) on the 3' end. GAPDH probes were labeled with carboxy-X-rhodamine fluorescent dye (ROX) as the fluorescent reporter on the 5' end and the quencher Black Hole quencher-2 (BHQ-2) on the 3' end.

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with a slight change in current when held at −80 mV, close to the equilibrium potential of K⁺. When held at less negative potentials, such as −60 or −40 mV, which increases the driving force for K⁺, these TRCs responded to acid stimulation with a sizable sustained inward current accompanied by a significant reduction of the membrane conductance (Fig. 1A), leading to cell depolarization in current-clamp configuration (Fig. 1B). The majority of cells (120) responded to acid stimulation with a large rapidly activating and desensitizing inward current, which depolarized cells by increasing membrane conductance. ASICs have been proposed to mediate this response (Lin et al. 2002a). Some of these cells (3 of 9 tested) also possessed the acid-suppressive conductance with the same response profile as Fig. 1A. This could be observed when TRCs were held at 20 mV, where the ASIC-like current reached its reversal potential and was largely diminished (data not shown).

Membrane depolarization resulting from the blockage of a steady-state conductance most likely involved a K⁺ conductance, since the equilibrium potentials for other ions, such as Cl⁻, Na⁺, and Ca²⁺ were more positive; blocking the conductance of these ions would only result in hyperpolarization. To examine if acid blocked a K⁺ conductance, we recorded acid responses at different holding potentials to determine the reversal potential of this acid-sensitive current. The mean current-voltage (I-V) relationship (n = 3–5 cells) was plotted (Fig. 1C), showing the acid-sensitive current reversed at −90 mV, a potential that was close to the equilibrium potential of K⁺ (E_K), which was calculated to be −94 mV. The I-V curve was fitted well to the Goldman-Hodgkin-Katz constant field equation, indicating that acid blocked a background or leak K⁺ channel (K_leak), which lacked intrinsic voltage sensitivity and showed open or slightly outward rectification under asymmetrical physiological K⁺ gradients. Since some members of the K₂P family of channels (including TASKs and TALK-1) conduct K_leak currents and are sensitive to extracellular low pH (Goldstein et al. 2001; Lesage and Lazdunski 2000), we examined TRCs for the presence of TASK1-3 and TALK-1 channels.

The high H⁺ sensitivity is a hallmark feature of TASKs (Goldstein et al. 2001; Lesage and Lazdunski 2000; O’Connell et al. 2002). We therefore examined the pH dependence of the K_leak by holding TRCs at −60 mV and stimulating with acidic solutions ranging from pH 7.18 to 4. As shown in Fig. 1D, the K_leak was highly sensitive to extracellular pH. A drop of pH from pH 7.4 to below 7.18 inhibited the conductance and resulted in a sustained current, which was about 90% reached by pH 6 and saturated at pH 5. This pH sensitivity was similar to cloned TASK-2 tested at similar resting bath pH conditions (Morton et al. 2003), but markedly different from the ASIC-like current in TRCs (Lin et al. 2002a). Our data thus suggested that TASK-like channels might be present in TRCs and involved in sour taste transduction.

