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Taste Function in Mice with a Targeted Mutation of the *Pkd1l3* Gene

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Abstract

Recent studies, both in vitro and in vivo, have suggested the involvement of the polycystic kidney disease-1 and -2 like genes, *Pkd1l3* and *Pkd2l1*, in acid taste transduction. In mice, disruption of taste cells expressing PKD2L1 eliminates gustatory neural responses to acids. However, no previous data exist on taste responses in the absence of PKD1L3 or on behavioral responses in mice lacking either of these proteins. In order to assess the function of PKD1L3, we genetically engineered mice with a targeted mutation of the *Pkd1l3* gene. We then examined taste responsiveness of mutant and wild-type mice using several different approaches. In separate groups of mice, we measured preference scores in 48-h 2-bottle tests, determined NaCl or citric acid taste thresholds using a conditioned taste aversion technique, and conducted electrophysiological recordings of activity in the chorda tympani and glossopharyngeal nerves. Multiple taste compounds representing all major taste qualities were used in the preference tests and nerve-recording experiments. We found no significant reduction in taste responsiveness in *Pkd1l3* mutant mice in behavioral or electrophysiological tests when compared with wild-type controls. Therefore, further studies are needed to elucidate the function of PKD1L3 in taste bud cells.

Key words: gene knockout, gustatory nerves, polycystic kidney disease–like ion channel, preference, taste, threshold

Introduction

Discrimination between different taste modalities, that is, sweet, umami, bitter, salt, and acid, begins in the periphery when individual tastants interact with an organism at the apical ends of taste cells exposed to the oral cavity (Lindermann 2001; Chandrashekar et al. 2006). Molecular mechanisms of reception and transduction of sweet, umami, and bitter tastes have been largely decoded in the recent years (reviewed in Chandrashekar et al. 2006; Bachmanov and Beauchamp 2007; Roper 2007). It has long been hypothesized that ionic taste stimuli are able to pass into taste bud cells through specific ion channels (Scott 2005). Several molecules have been implicated in salt taste, including, transient receptor potential cation channel, subfamily V, member 1 (TRPV1) (Lyall et al. 2004; Ruiz et al. 2006; Treesukosol et al. 2007; Katsumata et al. 2008), SNMX-29 (Senomyx taste-specific gene #29) (Moyer et al. 2008), and the selective epithelial amiloride-sensitive sodium channels, or ENaCs (Bosak et al. 2010; Chandrashekar et al. 2010). However, decoding the reception and transduction mechanisms of acid taste has proven to be more complex.

In the past, a number of candidate acid taste receptors have been proposed including, the amiloride-sensitive cation channel 1 (ACCN1) (Ugawa et al. 1998, 2003; Liu and Simon 2001; Lin et al. 2002; Ugawa 2003), members of the hyperpolarization-activated cyclic nucleotide-gated (HCN1 and HCN4) channels (Stevens et al. 2001), 2-pore domain

potassium leak conductance channels, for example, TASK-1 (Lin et al. 2004; Richter et al. 2004), and the Na^+ –H^+ exchanger isoform 1 (Vinnikova et al. 2004).

More recently, results from several studies have revealed 2 members of the polycystic kidney disease (*Pkd*)–like gene family, *Pkd1l3* and *Pkd2l1*, which are coexpressed selectively in a subset of taste cells suggesting that they may function as a heteromer taste receptor (Huang et al. 2006; Ishimaru et al. 2006; LopezJimenez et al. 2006). Additionally, cells which express PKD2L1 do not coexpress bitter, sweet, or umami taste receptors or transduction machinery (Huang et al. 2006; Ishimaru et al. 2006), suggesting PKD2L1 is not involved in these tastes and leaving a possibility that it is involved in acid or salt taste reception. A study using a heterologous expression system supported a hypothesis that PKD1L3 and PKD2L1 interact to form a functional receptor: Coexpression of the 2 proteins was necessary for effective translocation to the cell membrane, and once expressed these proteins form ion channels which are responsive to acids. However, activation of these channels evoked an off-response but not on-response (Ishimaru et al. 2006; Inada et al. 2008), meaning the channel is opened only after the acid stimulus is removed. Although off-responses have been associated with acid taste stimuli in mammals previously (DeSimone et al. 1995; Danilova et al. 2002; Lin et al. 2002), this channel cannot account for acid stimulus induced on-responses. These data lead to the hypothesis that perhaps there are 2 different molecular mechanisms for acid taste transduction, one responsible for on-responses and the other responsible for off-responses (Inada et al. 2008).

Several other questions surround the involvement of PKD2L1 and PKD1L3 in acid taste. The 2 proteins are expressed in ~20% of overlapping taste cells, in the foliate and vallate papillae, likely Type III cells (Kataoka et al. 2008). However, PKD1L3 is not expressed in taste cells of the fungiform and palate papillae of mice, whereas PKD2L1 is expressed in taste cells of these papillae (Huang et al. 2006; Ishimaru et al. 2006). Because mouse fungiform papillae taste bud cells respond to acids (Yoshida et al. 2009) but do not express PKD1L3 required for acid responsiveness in vitro (Ishimaru et al. 2006; Inada et al. 2008), it is not clear what the acid receptor may be in these taste buds.

Evidence from in vivo studies has previously demonstrated that responses to acid stimuli can be eliminated by ablating those cells that express PKD2L1 (Huang et al. 2006; Chandrashekar et al. 2009). Additionally, a recent study has shown that 2 patients with acid-specific ageusia do not express PKD2L1, PKD1L3, or several acid-sensitive ion channels (Huque et al. 2009). However, besides this observation, there is no direct evidence linking acid (or any other) taste to PKD1L3 in vivo.

To examine what, if any, role PKD1L3 may have in taste function in living animals, we produced mice with a targeted mutation of the *Pkd1l3* gene and characterized their behavioral and neural taste responses. Mouse *Pkd1l3* encodes a >2100 amino acid protein with 36 coding exons and several splice variants. Like other PKD1 family members, PKD1L3 has 11 transmembrane (TM) domains and a very long extracellular N-terminal. This N-terminal contains a C-type lectin-binding domain, which is indicative of protein–protein or protein–carbohydrate interactions, a repetitive domain (13 amino acids repeated 28 times), and a G protein-coupled receptor proteolytic site. In addition, PKD1L3 has a Polycystin-1, Lipoxygenase, Alpha-Toxin or LH2 Lipoxygenase homology domain in its first intracellular loop, which may be predictive of protein interactions, and an ion channel pore region between TM domains 10 and 11 (Li et al. 2003; LopezJimenez et al. 2006). To produce genetically engineered mice with a targeted mutation of the *Pkd1l3* gene, we deleted *Pkd1l3* exons 17 through 21, which encode TM domains TM2–TM5. This shifted the reading frame downstream of the deleted exons with the consequent appearance of stop codons, resulting in a truncated protein with no ion channel pore, and therefore nonfunctional.

