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ACTIVATION OF A CATION CONDUCTANCE BY ACETIC ACID IN TASTE CELLS ISOLATED FROM THE BULLFROG

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Summary

The ionic mechanism of the conductance activated by acetic acid was analyzed in isolated bullfrog taste cells under whole-cell voltage-clamp. Bath-application of acetic acid (pH 3.9–4.7) induced an inward current in about 80 % of the taste cells. The current occurred in external 80 mmol l^{-1} Ba^{2+} and internal 100 mmol l^{-1} Cs^{+}, which completely blocked the delayed outward K^{+} current. The concentration–response relationship for the acid-activated current was consistent with that of the gustatory neural response. Prolonged adaptation of the surface of the tongue to HCl prior to taste cell isolation decreased the acid-induced current to about 20 % of the control value without decreasing NaCl-induced neural responses and voltage-activated Na^{+} currents. The results suggest that the transduction mechanism of the acid response might be different from that of the response to salt. The *I–V* relationship of the acid-induced response was nearly linear at membrane potentials between −80 and 80 mV. The acid-induced conductance was permeable to alkali metal and alkali earth metal ions. The permeability ratios were P_{Ca}:P_{Ba}:P_{Sr}:P_{Na}:P_{Cs}=1.87:1.17:0.73:0.99:1.00. The present study suggests that the acid-induced receptor current in bullfrog taste cells is generated by an increase in a cation conductance in the apical taste membrane.

Introduction

Taste cells in vertebrates transmit external gustatory information to primary neurones through synaptic transmission elicited by depolarization or by other intracellular signals elicited by taste stimuli (Kinnamon and Getchell, 1991).

We reported that cation channels in bullfrog taste cells could be activated by acid stimuli (Miyamoto *et al.* 1988*a*). The ionic selectivity of the acid-induced intracellular receptor potential was consistent with that of the acid-induced neural response (Okada *et al.* 1987). Acid stimuli could also induce a transepithelial voltage response accompanying a conductance increase in the dorsal lingual epithelium of the bullfrog (Soeda and Sakudo, 1985). Kinnamon and Roper (1988*a*,*b*) claimed that acid stimuli might directly block the resting K^{+} conductances localized in the apical membrane of mudpuppy taste cells. The block would result in the depolarization of the cells. Similar

Key words: frog, taste cell, acid-induced current, whole-cell patch-clamp, *Rana catesbeiana.*

results were obtained in taste cells of the larval tiger salamander (Sugimoto and Teeter, 1991). Recently, in mammals, it has been suggested that protons could pass through amiloride-sensitive Na^+ channels (Gilbertson *et al.* 1992, 1993).

For other taste stimuli, a variety of transduction mechanisms have been proposed. Depolarization in response to salty stimuli may be induced by amiloride-sensitive and amiloride-insensitive passive cation transport in the apical and basolateral membranes of taste cells (DeSimone *et al.* 1984; Avenet and Lindemann, 1988; Miyamoto *et al.* 1989) and by passive paracellular transport (Ye *et al.* 1991). Bitter stimuli may elicit a direct block of the apical resting K^+ conductance (Kinnamon and Roper, 1988*a*,*b*), Cl^- secretion through the apical membrane (Okada *et al.* 1988) and an increase in intracellular Ca^{2+} concentration (Akabas *et al.* 1988). The depolarization induced by sweet stimuli may be attributed to Na^+ entry through apical amiloride-sensitive Na^+ channels (Mierson *et al.* 1988), to the block of basolateral resting K^+ conductance mediated through cyclic-nucleotide-dependent phosphorylation (Tonosaki and Funakoshi, 1988; Béhé *et al.* 1990) and to proton entry through the apical membrane (Okada *et al.* 1992*b*). The water response may be due to 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS)-sensitive Cl^- secretion through the apical membrane and the block of basolateral K^+ conductance (Okada *et al.* 1993).

In the present experiments, we show that acid stimuli activate a cationic conductance in isolated bullfrog taste cells. Frogs may be able to discriminate between sour and other stimuli using this increased cationic conductance.

A preliminary report of some of these data has been presented (Okada *et al.* 1992*a*).