TASK channels exhibit distinct pharmacological characteristics. Unlike the voltage-gated K⁺ channels, they are insensitive to the “classical” K⁺ channel blocker TEA, but are blocked by Ba²⁺, a common inhibitor of two-pore domain channels (Ashmole et al. 2001; Duprat et al. 1997; Girard et al. 2001; Kim et al. 1998, 2000; Leonoudakis et al. 1998; Rajan et al. 2000; Reyes et al. 1998). The K_leak conductance in taste cells reported by Bigiani (2001) also is insensitive to TEA but blocked by Ba²⁺. We therefore examined effects of TEA and Ba²⁺ on the acid-sensitive K_leak in TRCs. As shown in Fig. 2B, TEA (10 mM) added to the bath solution did not inhibit either the resting conductance or the acid-induced current (n = 3). Instead, the acid responses increased slightly. However, when BaCl₂ (5 mM) was added to the bath, the resting conductance was greatly reduced, leading to an abolishment of acid responses (n = 4; Fig. 2C). Similar to Ba²⁺ block, bath application of quinidine (1 mM) suppressed the conductance and the acid-induced responses (data not shown). Additionally, we examined the effect of the Cl⁻ channel blocker NPPB (0.1 mM), which reportedly blocks an acid-sensitive Cl⁻ current.
K<sub>leak</sub> channels are involved in setting V<sub>rest</sub> (Hille 2001). Cloned TASKs functionally expressed in heterologous cells contribute significantly to V<sub>rest</sub>, which reaches a value close to E<sub>K</sub>, since these channels are conductive at all potentials (Ashmole et al. 2001; Duprat et al. 1997; Girard et al. 2001; Kim et al. 1998, 2000; Leonoudakis et al. 1998; Rajan et al. 2000; Reyes et al. 1998). We reasoned if TASKs are molecular substrates for the pH-sensitive K<sub>leak</sub>, TRCs that possess such current should have a relative large resting current and a negative V<sub>rest</sub> close to E<sub>K</sub>. We tested whether V<sub>rest</sub> in these cells correlated with the pH-sensitive K<sub>leak</sub> current. To determine this, we used depolarizing voltage steps from −80 to 60 mV to induce currents in cells expressing K<sub>leak</sub> (Fig. 3A). Both voltage-gated Na<sup>+</sup> and K<sup>+</sup> currents were present, an indication of TRCs, which are distinct from epithelial cells and some glial-like taste buds cells that do not possess voltage-gated currents (Akbas et al. 1990; Bigiani 2001). The outward component, including both leak currents and voltage-gated currents was plotted in Fig. 3B. The voltage-gated K<sup>+</sup> current was blocked by TEA (data not shown). The leak current was present at all potentials in these cells and the amplitudes at potentials of −60 and −40 mV, where both voltage-gated K<sup>+</sup> and inward rectifier currents were barely activated (Chen et al. 1996), were measured. On average, they were 47.5 ± 7.5 and 111.2 ± 14.4 pA (n = 13), respectively, which were different in cells where the leak current at such voltages was minimal. Another noticeable feature of these cells was the V<sub>rest</sub> which averaged −70 ± 1.3 mV (n = 13). This was significantly different from TRCs that did not show pH-sensitive K<sub>leak</sub> (−42.2 ± 1.5 mV, n = 20; t-test, P < 0.001). As shown in Fig. 3C, V<sub>rest</sub> closely correlated with leak currents (r = −0.77, SD = 34, n = 13, P = 0.002), suggesting leak currents were responsible for setting the V<sub>rest</sub>. Because leak currents at −40 mV might not exclusively be K<sub>leak</sub>, we further examined if the pH-sensitive K<sub>leak</sub> conductance correlated to the V<sub>rest</sub>. Decreases in membrane conductance induced by pH 5 at −60 mV were measured and correlated with V<sub>rest</sub>. As shown in Fig. 3D, the pH-sensitive K<sub>leak</sub> and V<sub>rest</sub> were correlated significantly (r = −0.78, SD = 0.38, n = 15, P = 0.0006). The larger the pH-sensitive K<sub>leak</sub>, the more negative V<sub>rest</sub>. Thus these data provide strong evidence that the pH-sensitive K<sub>leak</sub> is a major contributor of leak current and plays a significant role in setting V<sub>rest</sub> in these TRCs. Since pH-sensing and setting resting potentials are two important functions of TASK channels, our electrophysiological data suggested that either TASKs or closely related channels (like TALK-1, see Immunochemistry) mediated the K<sub>leak</sub> Current in TRCs.