Design of our taste phenotyping experiments took into account the following 2 aspects. First, patterns of PKD1L3 coexpression with other taste-related molecules suggest that it is not involved in sweet, umami, or bitter taste. We therefore used an extended selection of salt and acid taste stimuli, although prototypical taste stimuli representing the main taste qualities were included. Second, in wild-type mice PKD1L3 is not expressed in taste buds of the fungiform and palate papillae. Because taste bud cells of the fungiform (Yoshida et al. 2009) and probably palate papillae respond to taste stimuli of all qualities, including acid and salt, we anticipated that PKD1L3 ablation will not necessarily alter taste input from this receptive field. We therefore designed experiments that would either detect changes in responsiveness despite residual input from fungiform and palate taste buds (i.e., taste thresholds tests, as detailed below) or that would detect changes in responsiveness from other receptive fields that include *Pkd1l3*-expressing taste buds (i.e., glossopharyngeal nerve responses).

We used 2 different behavioral measures, the first of which, long-term 2-bottle preference tests of naive mice, has been used to identify genetic differences in taste and recently has been used to help identify the function of taste-related genes (e.g., Whitney and Harder 1994; Wong et al. 1996; Bachmanov et al. 2001; Damak et al. 2003, Hisatsune et al. 2007). We have shown that acid taste responses in the long-term 2-bottle tests are determined by taste perception rather than postingestive factors (Boughter et al. 2001). In addition, we measured salt and acid taste thresholds using a conditioned taste aversion based method. This approach involves conditioning animals to avoid a suprathreshold concentration of a taste solution and then testing various concentrations of the same tastant. The obtained values correspond to recognition thresholds (Ishiwatari and Bachmanov 2009). Taste thresholds reflect changes in

peripheral taste input, which was shown, for example, in experiments with gustatory nerve cut (Spector et al. 1990; Slotnick et al. 1991; Kopka and Spector 2001; Golden GJ and Bachmanov AA, unpublished data). Correspondingly, we expected that if taste thresholds are affected by partial gustatory deafferentation in nerve section experiments and if PKD1L3 is indeed involved in taste reception, then taste thresholds also should be affected by partial elimination of gustatory input from *Pkd1l3*-expressing vallate and foliate taste papillae in *Pkd1l3* mutant mice. Finally, we conducted electrophysiological recordings of taste-evoked activity in both the chorda tympani and the glossopharyngeal nerves. Although PKD1L3 is not expressed in the fungiform buds innervated by the chorda tympani, the chorda tympani also innervates PKD1L3-expressing foliate papillae, at least in rats (Yamamoto and Kawamura 1975), and given the anatomical similarity, likely mice. The glossopharyngeal nerve innervates vallate and foliate papillae, both of which express PKD1L3. Therefore, if PKD1L3 is involved in taste function, its elimination in mutant mice could change taste responsiveness in the chorda tympani nerve and should change responses in the glossopharyngeal nerve.

Materials and methods

All animal experimentation was conducted in National Institute on Deafness and Other Communication Disorders (NIDCD) and Monell animal facilities, which are approved by the American Association for Accreditation of Laboratory Animal Care and meet all federal and state requirements for animal care. All experimental procedures were approved by institutional animal care and use committees before the onset of the experiments.

Generation of *Pkd1l3* mutant mice

Targeting vector

To construct the targeting vector for the *Pkd1l3*^{tm1Sul} targeted allele, we purchased bacterial artificial chromosome (BAC) clones from a C57BL/6J mouse genomic DNA BAC library that contained the *Pkd1l3* gene (RPCI 23 85g03 and 178p21). Using recombineering, we retrieved a genomic *Pkd1l3* 12.5-kb fragment containing the exons 17 through 22 (which encode TM2–TM5 domains) from the BAC DNA into a thymidine kinase gene containing plasmid (pLMJ235). Primers used were: 1107F-5′ ACGCGTCGACACGAACGAACGAACGAAAGAAAG; 1108R-5′ CC GGAATTCCTACCAGCTGTCAAACAATGTGT; 1117 F-5′ CCGGAATTCTGTGTATCCCTGGGCTGCCCTG; and 1123R-5′ ATAAGAATGCGGCCGCCCAGGTGCCGACCAAGGACACT.

A phosphoglycerate kinase promoter-neomycin resistant cassette was amplified by PCR from pLMJ260 vector. A Pvu I restriction site was attached to each end, and the neo fragment was digested with Pvu I enzyme and ligated into the Pac I digested pLMJ235 containing the *Pkd1l3* 12.5-kb genomic fragment (1243F-5′GCTCGATCGATATCAAGCTGAAGTTCCTA and 1244R-5′CAGCGATCGCACCGCGGTGGTACCATA). The neo cassette therefore replaced exons 17 through 21 of *Pkd1l3* (Figure 1A). The targeting construct was designed to eliminate several TM domains in the protein and to shift the reading frame downstream of the deleted exons with the consequent appearance of stop codons, resulting in a truncated protein with no ion channel pore. The sequence of the full construct (denoted SS513) was verified by dideoxysequencing method.

Electroporation of the targeting vector in ES cells

SS513 DNA was linearized with Not I enzyme, twice phenol-chloroform-isoamyl alcohol extracted, precipitated, redissolved in sterile water, and electroporated into a hybrid line: V6.4 (C57BL/6J × 129S4/SvJae) embryonic stem (ES) cells according to standard procedures. ES cell clones positive for homologous recombination were chosen by Southern blotting (described in detail in Supplementary material) from DNA digested with Spe I and probed with both 5′ and 3′ probes chosen outside the sequence used to generate the construct (Figures 1A,B). Expected sizes for wild-type *Pkd1l3* and mutant *Pkd1l3* alleles are 10.7 and 9.3 kb, respectively, with 5′ probe, and 8.9 and 6.8 kb for the 3′ probe respectively (Figure 1B). Among 132 independent clones selected for analysis, 5 clones were positive for homologous recombination event.

Blastocysts injection and production of mutant mice

ES cell clone 1044, heterozygous for the targeted mutation, was injected into C57BL/6 blastocysts to generate chimaeras. The resulting male chimaeras were mated with female C57BL/6 mice to produce *Pkd1l3* +/– heterozygous F_1 offspring. Germline transmission of injected ES cells was confirmed by the inheritance of agouti coat color in the F_1 animals, and all F_1 offspring were tested for the presence of the mutated *Pkd1l3* allele by both Southern blot analysis and by multiplex polymerase chain reaction (PCR) (described in Supplementary material). F_1 (*Pkd1l3* +/–) males and females were interbred to generate F_2 generation that included homozygous (*Pkd1l3* –/–) mutants as well as wild-type (*Pkd1l3* +/+) and heterozygous (*Pkd1l3* +/–) mice. All F_2 mice were genotyped by PCR analysis of genomic DNA using the multiplex protocol described below. Mice homozygous for this targeted mutation are viable and fertile and do not display any gross physical or behavioral abnormalities. The F_2 mice have an expected genetic background of 25% 129S4/SvJae and 75% C57BL/6. The F_2 mice were used for behavioral and neurophysiological studies. This strain was deposited at The Jackson Laboratory (official strain name B6;129S4-*Pkd1l3*^{tm1Sull}J; stock number: 008419).