Materials and methods

Preparation

Thirty-seven bullfrogs (*Rana catesbeiana*) weighing 245–570 g were used for the experiments over the course of a year. Isolated taste cells were obtained from the tongue surface of decapitated and pithed animals as described before (Miyamoto *et al.* 1988*b*). Briefly, the fungiform papillae were dissected out from the tongue in Ca^{2+}-free saline (in $mmol\,l^{-1}$: NaCl, 115; KCl, 2.5; sodium Hepes, 5; pH 7.2). The papillae were bathed in Ca^{2+}-free saline containing $2\,mmol\,l^{-1}$ EDTA for 10 min and were incubated in the same saline containing L-cysteine ($10\,mmol\,l^{-1}$) and papain ($10\,i.u.\,ml^{-1}$, Sigma, St Louis, USA) for 10 min. The papillae were then rinsed with normal saline (see below), and individual cells were dissociated by gentle trituration in normal saline. Isolated taste cells were readily distinguished from other types of cell (Miyamoto *et al.* 1988*b*). For the whole-nerve response experiments, the animals were anaesthetized with an intraperitoneal injection of 50 % urethane saline solution at $3\,g\,kg^{-1}$ body mass. To prevent spontaneous contraction of the tongue, the hypoglossal nerve and the hyoglossal muscle were cut bilaterally. The tongue was fully pulled from the mouth and its base was fixed with steel pins onto a cork plate in an experimental chamber.

Recording

Voltage-clamp recording was performed using the whole-cell mode of the patch-clamp

technique (Hamill *et al.* 1981). Patch pipettes were pulled from thick-walled glass capillaries containing a fine filament (outer diameter, 1.4 mm; Summit Medical, Tokyo, Japan) on a two-stage puller (Narishige PD-5, Tokyo, Japan). The tips of the electrodes were heat-polished with a microforge (Narishige MF-83). The resistance of the resulting patch electrodes was 3–6 MΩ when filled with internal solution. Recordings were made from taste cells settled on the bottom of a chamber placed on the stage of an inverted microscope (Olympus IMT-2, Tokyo, Japan). The recording pipette was positioned with a water-driven micromanipulator (Narishige WR-88). Initial sealing of the pipette on the cell surface was facilitated by applying weak suction. After adjusting the holding voltage, the patch membrane was broken by applying strong suction, resulting in a sudden increase in capacitance. In voltage-clamp mode, whole-cell current was measured with a whole-cell clamp amplifier (ACT ME Laboratory TM-1000, Tokyo, Japan). The current signal was low-pass filtered at 5 kHz, digitized at 100 kHz, acquired at a sampling rate of 0.5–20 kHz and stored on a floppy disc through a personal computer (NEC PC-9801 UV, Tokyo, Japan) running DAAD-12 software (ACT ME Laboratory), which was also used to control the D/A converter for generation of clamp protocols. The indifferent electrode contained a 3% agar solution. Although the electrode could cancel the liquid junction potential of the external solution with respect to the patch pipette, there was another liquid junction potential when the external solution was changed (Ohmori, 1985). The measured reversal potentials for acid-induced currents were corrected for these junction potentials. The series resistance value after 80% compensation was 3–10 MΩ. Input resistance was calculated from the current generated by a 20 mV hyperpolarizing voltage step from the holding potential.

The glossopharyngeal nerves from both sides were dissected from the surrounding connective tissues and cut near the hyoid bone. The nerves were placed over bipolar silver wires for recording impulses and immersed in liquid paraffin. The gustatory neural impulses were amplified with an a.c. amplifier, integrated with a time constant of 0.3 s and recorded with a pen recorder. Amplitudes 5 s after the onset of stimulation were used as measurements of acid- and NaCl-induced responses.