Immunochemistry

To determine which, if any, cloned TASKs were present in TRCs, three commercially available antibodies against TASK-1, TASK-2, and TASK-3 were used in immunocytochemical experiments. No antibodies were available for TALK-1 channels. The anti-TASK-1 antibody at 1:100 or 1:50 final dilutions failed to label TRCs, although a few nerve fibers adjacent to taste buds were immunopositive (Fig. 4, C and D). In contrast, anti-TASK-1 labeled control tissues, including a subset of neurons in the motor trigeminal nucleus of the brain (Miyamoto et al. 1998) and the volume-sensitive Cl<sup>−</sup> current (Gilbertson 2002) in TRCs, and found no effect on the pH-sensitive current in these cells (data not shown).

FIG. 2. Pharmacological effects of K<sup>+</sup> channel blockers. A: a TRC responded to pH 5 at a holding potential of −40 mV. B: bath application of the “classical” K<sup>+</sup> channel blocker tetraethylammonium (TEA; 10 mM) suppressed neither steady-state conductance nor acid-induced response. C: BaCl<sub>2</sub> (5 mM) applied to the bath reversibly blocked the steady-state conductance, inhibiting response to acid (n = 4). All 3 traces were recorded from the same cell.

FIG. 3. Correlation of the pH-sensitive K<sub>leak</sub> with the resting membrane potential (V<sub>rest</sub>). A: depolarizing voltage steps from −80 to 60 mV in 10-mV increments were applied to a cell expressing the pH-sensitive K<sub>leak</sub>. Traces are shown for every 20 mV. Note the presence of both voltage-gated inward and outward currents, which indicated that the cell was a TRC. V<sub>rest</sub> of the TRC was −76 mV. B: peak outward current at different potentials, including both leak and voltage-gated components is plotted. Leak currents were present at all potentials tested and could be observed easily at voltages between −40 to 60 mV, where there were no voltage-activated K<sup>+</sup> currents under our recording conditions. C: amplitude of leak current measured at −40 mV were plotted vs. V<sub>rest</sub> of the same cells showing significant correlation (r = −0.77, SD = 34, P = 0.002, n = 13). D: cells expressing the pH-sensitive K<sub>leak</sub> all had highly negative V<sub>rest</sub> near E<sub>K</sub>. The V<sub>rest</sub> closely correlated to the amount of conductance reduced by acid stimulation (r = −0.78, SD = 0.38, P = 0.0006, n = 15). The larger the pH-sensitive conductance, the more negative the V<sub>rest</sub>, which suggested that the pH-sensitive K<sub>leak</sub> was responsible for setting V<sub>rest</sub> in these cells.
FIG. 4. Expression of TASK channel proteins in TRCs. Antibodies against TASK-1, -2, and -3 were applied to control tissues and to foliate and vallate papillae containing TRCs. Photomicrographs were obtained using a laser-scanning confocal microscope. A and B: anti-TASK-1 antibody at 1:100 final dilution reacted positively to neurons in motor trigeminal nucleus of brain stem and granule layer of cerebellum, respectively. C: anti-TASK-1 antibody did not label TRCs in vallate papillae. D: transmission image of C, showing presence of many taste buds. E: low magnification image showing presence of immunoreactive TRCs to anti-TASK2 antibody (1:100) in many taste buds of a foliate papilla. F and G: high magnification showing labeled individual TRCs in foliate and vallate papillae. Immunoreactive TRCs were elongate and often had round- or oval-shaped nuclei. H: antibody against TASK-3 at working dilutions of 1:500 to 1:50 apparently did not react with TRCs. J and J: positive reaction for the anti-TASK-3 antibody is present in adjacent nerve fibers and a few nontaste cells. Negative controls consisted of omitting the primary antibody and preabsorbing the primary antibody with antigen peptide overnight before application to taste tissues (data not shown). Scale: 20 μm. K: Western blot showing an anti-TASK-2 antibody labeled an ~45 kD band indicating the presence of TASK-2 protein in rat foliate and vallate taste buds (taste). Negative control was rat cortex (brain).