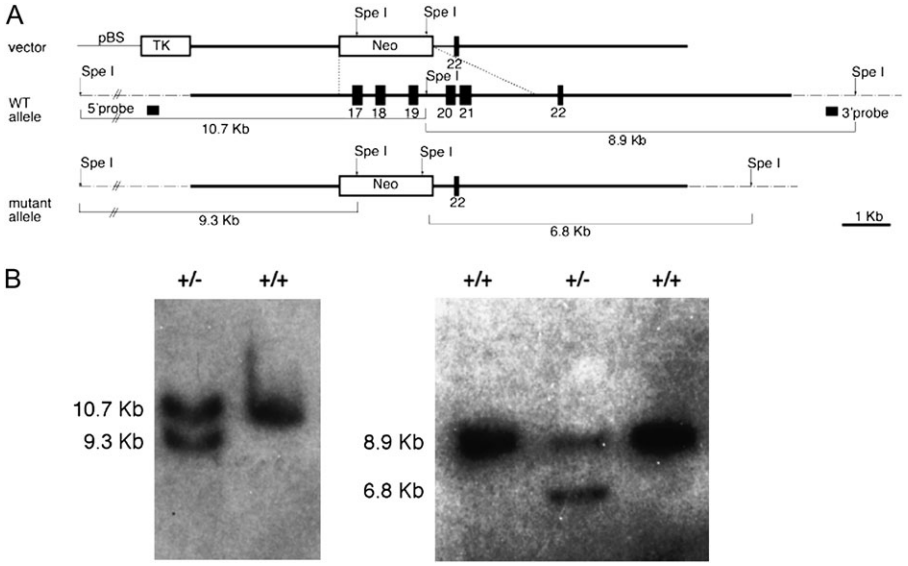

Figure 1 Successful generation of mice lacking the channel pore region of the *Pkd1l3* gene. **(A)** A vector containing a neo cassette was used to replace exons 17 through 21 (which encode TM domains 2 through 5) of *Pkd1l3*. The targeting construct was designed to eliminate several TM domains in the protein and to shift the reading frame downstream of the deleted exons with the consequent appearance of stop codons, resulting in a truncated protein with no ion channel pore. **(B)** ES cell clones positive for homologous recombination (*Pkd1l3* genotype +/–) were chosen by Southern blotting from DNA digested with Spe I and probed with both 5′ and 3′ probes (shown in Figure 1A). Expected sizes for wild-type (+) and mutant (–) *Pkd1l3* alleles are 10.7 and 9.3 kb, respectively, for the 5′ probe and 8.9 and 6.8 kb, respectively, for the 3′ probe.

In situ hybridizations

The procedure for in situ hybridization has been described elsewhere (LopezJimenez et al. 2005). In short, cryostat sections of vallate papillae were processed for fluorescent in situ hybridization, hybridized overnight with cRNA probes labeled with digoxigenin using a DIG RNA labeling Kit following the recommended protocol (Roche), and the positive signals detected with the Tyramide Signal Amplification kit (Molecular Probes). The in situ hybridization probes used in these studies corresponded to nucleotide positions +3777 to 4387 (including exons 18 and 19) of NM_181544 cDNA (*Pkd1l3*); and +1631 to 2340 of NM_181422 cDNA (*Pkd2l1*).

Behavioral tests

Animals

F_2 mice were born in the NIDCD animal facility and shipped to the Monell Center for experiments. Mice had at least 5 days to acclimate between arrival to Monell and the start of the tests. During behavioral testing at Monell, mice were housed in individual cages in a temperature-controlled room at 23 °C on a 12:12 h ligh:dark cycle and had free access to Teklad Rodent Diet 8604 (Harlan Teklan).

Mice used for behavioral experiments were divided into 2 testing groups. Group 1 mice were used for 2-bottle preference testing. Group 1 included 7 (4 male, 3 female) *Pkd1l3* mutant mice (–/–), 10 (5 male, 5 female) heterozygous (+/–), and 9 (5 male, 4 female) wild-type (+/+) littermate controls. Group 1 mice were 7–10 weeks old upon the initiation of testing and were age matched across the subgroups. Group 2 mice were used to measure taste thresholds using methods described previously (Ishiwatari and Bachmanov 2009) and were divided into 2 subgroups: 2a, 9 (5 male, 4 female) *Pkd1l3* –/– and 10 (6 male, 4 female) *Pkd1l3* +/+ mice and 2b, 9 (6 male, 3 female) *Pkd1l3* –/– and 10 (6 male, 4 female) *Pkd1l3* +/+ mice. Group 2a mice were tested with NaCl, and Group2b mice were tested with citric acid. Group 2 mice were 12–15 weeks old at the start of testing and were age matched across the subgroups.

Taste solutions

Taste solutions were prepared in deionized water using reagent-grade chemicals purchased from Sigma Chemical Company, with the exception of HCl which was purchased from Fischer Scientific. All taste stimuli were presented at room temperature.

Procedures for 2-bottle preference tests of naive mice

Fluid intake measurements are described in Supplementary material. Prior to testing, mice from Group 1 were given deionized water in a single tube for 4 days; after this acclimation period, the experiment began. Concentration series for each taste substance were tested in ascending order, staring with presentation of water in both tubes (concentration 0). Group 1 mice were tested in the following order with: 0, 0.1, 0.3, 1, 3, 10, and 30 mM citric acid; 0, 9.375, 18.75, 37.5, 75, 150, 300, 450, and 600 mM NaCl; 0, 0.3, 1, 3, 10, 30, and 100 mM inosine monophosphate

(IMP); 0, 0.003, 0.01, 0.03, 0.1, 0.3, and 1 mM quinine; 0, 0.03, 0.1, 0.3, 1, 3, and 10 mM sucralose; 0, 0.1, 0.3, 1, 3, 10, and 30 mM HCl; 0, 12.5, 25, 50, 100, 200, 300, and 400 mM KCl; 0, 3.125, 6.25, 12.5, 25, 50, and 100 mM $CaCl_2$; 0, 6.25, 12.5, 25, and 50 mM NH_4Cl; 0, 6.25, 12.5, 25, and 50 mM $MgCl_2$; and 0%, 1%, 3%, 10%, 20%, and 30% (v/v) ethanol. There were no breaks between testing different concentrations of the same compound. Mice received only deionized drinking water in both drinking tubes for at least 5 days between testing different compounds. Body weight was measured at the beginning and the end of the experiment and prior to testing the concentration series of each compound.

Procedures for taste threshold tests

Prior to testing, mice from Group 2 were given deionized water in 2 drinking tubes for 4 days; after this acclimation period, the experiment began. On experimental days 1 and 3, mice were exposed for these two 24-h periods to an LiCl-containing conditioned stimulus (CS) solution available in both tubes and as the only source of liquid. The 2 CS exposures were separated by a 24-h presentation of deionized water in both tubes (day 2) and a second 24-h presentation of deionized water (day 4) followed the second period of LiCl conditioning. Starting from day 5, mice were tested with ascending concentration series of either NaCl or citric acid in 48-h 2-bottle preference tests using procedures described in the previous section.

Mice from Group 2a were used to measure NaCl taste thresholds. They received 150 mM LiCl as the CS and were then tested with 0, 0.5, 1, 2, 4, 8, 16, 32, 64, and 150 mM NaCl. Mice from Group 2b were used to measure citric acid taste thresholds. They received a mixture of 150 mM LiCl and 10 mM citric acid as the CS and were then tested with 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 10 mM citric acid.

