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Acetic acid activates PKD1L3–PKD2L1 channel—A candidate sour taste receptor

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ABSTRACT

The polycystic kidney disease (PKD) 1L3–PKD2L1 channel is a candidate sour taste receptor expressed in mammalian taste receptor cells. Various acids are reported to activate PKD channels after the removal of the acid stimuli, but little information is available on the activation of these channels by acetic acid. It was difficult to analyze the PKD channel activation by acetic acid using Ca^{2+} imaging experiments because this acid induces a transient and nonspecific response in cultured cells. Here, we developed a novel method to evaluate PKD channel activation by acetic acid. Nonspecific responses were observed only over a short period after the application of acetic acid. In contrast, PKD channel activation evoked by acetic acid as well as citric acid was detected even at a later time point. This method revealed that PKD1L3–PKD2L1 channel activation by acetic acid was pH-dependent and occurred when the ambient pH was <3.1.

Introduction

Many organisms can detect sour compounds and respond by exhibiting aversive behavior [1]. Sour taste is the key element in the flavor profile of food acidulants [2]. Acetic acid is one of the most familiar acids in foods. The seasoning agent vinegar contains a high concentration of acetic acid (approximately 500–1000 mM), and many fruits also contain acetic acid whose sourness contributes considerably to their taste quality. Additionally, orally administered acetic acid is reported to exert a variety of effects with regard to preventing hypertension [3], enhancing glycogen repletion after fasting [4], evoking a hypoglycemic effect [5], and so on [6,7].

Polycystic kidney disease (PKD) 1L3 and PKD2L1 channels, related members of the transient receptor potential ion channel family, were recently reported as candidate sour taste receptors in mammals [8,9]. These channels were expressed in a subset of taste receptor cells (TRCs) that were distinct from sweet, bitter, and umami (taste of *L*-amino acids)-sensing TRCs in the taste tissue, and were also found to be coexpressed in the same TRCs in the circumvallate and foliate papillae. In addition, PKD2L1 proteins accumulate in the taste pore region where taste compounds are detected [8]. HEK293T cells that heterologously coexpressed PKD1L3 and PKD2L1 responded to various acids, including 2 strong and 5 weak acids, after the removal of the acid stimuli; this response was termed as an off-response and these channels functioned as cation channels with a relatively high permeability to Ca^{2+} [8,10]. It has also been shown that transgenic mice lacking PKD2L1-expressing cells, generated by the expression of the attenuated diphtheria toxin, exhibited no gustatory nerve response to acidic stimuli in the chorda tympani nerves, indicating that PKD2L1-expressing cells function as sour taste sensors [9].

To date, 7 acid solutions, namely, inorganic acids (hydrochloric, sulfuric, and phosphoric acid), dicarbonic acids (malic, succinic, and tartaric acid), and tricarbonic acid (citric acid), have been reported to activate the PKD1L3–PKD2L1 channel [8,10]. Other acid solutions, including monocarbonic acids, may also be able to activate PKD1L3–PKD2L1 channels. However, methodological problems such as the occurrence of nonspecific responses make it difficult to examine the activation of PKD channels by acetic acid [10].

The present study aims to develop a method for evaluating the activation of heterologously expressed PKD1L3–PKD2L1 channels by acetic acid. Our results strongly suggest that these channels are activated by acetic acid in a pH-dependent manner, as in the case of other acids such as citric acid.

Materials and methods

Sample solutions. The Ca^{2+}-containing buffer was composed of 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 130 mM NaCl, 10 mM glucose, 5 mM KCl, 2 mM $CaCl_2$, and 1.2 mM $MgCl_2$ (pH adjusted to 7.4 by using NaOH). The acids were diluted with the assay buffer to produce 1, 2, 3, and 4 mM solutions of citric acid (corresponding to pH 3.4, 3.1, 2.9, and 2.8, respectively) and 10, 40, 50, 80, and 100 mM solutions of acetic acid

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(corresponding to pH 3.5, 3.1, 3.0, 2.9, and 2.8, respectively). Another assay buffer that did not contain Ca^{2+} was prepared by using 0.2 mM ethylene glycol tetraacetic acid (EGTA) instead of $CaCl_2$.

Acid solutions that contained the same concentration of acetic acid but with different pH values were prepared by varying the concentration of HEPES. Solutions containing 100 mM acetic acid were diluted with assay buffer containing 1, 2, 5, 10, and 20 mM HEPES (pH 7.4) to prepare solutions with final pH values of 2.8, 2.9, 3.1, 3.4, and 3.6, respectively.

Cell culture and transfection. The expression vectors for mouse PKD1L3 and PKD2L1 channels were generated by subcloning the coding sequences of the channels into pDisplay (Invitrogen, Carlsbad, CA) and pCI (Promega, Madison, WI) vectors, respectively, as described previously [8]. HEK293T cells were cultured at 37 °C in Dulbecco's modified Eagle's medium (Sigma–Aldrich Japan, Tokyo, Japan) supplemented with 10% fetal bovine serum (Invitrogen). Cells were seeded onto glass-base dishes (35 mm in diameter), and were transiently transfected with the expression vectors for PKD1L3, PKD2L1, and red fluorescent protein (DsRed2) (pDsRed2-N1; Takara Bio Inc., Shiga, Japan) at a ratio of 1:1:0.04, using the Lipofectamine 2000 reagent (Invitrogen). Cells expressing PKD2L1 alone were prepared by transfection with the expression vectors for PKD2L1 and DsRed2 at a ratio of 1:0.04.