RT-PCR and qPCR

To verify expression of TASK channels in rat taste buds, a series of RT-PCR assays were performed on mRNA isolated from fungiform, foliate, and vallate taste buds. PCR products for both TASK-1 and TASK-2 messages could be found in all three lingual taste bud types in a minimum of three independent experiments (Fig. 5). To further confirm their identity, the PCR products were sequenced, and the resulting sequences were compared with published sequences using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Over the regions sequenced (461 bp for TASK-1 and 302 bp for TASK-2), there was 100% identity between our PCR product for TASK-1 and TASK-1 from rat cerebellar granule cells and 95% homology between rat taste bud TASK-2 and TASK-2 from mouse kidney (data not shown). RT-PCR analysis for TASK-3 expression in rat taste buds produced more equivocal results. In most assays, no bands representing the appropriately sized PCR product were found consistently in any taste bud type, although bands in the positive control lanes (e.g., from brain) were found (Fig. 5). Rarely, however, a band was found for TASK-3 in one or more of the lanes containing one of the three taste bud types (data not shown). Considering the immunocytochemical results for TASK-3, it is possible this may have represented contamina-

FIG. 5. RT-PCR reveals the presence of several K_{L} channels in mRNA from the 3 lingual taste bud types. Primers for TASK-1 and TASK-2 amplify ethidium bromide-stained PCR products of expected sizes (TASK-1: 333 bp; TASK-2: 359 bp), but TASK-3 in most cases was negative (expected product: 427 bp). Positive controls (rat kidney or brain RNA) are shown for TASK-1, TASK-2, and TASK-3 with each set of primers. Negative (−) control lanes represent those in which cDNA was omitted from the PCR reaction. This top gel was overexposed to show the presence of TASK-1 in rat taste buds. Inset: RT-PCR assay revealing expression of TALK-1 in 3 lingual taste buds and pancreas (expected product size: 360 bp), but not in liver and kidney. Negative (−) control represents the omission of template in the PCR reaction.

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tion of our taste buds with surrounding nontaste cells on these rare occasions.

Given that TASK-2 expression was significant as indicated by RT-PCR, Western blotting, and immunocytochemistry, we probed for an additional member of the K<sub>2p</sub> family of channels whose functional and pharmacological properties are qualitatively similar to TASK-2 (Girard et al. 2001; Han et al. 2003; O’Connell et al. 2002). Like TASK-2, TALK-1, whose expression has been reported predominantly in the pancreas (Han et al. 2003), may be inhibited by decreases in extracellular pH near the physiological pH range, is insensitive to TEA and blocked by Ba<sup>2+</sup> and quinidine (Han et al. 2003), and possesses more sequence similarity to TASK-2 than either TASK-1 or TASK-3 (Kang and Kim 2004; Kim 2003). RT-PCR analysis for TALK-1 revealed its presence in rat pancreas and all three lingual taste buds (Fig. 5, inset). Consistent with earlier reports (Han et al. 2003), TALK-1 was not expressed in liver or kidney. Sequencing of the PCR products (361 bp) from the TALK-1 assay showed 96% identity with mouse TALK-1 (accession no. AY404471).

Since the immunocytochemical data indicated that TASK-2 expression was greater than that of TASK-1 (or TASK-3), a series of multiplexed Taqman-style quantitative real-time PCR reactions were run on pooled cDNA isolated from the three lingual taste buds of several rats. In a single tube, primer sets for one of the three TASK channels or TALK-1 and a dual-labeled fluorogenic probe specific for a region within the K<sub>2p</sub> primer boundaries was multiplexed with primers and a probe for the housekeeping gene GAPDH. Each of the four K<sub>2p</sub> channels was analyzed in this manner to determine their expression relative to GAPDH by calculating the ΔC<sub>T</sub> values for each replicate as described. To compare expression among the various K<sub>2p</sub> channels and taste bud types, the relative expression of each K<sub>2p</sub> channel within each taste bud type was determined with respect to an internal calibrator (i.e., the most highly expressed K<sub>2p</sub> channel). As shown in Fig. 6, relative