Data analysis

Data from both the 2-bottle preference tests and the taste threshold tests are reported in the form of percent preference scores, which were calculated using average daily fluid intakes of individual mice (solution intake/total fluid (solution + water) intake) × 100. A preference score of 50% indicates no preference or avoidance of the solution in comparison with water, whereas a score >50% suggests preference of the solution to water, and a score <50% suggests avoidance of the solution in comparison with water. Preference scores for both Group 1 and Group 2 mice were analyzed using repeated measures analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) post hoc tests using Statistica software (StatSoft, Inc.). Analyses were conducted using 3-way ANOVA to estimate effects of concentration (within-group factor), sex, and genotype (between-subjects factors). Preference ratios for water (0 concentrations) were not included in ANOVA analyses.

We calculated taste thresholds for Group 2 mice by fitting the NaCl or citric acid preference scores of each mouse or a whole group to a sigmoidal concentration–response regression curve using the function: $f(x) = 50/(1 + \exp(b(\log(x) - \log(c)))$, where (x) is the stimuli concentration, (b) is the slope, and (c) is the stimulus concentration at 25% preference score as described previously (Ishiwatari and Bachmanov 2009; additional details are provided in Supplementary material). Thresholds of individual mice were used for statistical comparisons, and group data were used to generate the regression plots in Figure 4. Calculations were conducted using the statistical language and environment R. Threshold values of mice with different *Pkd1l3* genotypes were compared using *t*-tests.

Statistical rejection was set at the 0.05 level for all data analysis.

Neurophysiology

Animals

Male mice were used in experiments. *Pkd1l3* –/– mice were born in the NIDCD animal facility and shipped to Monell for experiments. Mice from the C57BL/6 (B6) inbred strain were obtained from The Jackson Laboratory and used as a wild-type control (*Pkd1l3* +/+). The mice were 11–30 weeks old at the time of recordings. Ages of *Pkd1l3*–/– and *Pkd1l3* +/+ mice were similar (17 ± 3 and 20 ± 1 weeks, respectively, means and standard errors; $P = 0.4$, *t*-test). The mice were kept at a Monell animal facility and had free access to deionized water and Teklad Rodent Diet 8604 (Harlan Teklad). Chorda tympani recordings were obtained from 3 *Pkd1l3* –/– mice and 22 *Pkd1l3* +/+ mice. Glossopharyngeal nerve recordings were obtained from 7 *Pkd1l3* –/– mice and 8 *Pkd1l3* +/+ mice.

Taste stimuli

The following taste stimuli were used: NH_4Cl (100 mM); HCl (1, 10, and 100 mM); citric acid (1, 10, and 100 mM); acetic acid (1, 10, and 100 mM); NaCl (1, 10, 100, 300, and 1000 mM); KCl (1, 10, 100, and 1000 mM); $CaCl_2$ (100 and 1000 mM); quinine HCl (20 mM); strychnine (10 mM); sucrose (100, 300, and 1000 mM); sucralose (10 mM); glycine (1000 mM); monosodium glutamate (MSG) (100 and 1000 mM); IMP (10 mM). All taste compounds were purchased from Sigma except HCl and acetic acid, which were purchased from Fisher Scientific. All were dissolved in deionized water.

Electrophysiological recording of taste responses

Techniques for surgery, taste stimulation, and recordings were described previously (Inoue, Li, et al. 2001; Inoue, McCaughey et al. 2001; Inoue, Beauchamp, and Bachmanov 2004; Inoue, Reed, et al. 2004; Inoue et al. 2007) and can be found in detail in Supplementary material.

Data analyses

Differences between *Pkd1l3* –/– and *Pkd1l3* +/+ mice in responses to each taste stimulus in each nerve were assessed using *t*-tests. Data for compounds tested at more than one concentration were analyzed using ANOVA (2-way ANOVA when multiple concentrations were tested in both *Pkd1l3* –/– and *Pkd1l3* +/+ mice and one-way ANOVA when they were tested only in *Pkd1l3* –/– mice). These statistical tests used a criterion for significance of $P < 0.05$.

Results

In situ hybridization

We used in situ hybridization to verify the presence or absence of *Pkd1l3* transcript in slices of vallate papillae from *Pkd1l3* –/–, *Pkd1l3* +/–, and *Pkd1l3* +/+ mice. As predicted, *Pkd1l3* –/– mice showed a lack of the *Pkd1l3* transcript. However, *Pkd2l1* which is coexpressed with *Pkd1l3* is still expressed in the *Pkd1l3* –/– animals, indicating the taste bud cells are intact (Figure 2).

Behavioral tests

Body weight and water consumption

Because intakes of taste solutions can depend on animals' body size and habitual fluid intake, in our preliminary analyses, we analyzed whether these traits are influenced by the *Pkd1l3* genotype. During 2-bottle preference tests of naive mice, body weights were collected a total of 12 times throughout the taste tests and were not affected by the *Pkd1l3* genotype ($F_{2,23} = 0.6$, $P = 0.54$) or its interaction with time ($F_{22,253} = 0.6$, $P = 0.94$) (2-way ANOVA; where time was the within-group factor and genotype was the between-group factor). There was an effect of time ($F_{11,253} = 57.5$, $P < 0.001$) as mice of all genotypes increased their body weight over the course of the experiment. Body weights for *Pkd1l3* +/+ and *Pkd1l3* –/– mice also did not differ significantly for mice in taste threshold tests ($P > 0.5$, *t*-test). We conclude that *Pkd1l3* genotype does not affect body weight.

We also analyzed intake of water presented in both drinking tubes during the first 2 test days of concentration series for each taste compound (i.e., concentration 0). Water intake was calculated as the average daily water intake from drinking tube #1 plus the average daily water intake from drinking tube #2. In 2-bottle preference tests of naive mice, water intake was analyzed using 2-way ANOVA (with *Pkd1l3* genotype as a between-group factor and test as a within-group factor). Water intake was affected by genotype ($F_{2,22} = 4.9$, $P = 0.018$), test ($F_{10,220} = 4.3$, $P < 0.001$), and an interaction between genotype and test ($F_{20,220} = 3.5$, $P < 0.001$). Overall across all tests, *Pkd1l3* –/– mice had significantly higher ($P < 0.05$, Tukey HSD post hoc tests) water intakes than *Pkd1l3* +/– and *Pkd1l3* +/+ mice. When genotypes were compared in individual tests, *Pkd1l3* –/– mice had significantly higher water intakes before the test with NH_4Cl than either *Pkd1l3* +/– or *Pkd1l3* +/+ mice, whereas *Pkd1l3* +/– and *Pkd1l3* +/+ mice did not differ (the daily intake means were 11.9, 5.9, and 6.5 mL, respectively; $P < 0.05$, Tukey HSD post hoc tests). In taste threshold tests, there were no genotype differences in water intake for *Pkd1l3* –/– and *Pkd1l3* +/+ mice ($P > 0.4$, *t*-test). Therefore, *Pkd1l3* genotype had only transient effect on water intake. This variation in water intake did not compromise our analyses of taste responsiveness because we used preference scores that are independent of variation in habitual fluid intake.