Ca^{2+} imaging. Transfected cells were washed 30–38 h after transfection, and nontransfected cells were washed 53–57 h after seeding onto glass-base dishes. The cells loaded with 5 μM of fura-2/AM (Invitrogen) for 30 min at room temperature. The cells were then rinsed and incubated in the assay buffer for at least 10 min. The assay buffer, in which the cells were rinsed, incubated, and subsequently perfused, contained HEPES at concentrations of 1, 2, 5, 10, or 20 mM corresponding to concentration of the acid solution applied.

A perfusion device was used to apply the acid solutions and wash the fura-2-loaded cells. The cells were exposed to a constant flow (approximately 10 mL/min) of the assay buffer, and the acid solutions were applied for approximately 6 s. The cells were washed with the assay buffer immediately after the application of an acid solution, and the intensity of the fura-2 fluorescence was monitored for at least 200 s.

The fura-2 fluorescence intensity at the excitation wavelengths 340 and 380 nm was measured at 510 nm by using a computer-controlled filter exchanger (Lambda 10-2; Sutter, San Rafael, CA), a MicroMax cooled charge-coupled device (CCD) camera (Princeton

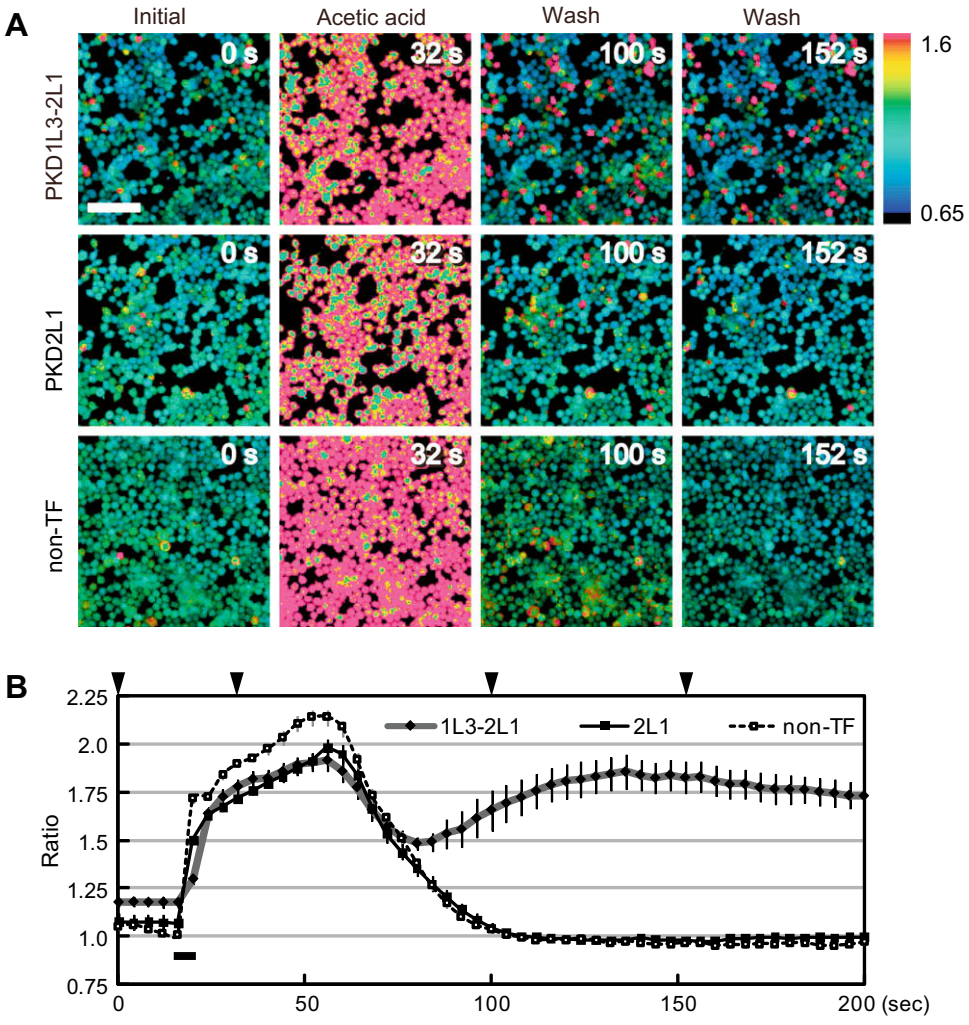

Fig. 1. Nonspecific response of HEK293T cells to the application of acetic acid. The fluorescence of the fura-2-loaded HEK293T cells, which were transfected with either both PKD1L3 and PKD2L1 or with PKD2L1 alone or were nontransfected cells, was recorded during the application of acetic acid and the subsequent washing with the assay buffer. (A) Representative ratiometric images of the fura-2-loaded cells at the time points indicated in B. The color scale indicates the F340/F380 ratio. Scale bar, 100 μm. (B) Sequential measurement of the F340/F380 ratiometric values. The thick, solid and dashed lines indicate the mean of the ratiometric values of 10 cells transfected with either both PKD1L3 and PKD2L1 (1L3–2L1) or with PKD2L1 alone (2L1) or of 10 nontransfected cells, respectively. Note that 10 cells were selected from the PKD1L3–PKD2L1 transfected cells that showed high values at 152 s. The black bar indicates the time point of the application of the 100 mM acetic acid (pH 2.8) stimulus. Arrowheads indicate the time point at which the images shown in A were recorded.