![Relative expression of TASK and TALK channel mRNA in 3 lingual taste bud types. Expression was determined relative to an internal calibrator as described in Eqs. 1–4.](http://jn.physiology.org/.../55100905900/.../jn.org)
TASK-2 expression in all three taste bud types is conservatively 10–100 times more highly expressed than TASK-1 and TALK-1, which, in turn, is from 2 to 80 times more highly expressed than TASK-3. Expression of TASK-2 was significantly higher than TASK-1, TALK-1, or TASK-3 within a taste bud type (e.g., TASK-2 > TASK-1 and TASK-2 > TASK-3; P < 0.05); however, TASK-1 expression was not significantly different from TASK-3 expression. There were no significant differences for TASK-1, TASK-2, or TALK-1 expression within each of the three taste bud types as determined by ANOVA. Because of its exceedingly low expression, differences in TASK-3 expression among the three taste bud types were not statistically analyzed.

**Discussion**

In this study, we employed multiple techniques to identify a background or leak K⁺ channel and to examine its potential function in setting V_{rest} and transducing sour taste in TRCs of rat vallate papillae. Our results from whole cell patch-clamp recordings showed that a K_{leak} current in a subset of TRCs exhibited high sensitivity to acidic extracellular pH. Ba²⁺, a common blocker for two-pore domain leak K⁺ channels, but not the “classical” K⁺ channel blocker TEA, inhibited the conductance. The amplitude of pH-sensitive K_{leak} correlated significantly with V_{rest}, suggesting that K_{leak} was the major determinant for setting V_{rest} in these cells. These three important features suggested the presence of TASKs or TASK-like K⁺ channels in TRCs. Consistent with this, we found strong TASK-2 immunoreactivity in a subset of TRCs and verified TASK-2 protein expression with Western blotting. Both RT-PCR and qPCR assays were consistent with the expression of TASK-2 and, to a lesser extent, TASK-1 and TALK-1 in rat taste buds. TASK-3 expression seems minimal and may be indicative of contamination from nontaste cells. Quantitative assays showed that TASK-2 was expressed at significantly higher levels than TASK-1 and TALK-1 in all taste bud types. Together, our data provide strong evidence that several K_{gp} channels (predominantly TASK-2) are present in rat TRCs and may be involved in transducing sour taste, setting resting potentials, and regulating cell excitability.

Several K⁺ channels are reportedly present in TRCs of various species and perform multiple functions, such as controlling resting potentials and the firing rate of action potentials, maintaining K⁺ homeostasis, and participating in taste transduction for salty, sour, sweet, bitter, and fatty acid stimuli (Bigiani et al. 2001, 2002; DeSimone et al. 2001; Gilbertson et al. 2000; Herness and Gilbertson 1999; Kinnamon and Margol-skee 1996; Lindemann 1996, 2001; Margolskee 2002; Miyamoto et al. 2000). The subset of K_{gp} channels examined in this study in TRCs share several important features with the leak K⁺ channels reported by Bigiani (2001) in taste bud cells in that they are voltage-independent, K⁺-selective, and sensitive to Ba²⁺. While Bigiani (2001) emphasized its possible glial-like function in maintaining K⁺ homeostasis in mouse taste bud cells, some of his recordings were from cells that did not express voltage-gated currents, suggesting they may not be TRCs. We concentrated only on TRCs and examined their roles in control of V_{rest} and in sour taste transduction. We showed that the K_{leak} current was blocked by small extracellular licular acidification and was not voltage-dependent, both of which are properties that are diagnostic of TASKs.