Sex effects

We found only a few relatively weak effects of sex in behavioral tests. Preference ratios for quinine, $MgCl_2$, and ethanol were affected by the interaction between sex and genotype ($P < 0.05$, see Table 1). For both quinine and ethanol, male *Pkd1l3* –/– ($n = 4$) and female *Pkd1l3* +/+ mice ($n = 4$) tended to have slightly higher preference ratios than other groups, but no comparisons between genotypes were significant in post hoc tests (Tukey HSD). For $MgCl_2$, female *Pkd1l3* +/+ mice had higher preference ratios than females of other genotypes and males of any genotype ($P < 0.05$, Tukey HSD post hoc tests). With NH_4Cl there was a significant effect for sex ($F_{1,20} = 5.7$, $P = 0.026$), and in post hoc tests, female mice had higher preference ratios than male mice. Because there were no strong and consistent effects of sex, the subsequent analysis presented in Results includes only genotype and concentration effects.

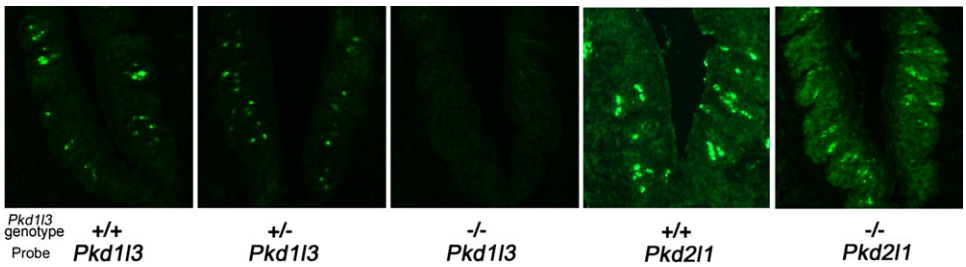

Figure 2 Lack of expression of *Pkd1l3* and normal expression of *Pkd2l1* transcripts in *Pkd1l3* –/– mice. In situ hybridization of *Pkd1l3* (left 3 panels) and *Pkd2l1* (right 2 panels) to vallate papillae. As predicted, *Pkd1l3* message was found in *Pkd1l3* +/+ and *Pkd1l3* +/– mice but not *Pkd1l3* –/– mice. This disruption of *Pkd1l3* did not interfere with normal expression of *Pkd2l1* as compared with *Pkd2l1* in wild-type mice.

Table 1 Significant ANOVA results ($P < 0.05$) in behavioral tests

Taste compound	Effect	df	*F* value	*P* value
Group 1				
Citric acid	Concentration	5, 100	155.8	<0.00001
	Concentration × genotype	10, 100	2.3	0.019
NaCl	Concentration	7, 140	131.3	<0.00001
IMP	Concentration	5, 100	34.3	<0.00001
Quinine	Sex × genotype	2, 20	3.6	0.046
	Concentration	5, 100	133.9	<0.00001
Sucralose	Concentration	5, 100	107.7	<0.00001
HCl	Concentration	5, 95	128.6	<0.00001
KCl	Concentration	6, 120	59.8	<0.00001
$CaCl_2$	Concentration	5, 100	45.8	<0.00001
NH_4Cl	Sex	1, 20	5.7	0.026
	Concentration	6, 120	50.6	<0.00001
$MgCl_2$	Genotype	2, 19	5.3	0.015
	Sex × genotype	2, 19	5.7	<0.00001
	Concentration	3, 57	11.8	<0.00001
Ethanol	Sex × genotype	2, 19	3.9	0.039
	Concentration	4, 76	20.7	<0.00001
Group 2a				
NaCl	Concentration	8, 120	35.0	<0.00001
Group 2b				
Citric acid	Concentration	7, 105	55.6	<0.00001

All other effects were not significant ($P \geq 0.05$); df, degrees of freedom.

Two-bottle preference tests of naive mice

In 2-bottle preference tests, preferences of naive mice scores for each of 11 tastants were significantly affected by concentration ($P < 0.05$, see Table 1) but typically not genotype or their interaction (Figure 3). Mice of all genotypes avoided the majority of compounds in a concentration-dependent manner, meaning as concentration increased preference scores decreased. There was an increase in preference scores as concentrations increased only for sucralose and IMP, although at the highest concentration of IMP mice of all genotypes had preference scores close to 50%.

Only for citric acid was there an effect of the interaction of genotype and concentration on preference score ($F_{10,100}$ = 2.3, $P = 0.019$). Contrary to expectations, *Pkd1l3* –/– mice tended to have had the lowest preference scores (or highest avoidance) at mid-range concentrations of citric acid (3 and 10 mM) and *Pkd1l3* +/– mice had the highest scores (weakest avoidance). However, mice with different genotypes did not differ significantly at any concentration in post hoc tests (Tukey HSD). There was one additional effect of genotype alone for $MgCl_2$ ($F_{2, 57} = 5.3$, $P = 0.015$), the *Pkd1l3* –/– mice tended to have lower average preference scores than the *Pkd1l3* +/+ or *Pkd1l3* +/– mice, although these comparisons (strain means collapsed across all concentrations) were not significant in post hoc testes (Tukey HSD). Therefore, none of the tests detected any taste deficiency of *Pkd1l3* –/– mice.

Taste threshold tests

Analysis of preference ratios from mice tested with a 9 concentration series of NaCl following LiCl conditioning revealed significant effects only for concentration ($F_{8,64}$ = 17.8, $P < 0.05$; Figure 4). Thresholds were 9.5 ± 2.5 mM NaCl for *Pkd1l3* –/– mice and 14.4 ± 3.3 mM NaCl for *Pkd1l3* +/+ mice. These thresholds did not differ significantly ($P = 0.3$, *t*-test).

For mice tested with an 8 concentration series of citric acid following LiCl + citric acid conditioning, there were significant effects for only concentration ($F_{7,63} = 43.6$, $P \leq 0.05$; Figure 4). Thresholds were 2.2 ± 0.8 and 2.5 ± 0.3 mM citric acid for *Pkd1l3* –/– and *Pkd1l3* +/+ mice, respectively, which did not differ statistically ($P = 0.7$, *t*-test).

Neurophysiology

In both *Pkd1l3* –/– and *Pkd1l3* +/+ mice, we obtained strong integrated chorda tympani and glossopharyngeal nerve responses to higher concentrations of taste stimuli representing different taste qualities, as illustrated by the representative traces in Figures 5 and 6. In both gustatory nerves, responses to acids, salts, bitter, sweet, and umami taste stimuli did not differ significantly between *Pkd1l3* –/– and *Pkd1l3* +/+ mice (Tables 2 and 3). For stimuli tested at multiple concentrations, the neural responses typically increased with increasing solution concentration.

Discussion

Previous studies suggested that PKD1L3 couples with PKD2L1 to form a heteromeric acid taste receptor, at least in the taste cells of the foliate and vallate papillae. If this is true, then genetic disruption of the *Pkd1l3* gene should result in acid taste deficiency. To test this hypothesis, we mutated the *Pkd1l3* gene in the mouse genome, which resulted in a truncated PKD1L3 protein with several TM domains and the ion channel pore eliminated. As expected, the resulting *Pkd1l3* mutant mice lacked detectable channel pore–containing *Pkd1l3* transcript in their taste cells. We then conducted a series of experiments to thoroughly examine the effect of a disrupted *Pkd1l3* gene on taste function in these mice.