The TASK group consists of five subtypes, of which three mammalian representatives, TASK-1, -2, and -3, are known to be sensitive to low extracellular pH (Duprat et al. 1997; Kim et al. 1998, 2000; Leonoudakis et al. 1998; Rajan et al. 2000; Reyes et al. 1998). The TASK-4 subunit, also called TASK-2, is active only at alkaline pH (Decher et al. 2001; Girard et al. 2001) and therefore is not considered a likely candidate for the pH-sensitive K_{leak} in rat TRCs. Except for TASK-5, which does not form a functional homomeric channel (Kim and Gnatentco 2001), other TASKs conduct K⁺ currents that possess all the characteristics of mammalian background or leak conductance, i.e., they generate essentially instantaneous, non-inactivating, voltage-insensitive currents that have an open or weakly outwardly rectifying current-voltage relationship in asymmetric K⁺ gradients as predicted by the Goldman-Hodgkin-Katz constant field equation. Recently, identification of an additional member of the K_{gp} family, TALK-1, was reported with qualitatively similar properties to TASK-2 (Han et al. 2003). Presently, much less is known about the physiological and pharmacological properties of rat TALK-1; however, its inhibition by decreases in extracellular pH in the physiological range also makes it a candidate for the types of acid responses we report.

The activity of TASK-like channels (TASKs and TALK-1) is strongly dependent on external pH and also is regulated tightly by many transmitters, neuropeptides, and other factors (Maingret et al. 2001; Niemeyer et al. 2001; Patel et al. 1999; Sirois et al. 2000; Talley et al. 2000; Washburn et al. 2002). Thus TASKs present a constellation of functional properties that is unique among all K⁺ channels cloned to date. Moreover, our data showing the inhibition of acid responses by Ba²⁺ are consistent with TASK-2 and TALK-1 channels, but not TASK-1 or TASK-3 (O’Connell et al. 2002). TASK-2 is present primarily in epithelial tissues such as lung, colon, kidney, intestine, and stomach (Reyes et al. 1998); the latter two also seem to express TALK-1 in rat (Kang and Kim 2004). Functions of TASKs have been shown in controlling resting potentials, cell volume, cell excitability and chemoreception for pH/or pCO₂ and anesthetics (Bayliss et al. 2001; Han et al. 2002; Millar et al. 2000; Niemeyer et al. 2001; Sirois et al. 2000; Talley et al. 2000).

Because of the functional similarity between TASK-2 and TALK-1, additional RT-PCR and qPCR assays using primers for TALK-1 were carried out in taste buds and both positive (pancreas) and negative (liver, kidney) control tissues. Expression of TALK-1 was found in all three lingual papillae, and the possibility remains that TALK-1 may be contributing to the acid-induced currents we report here. However, quantitative assays for TALK-1 mRNA expression showed that it is 10–100 times less prevalent than TASK-2 (cf. Fig. 6). Thus, of those K_{gp} channels with properties similar to those reported in this study, TASK-2 seems the most likely candidate based on its high relative expression, and we have focused our discussion on this channel accordingly.

The hallmark features of TASKs coupled with the high expression of TASK-2 in mammalian TRCs (Fig. 6) suggest that TASK-2 may play important roles in TRCs. First, TASK-2 could be a sour taste transducer because of the high extracellular pH sensitivity. The presence of a hyperpolarizing con-
ductance produced by TASK-2 at resting potentials provides the electrical basis for H\(^+\) block-induced depolarization. Sour taste transduction can occur at both the apical and basolateral membrane of TRCs. However, tight junctions between taste bud cells and between epithelial cells separate the apical and basolateral membranes so that only limited amounts of H\(^+\) normally reach the basolateral membrane of TRCs. The membrane localization of TASK-2 is not known. Since positive immunoreactivity was present in the cell body and blockage of a basolateral background K\(^-\) conductance by Ba\(^2+\) led to depolarization and increase in the input resistance (Miyamoto et al. 2000), it is likely that TASK-2 is expressed at the basolateral membrane. Regardless of its location, 90% of the TASK-mediated pH-sensitive current could be obtained by a slight reduction of extracellular pH from 7.4 to 6. Therefore TASKs may be especially important in sensing weak acidic stimuli.

Second, TASK-2 could be a major determinant for setting \(V_{\text{rest}}\) and controlling TRC excitability in some TRCs. K\(_{\text{ir}}\) family members like TASKs and TALK-1 conduct instantaneous current at all potentials. This is distinct from the delayed rectifier K\(^-\) channel, which opens only at depolarizing potentials, and the K\(_{\text{v}}\) channel, which opens mostly at hyperpolarizing potentials. At \(\approx -60\) mV, K\(_{\text{ir}}\) rarely conducts significant outward current due to a rapid and highly voltage-dependent block by intracellular polyamines and also by Mg\(^2+\) (Ruppersberg 2000). The K\(_{\text{ir}}\) channel has been proposed for inducing the negative \(V_{\text{rest}}\) (Sun and Herness 1996). However, in our study, the slightly outwardly rectifying current-voltage relationship of the pH-sensitive K\(_{\text{leak}}\) and sizable leak current recorded at \(-40\) mV suggests that K\(_{\text{ir}}\) may not be a major contributor to \(V_{\text{rest}}\) in these TRCs. Instead, our results that \(V_{\text{rest}}\) in these TRCs is correlated significantly with the leak current at \(-40\) mV and to the pH-sensitive K\(_{\text{leak}}\) suggests that TASK-2 or TALK-1 is a major determinant for \(V_{\text{rest}}\) in these cells. However, this by no means excludes possible contributions of K\(_{\text{v}}\) and other stationary conductances in control of \(V_{\text{rest}}\). The \(V_{\text{rest}}\) in TRCs varies over wide ranges (Miyamoto et al. 2000). The fact that TASK-2 is only weakly expressed in many TRCs and that \(V_{\text{rest}}\) is not identical to E\(_{\text{K}}\) even in cells showing strong pH-sensitive K\(_{\text{leak}}\) suggests that a number of other conductances may also contribute to setting \(V_{\text{rest}}\) of TRCs.

Acids or sour taste are known to modify other taste sensations (Frank et al. 1983; Sakurai et al. 2000). The broad tuning of TRCs to different taste qualities makes it possible that the acid modification could occur at peripheral receptor cells levels. However, mechanisms underlying these modifications are poorly understood. This is in part due to the fact that H\(^+\) ions can interact with many ion channels, transport proteins, and intracellular signaling components, and in part due to the functional heterogeneity of TRCs. Acid modification may occur by direct interaction of H\(^+\) on ion channels that function as taste transducers, such as the epithelial Na\(^+\) channel (ENaCs). Protons modulate its activity and salt sensation by interaction with either the extracellular (Zhang et al. 1999) or intracellular sides of the channel (Lyall et al. 2002), or the H\(^+\) modification may be indirectly altering activities of ion channels and proteins that influence cell excitability. The degree of modification depends on acid concentrations and the H\(^+\) sensitivity of these components.

Among many potential pH-sensitive molecular targets that could change cell excitability, K\(_{\text{ir}}\) channels are obvious candidates, since some channels in this family regulate membrane potential and input resistance, key determinants of cell excitability. Daily complex foods consist of mixtures of taste qualities and many are slightly acidified to stimulate appetite. By blockage of K\(_{\text{ir}}\) channels, acids that are subthreshold for sour taste sensation may modify TRCs membrane properties, thus influencing sensations produced by other taste stimuli. Immunocytochemical studies showed that the expression level of TASK-2 in TRCs was heterogeneous, with strong expression in some but weak expression in many other TRCs. Depending on the number of TASK-2 channels, concentrations of H\(^+\) ions and \(V_{\text{rest}}\), the shift in membrane potential induced by H\(^+\)-dependent blockage of TASK-like channels may have diverse outcomes. Shifts that bring membrane potentials closer to threshold potentials for firing of Na\(^+\)- and/or Ca\(^2+\)-dependent action potentials can enhance the excitability, whereas stronger depolarization may induce firing of action potentials or may actually suppress the firing rates if prolonged depolarization occurs and deactivates voltage-gated channels. Moreover, as activities of TASKs also are regulated tightly by neurotransmitters and neuropeptides, they also present a substrate for efferent modulation and cell-to-cell regulation of TRCs (Finger et al. 1990). Therefore K\(_{\text{ir}}\) channels, such as the highly expressed TASK-2 channel, may provide unique molecular substrates for dynamic modulation of cell excitability.

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