We measured behavioral and gustatory taste responses to multiple taste compounds representing all major taste qualities and measured acid and salt taste thresholds. With

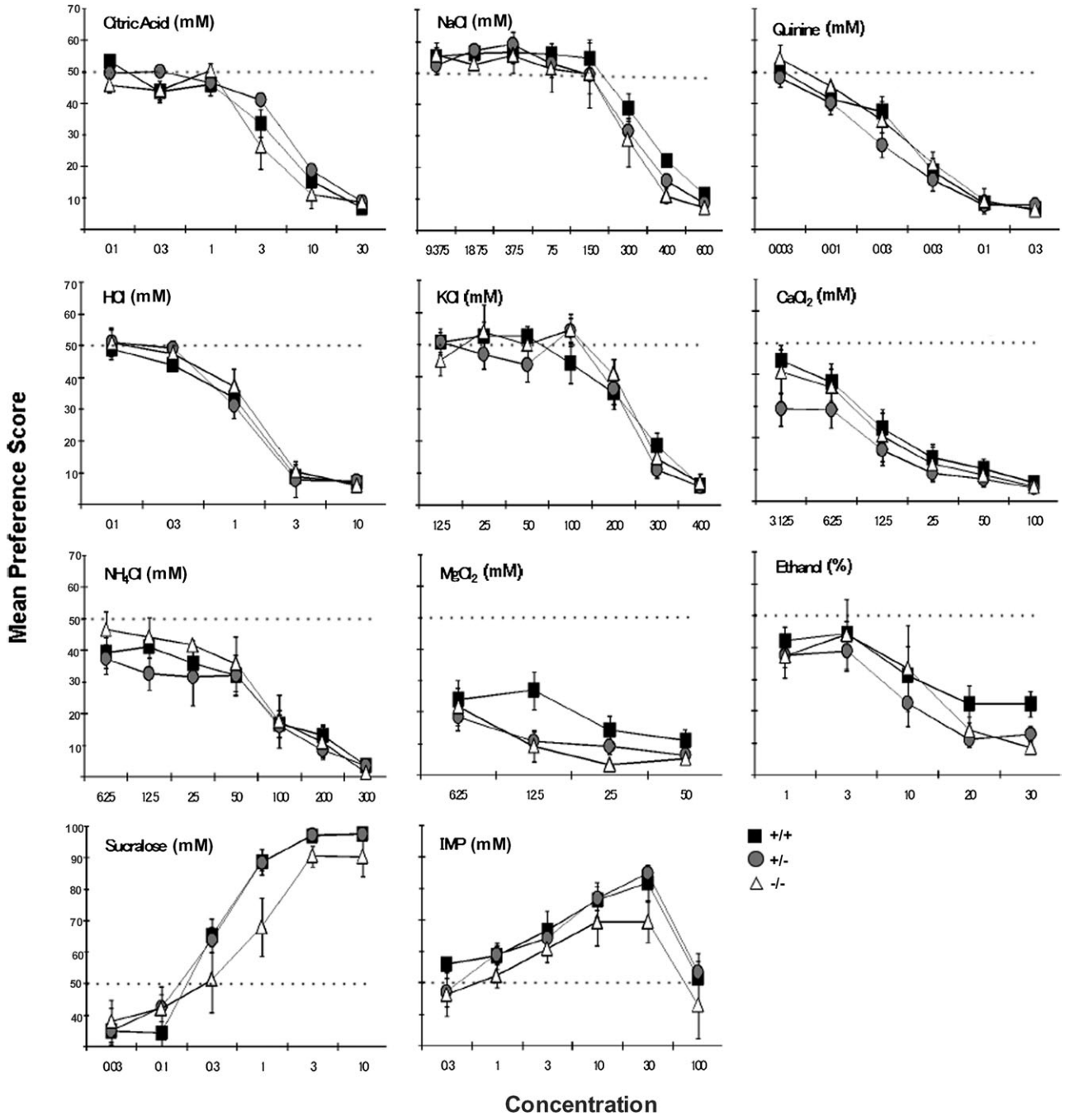

Figure 3 Similar taste preferences of naive *Pkd1l3* −/−, +/−, and +/+ mice for different tastants in 2-bottle preference tests (mean ± standard error). A preference ratio of 50 (dotted line) indicates no preference or avoidance of the solution in comparison with water. The first 9 panels represent compounds typically avoided in a concentration-dependent manner. Sucralose and IMP (bottom row) were preferred compounds.

2 exceptions, there were no differences between mice with different *Pkd1l3* genotypes. These exceptions were differences in 2-bottle preference tests of naive mice for responses to citric acid (significant effect of interaction between genotype and concentration) and $MgCl_2$ (significant effect of genotype). In both cases, *Pkd1l3* –/– mice were the most sensitive (showed the strongest aversion). These results are in contradiction with the hypothesis, based on evidence suggesting *Pkd1l3* may be part of an acid receptor channel in taste cells, which would suggest that *Pkd1l3* mutant mice should have a decreased response to acids.

There are 2 possible explanations for the genotype differences we did observe with citric acid and $MgCl_2$. First, and the most likely, is that these effects are false positives. Supporting this explanation is the fact that the effects were small. In both cases, the P values were >0.01, and neither was

significant in post hoc tests. Additionally, there is a lack of consistency between genotype difference in the citric acid preferences and the absence of genotype differences in the HCl preferences, citric acid taste thresholds, or in gustatory neural responses to a variety of acids.

A second and less likely possibility for these effects is that there could be some overcompensation in the *Pkd1l3* mutant mice making them more sensitive to citric acid and $MgCl_2$ in the 2-bottle preference tests with naive mice. For example, a recent hypothesis suggests that PKD1L3 is one of multiple channels and regulatory factors associated with acid taste, and individually each gene has only a limited impact on the overall response to acid (Huque et al. 2009). We could speculate that although PKD1L3 may be the main binding partner of PKD2L1 in vallate and foliate papillae (but not in fungiform and palate taste cells), a second binding partner for PKD2L1 may also be present at least in some of these taste cells. This second partner may be upregulated in the absence of PDK1L3, allowing these cells to either retain or overcompensate normal function. However, it is unclear how this effect would result in overcompensation only for taste responses to citric acid and $MgCl_2$ but not other taste stimuli. Should a second binding partner for PKD2L1 be identified it would be interesting to search for upregulation in *Pkd1l3* –/– mice.

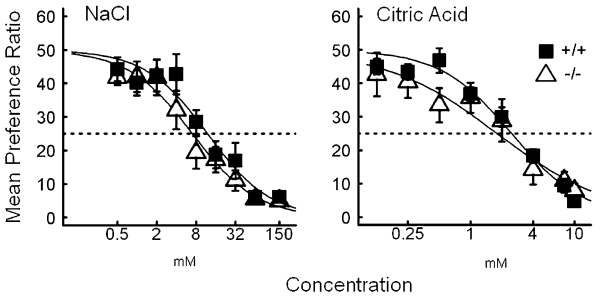

Figure 4 Similar taste thresholds of *Pkd1l3* –/– and +/+ mice. The figure shows preference ratios (mean ± standard error) in 48-h 2-bottle tests with NaCl (left) or citric acid (right) in mice conditioned by self-administration of LiCl or the LiCl + citric acid mixture, respectively. A curve for each genotype was produced using regression analysis of data from all mice of each genotype as a group using the function described in Materials and methods. The horizontal line at 25% indicates thresholds at the intersection of the regression curve with this line. Mean NaCl thresholds were 9.5 and 14.4 mM, and mean citric acid thresholds were 2.2 and 2.5 mM for *Pkd1l3* –/– and *Pkd1l3* +/+ mice, respectively (ns).

Is it possible that PKD1L3 is involved in the taste function, but our tests were not sensitive enough to detect changes in taste responsiveness of *Pkd1l3* mutant mice? In our study, we used standard assays (preference tests and recording of taste-evoked activity in gustatory nerves), which were sufficient to detect changes in taste responsiveness due to null or point mutations in genes such as *Tas1r3*, *Tas2r5*, gustducin, and *Trpm5* (Wong et al. 1996; Chandrashekar et al. 2000; Bachmanov et al. 2001; Damak et al. 2003; Zhang et al. 2003; Reed et al. 2004; Damak et al. 2006). This suggests that even if PKD1L3 is involved in taste, its contribution is less prominent compared with these other taste-related genes.

Furthermore, we have designed our taste tests so that we would be able to detect deficiency in the peripheral taste input in *Pkd1l3* mutants despite lack of expression of *Pkd1l3* in fungiform and palate papillae (see details in Introduction). We have measured taste thresholds, which are sensitive enough to detect only partial reduction in the gustatory input. We expected that if in *Pkd1l3* mutants gustatory input is eliminated only in *Pkd1l3*-expressing vallate and foliate taste papillae, but not in fungiform and palate papillae that lack *Pkd1l3* expression, we still would detect changes in taste thresholds. We have also measured taste-evoked activity in the glossopharyngeal nerve that innervates *Pkd1l3*-expressing vallate and foliate papillae and should have been affected if PKDL3 is involved in taste.

PKD1L3 was proposed to be involved in acid taste detection. However, acids can stimulate not only gustatory but

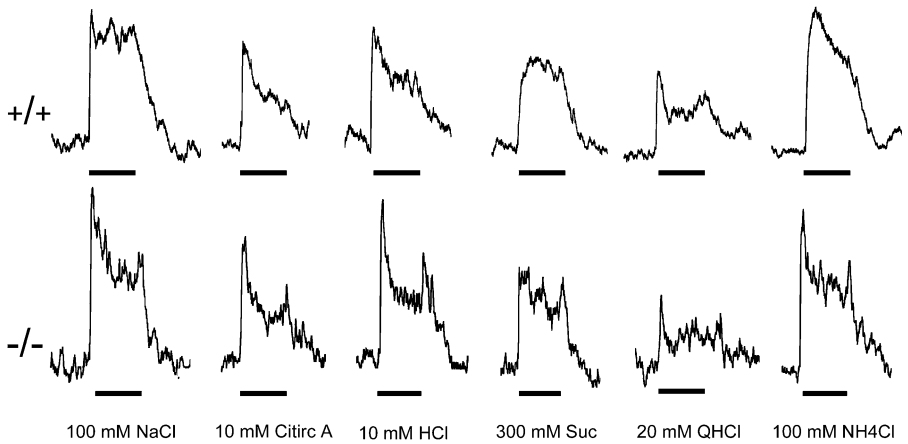

Figure 5 Sample recordings of integrated activity in the whole chorda tympani nerve show similar responses for *Pkd1l3* –/– and +/+ mice to oral application of NaCl, citric (Citric A) and hydrochloric (HCl) acids, sucrose (Suc), quinine hydrochloride (QHCl), and a reference stimulus, NH_4Cl. Horizontal bars under nerve recordings show 30-s periods of taste stimulus application to the tongue.

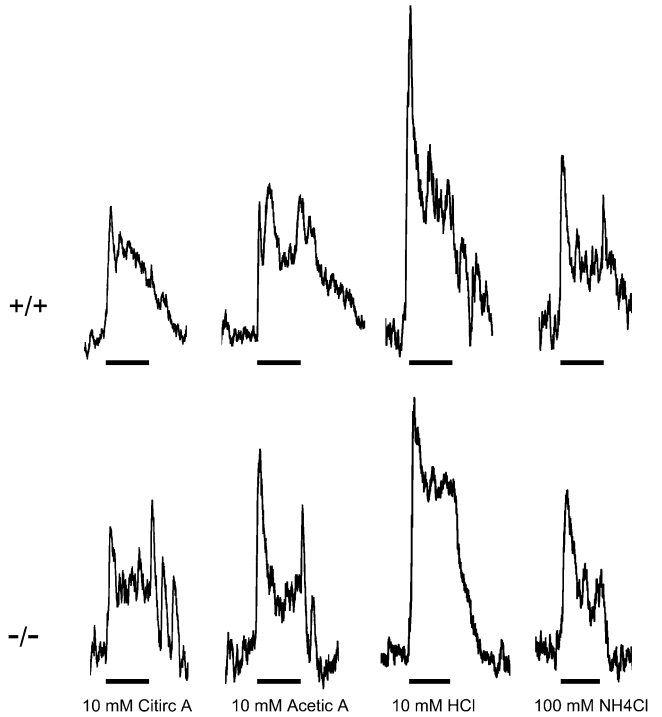

Figure 6 Sample recordings of integrated activity in the whole glossopharyngeal nerve show similar responses for *Pkd1l3* −/− and +/+ mice to oral application of citric (Citric A), acetic (Acetic A), and hydrochloric (HCl) acids, and a reference stimulus, NH_4Cl. Horizontal bars under nerve recordings show 30-s periods of taste stimulus application to the tongue.

Table 2 Chorda tympani responses (relative to 100 mM NH_4Cl) to taste stimuli in *Pkd1l3* −/− and *Pkd1l3* +/+ mice

Taste solution	*Pkd1l3* −/−	*Pkd1l3* +/+
	M (SE)	M (SE)
HCl 1 mM (pH = 3.05)	0.18 (0.02)	0.12 (0.03)
HCl 10 mM (pH = 2.11)	0.67 (0.12)	0.65 (0.05)
Citric acid 1 mM (pH = 3.25)	0.13 (0.07)	0.12 (0.05)
Citric acid 10 mM (pH = 2.63)	0.54 (0.11)	0.56 (0.07)
Citric acid 100 mM (pH = 2.08)	1.99 (0.46)	1.86 (0.15)
Acetic acid 1 mM (pH = 3.96)	0.06 (0.06)	0.03 (0.02)
Acetic acid 10 mM (pH = 3.47)	0.26 (0.07)	0.26 (0.07)
Acetic acid 100 mM (pH = 2.90)	0.98 (0.07)	1.07 (0.10)
NaCl 1 mM	0.01 (0.01)	
NaCl 10 mM	0.26 (0.07)	0.18 (0.04)
NaCl 100 mM	0.91 (0.15)	1.06 (0.05)
NaCl 300 mM	2.31 (0.39)	2.19 (0.17)
NaCl 1000 mM	3.03 (0.29)	
KCl 1 mM	0.08 (0.08)	
KCl 10 mM	0.10 (0.09)	
KCl 100 mM	0.65 (0.20)	0.65 (0.03)
KCl 1000 mM	1.94 (0.22)	
$CaCl_2$ 100 mM	0.83 (0.03)	0.98 (0.14)
Quinine HCl 20 mM	0.60 (0.15)	0.67 (0.05)
Strychnine 10 mM	0.42 (0.03)	
Sucrose 100 mM	0.19 (0.06)	0.33 (0.05)
Sucrose 300 mM	0.69 (0.10)	0.74 (0.06)
Sucrose 1000 mM	1.75 (0.37)	1.96 (0.15)
Sucralose 10 mM	0.54 (0.02)	
Glycine 1000 mM	1.14 (0.19)	0.84 (0.08)
MSG 100 mM	0.79 (0.02)	0.68 (0.06)
MSG 1000 mM	2.20 (0.08)	2.05 (0.11)
IMP 10 mM	0.31 (0.08)	0.39 (0.07)

Values are means (SE, standard error). There were no significant differences between *Pkd1l3* −/− and *Pkd1l3* +/+ mice for any of the taste stimuli tested in both genotypes ($P > 0.17$, *t*-tests). For compounds tested at multiple concentrations, effects of *Pkd1l3* genotype were also not significant ($F_{1,6-19} < 0.5$, $P > 0.4$, 2-way ANOVA). Effects of concentration were significant for all stimuli tested at multiple concentrations ($F_{1-3,7-24} > 47.8$, $P < 0.0002$, one- and 2-way ANOVAs).

also somatosensory chemoreceptors, and this could also have limited our ability to detect changes in acid taste responses in *Pkd1l3* mutant mice. For example, acid avoidance by *Pkd1l3* mutants in behavioral tests could have been due to aversive sensations evoked by stimulation of the trigeminal nerve (e.g., Finger et al. 2005; Hallock et al. 2009). Or, acid-evoked activity in somatosensory fibers of the glossopharyngeal nerve could have masked deficiency in acid-evoked activity in gustatory fibers of this nerve in *Pkd1l3* mutants. However, this seems unlikely because gustatory and somatosensory responses to acids could be distinguished based on acid concentrations. Typically, responses to dilute acid solutions are predominantly gustatory, and a somatosensory component requires higher acid concentrations (Kawamura et al. 1968; Silver and Finger 1991; Sostman and Simon 1991; Gilmore and Green 1993; Bryant and Moore 1995; Cerf-Ducastel et al. 2001). Consistent with this, Finger et al. (2005) have shown that elimination of gustatory input in P2X2/P2X3 double-knockout mice made their glossopharyngeal nerve completely unresponsive to 20 mM acid, although it still responded to other somatosensory stimuli. This indicates that responses of the glossopharyngeal nerve to acids with concentrations up to 20 mM are predominantly gustatory rather than somatosensory. We have observed behavioral and neural responses in *Pkd1l3* mutant mice to concentrations of acids as low as 1 mM (see Tables 2 and 3, Figures 3 and 4), which are most likely mediated by gustatory rather than somatosensory mechanisms. The fact that *Pkd1l3* mutants respond to such weak acid solutions and that these responses are similar to those of the wild-type mice

Table 3 Glossopharyngeal nerve responses (relative to 100 mM NH_4Cl) to taste stimuli in *Pkd1l3* −/− and *Pkd1l3* +/+ mice

Taste solution	*Pkd1l3* −/−	*Pkd1l3* +/+
	M (SE)	M (SE)
HCl 1 mM (pH = 3.05)	0.34 (0.09)	
HCl 10 mM (pH = 2.11)	1.66 (0.40)	1.26 (0.19)
HCl 100 mM (pH = 1.19)	3.54 (0.11)	
Citric acid 10 mM (pH = 2.63)	0.99 (0.10)	0.89 (0.10)
Citric acid 100 mM (pH = 2.08)	2.52 (0.49)	
Acetic acid 1 mM (pH = 3.96)	0.39 (0.04)	
Acetic acid 10 mM (pH = 3.47)	0.85 (0.18)	1.02 (0.13)
Acetic acid 100 mM (pH = 2.90)	2.41 (1.10)	
NaCl 100 mM	1.10 (0.21)	0.84 (0.06)
NaCl 1000 mM	3.26 (0.31)	
KCl 100 mM	0.56 (0.16)	
$CaCl_2$ 100 mM	1.75 (0.03)	2.01 (0.31)
$CaCl_2$ 1000 mM	2.52 (0.48)	
Quinine HCl 20 mM	1.84 (0.34)	1.45 (0.24)
Sucrose 300 mM	0.29 (0.06)	0.38 (0.05)
Sucrose 1000 mM	0.67 (0.09)	0.70 (0.12)
MSG 100 mM	1.05 (0.27)	0.70 (0.16)

Values are means (SE, standard error). There were no significant differences between *Pkd1l3* −/− and *Pkd1l3* +/+ mice for any of the taste stimuli tested in both genotypes ($P > 0.30$, *t*-tests). For sucrose (tested at 2 concentrations), the effect of *Pkd1l3* genotype was also not significant ($F_{1,10} = 0.5$, $P = 0.5$, 2-way ANOVA). Effects of concentration were significant for all stimuli tested at multiple concentrations ($F_{1-2,4-12} > 5.6$, $P < 0.04$, one- and 2-way ANOVAs), with the exception of $CaCl_2$ ($F_{1,2} = 8.2$, $P = 0.1$, one-way ANOVA).

indicates that gustatory acid responses are not altered by the *Pkd1l3* mutation.

Previous work in vitro has shown involvement of the PKD1L3 + PKD2L1 dimer in generation of off-responses to strong acids (5–30 mM) (Ishimaru et al. 2006; Inada et al. 2008). Our results have shown that *Pkd1l3* mutant mice have unaltered taste responses to a range of concentrations of both weak and strong acids and have unaltered neural on-responses. Together these data suggest that PKD1L3-dependent off-responses to acids are not important for neural and behavioral responses to acids, and that other, probably PKD1L3-independent on-responses are involved in the acid taste responsiveness in vivo.

Although we cannot entirely rule out the possibility of a truncated PKD1L3 protein being translated, our *Pkd1l3* –/– mice lack several structural components (ion channel pore and several TM domains) necessary for PKD channel function. Therefore, the most likely explanation for the normal taste function in our *Pkd1l3* mutant mice is that the function of PKD1L3 is independent of taste transduction. It has been suggested that PKD2L1 may be involved in pH detection in the spinal cord and brain (Huang et al. 2006). It is possible that PKD1L3 may have similar function in these or other tissues.

In summary, we found no deficiency in the taste responses of *Pkd1l3* –/– mice to acids or any other taste stimuli. Therefore, our results do not support a role for PKD1L3 in sensing of acids or other taste qualities. Further studies are needed to elucidate the function of PKD1L3 in taste bud cells.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

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