Solutions and stimulus applications

Normal saline solution consisted of (in mmol l^{-1}): NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Tris–Hepes, 10; glucose, 20 (pH 7.2). The external solution could be exchanged in 1–3 min. When the whole-cell configuration was obtained in normal saline, the external solution was replaced with 80 mmol l^{-1} $BaCl_2$, 80 mmol l^{-1} $CaCl_2$, 80 mmol l^{-1} $SrCl_2$, 120 mmol l^{-1} NaCl, 120 mmol l^{-1} choline chloride, 120 mmol l^{-1} tetraethylammonium chloride (TEACl) or 120 mmol l^{-1} sodium acetate, to obtain the control value for the *I–V* relationship, and was thereafter exchanged with solutions containing 0.03–0.5 mmol l^{-1} acetic acid (pH 3.9–4.7) to obtain the test value of the *I–V* relationship. The control solutions without acetic acid did not contain Hepes buffer and the pH values, other than with 120 mmol l^{-1} sodium acetate (pH 7.5), were between 5.5 and 6.5. The pipette solution (K^+ internal) contained (in mmol l^{-1}): KCl, 100; $MgCl_2$, 2; EGTA, 5; Tris–Hepes, 10 (pH 7.2). In some experiments, KCl was replaced with CsCl (Cs^+ internal). In the experiment on the desensitization of the acid-induced response, 1 mmol l^{-1} HCl (pH 3.0) dissolved in 100 mmol l^{-1} NaCl was perfused onto the tongue

surface at a rate of 0.1 ml s^{-1} for 60 min. The neural responses to 0.5 mmol l^{-1} acetic acid (pH 3.9) in 10 mmol l^{-1} $BaCl_2$ and 500 mmol l^{-1} NaCl were recorded just before, and 30 and 60 min after, the onset of HCl perfusion. When measuring the neural response, the adapting and stimulating solutions were perfused onto the tongue surface at a rate of 0.5 ml s^{-1} through a 10 ml syringe. After the desensitization treatment, acid-induced current in the whole-cell configuration was also recorded as described above.

All experiments were carried out at room temperature (20–25 °C).

Results

Response induced by acetic acid

When the whole-cell configuration was obtained with a pipette filled with K^+ internal solution, bullfrog taste cells isolated in the presence of papain had resting potentials ranging from −22 to −75 mV (−47.1±3.3 mV, mean ± S.E.M., *N*=24). The mean value was lower than that of cells isolated in the presence of protease (Boehringer, Mannheim) (Miyamoto *et al.* 1988*b*). A low resting potential in taste cells has been reported in *Rana esculenta* and *Rana ridibunda* (Avenet and Lindemann, 1988). The wide range of the resting potential suggests that Na^+/K^+ selectivity in resting frog taste cells varies widely (Avenet and Lindemann, 1988). The input resistance ranged from 0.8 to 10 GΩ (3.5±0.4 GΩ, *N*=24). All taste cells displayed transient inward and delayed outward currents in response to depolarizing voltage steps from a holding potential of −80 mV. The inward and outward currents were identified as Na^+ and K^+ currents, respectively (Miyamoto *et al.* 1991). When the taste cells dialyzed with Cs^+ internal solution were exposed to 80 mmol l^{-1} Ba^{2+} solution (pH 5.8) without Tris–Hepes buffer (control solution), the cells displayed an almost linear *I–V* relationship at membrane potentials between −80 and 80 mV and the input resistance increased to 11.5±3.1 GΩ (*N*=4). All other control solutions (pH 5.5–6.5) failed to induce any response in the taste cells. Exchanging the control Ba^{2+} solution with one containing acetic acid induced a sustained inward current at a holding potential of −40 mV (Fig. 1A). The lag of the response was due to the exchange of solutions. When the pH of the control solution was adjusted to 7.2, the lag was prolonged a little. This suggests that the pH of adapting solutions (control solutions) can affect the acid-induced response. The magnitude of the current induced by 0.1 mmol l^{-1} acetic acid (pH 4.3) in 80 mmol l^{-1} Ba^{2+} was −50.5±8.3 pA (*N*=4) at −40 mV. Sensitivity to 0.1–0.3 mmol l^{-1} acetic acid (pH 4.0–4.5) was examined in 44 cells under voltage-clamp. Thirty-six cells displayed an acid-induced current (>20 pA). Both acid-sensitive and acid-insensitive cells displayed the voltage-gated Na^+ current. The magnitude of the acid-induced current increased as the concentration of acid was increased (Fig. 1A). Because of the irreversible effects of low pH, cellular responses to stimuli above 0.5 mmol l^{-1} were not measured.

To find out whether the acetic acid response could be induced by the acetate anion or by an acetate-induced intracellular acidification (Thomas, 1984), taste cells were exposed to 120 mmol l^{-1} sodium acetate (pH 7.5). Even when external normal saline was replaced with 120 mmol l^{-1} sodium acetate without Tris–Hepes, taste cells displayed no response, suggesting that acetate cannot stimulate the cells.

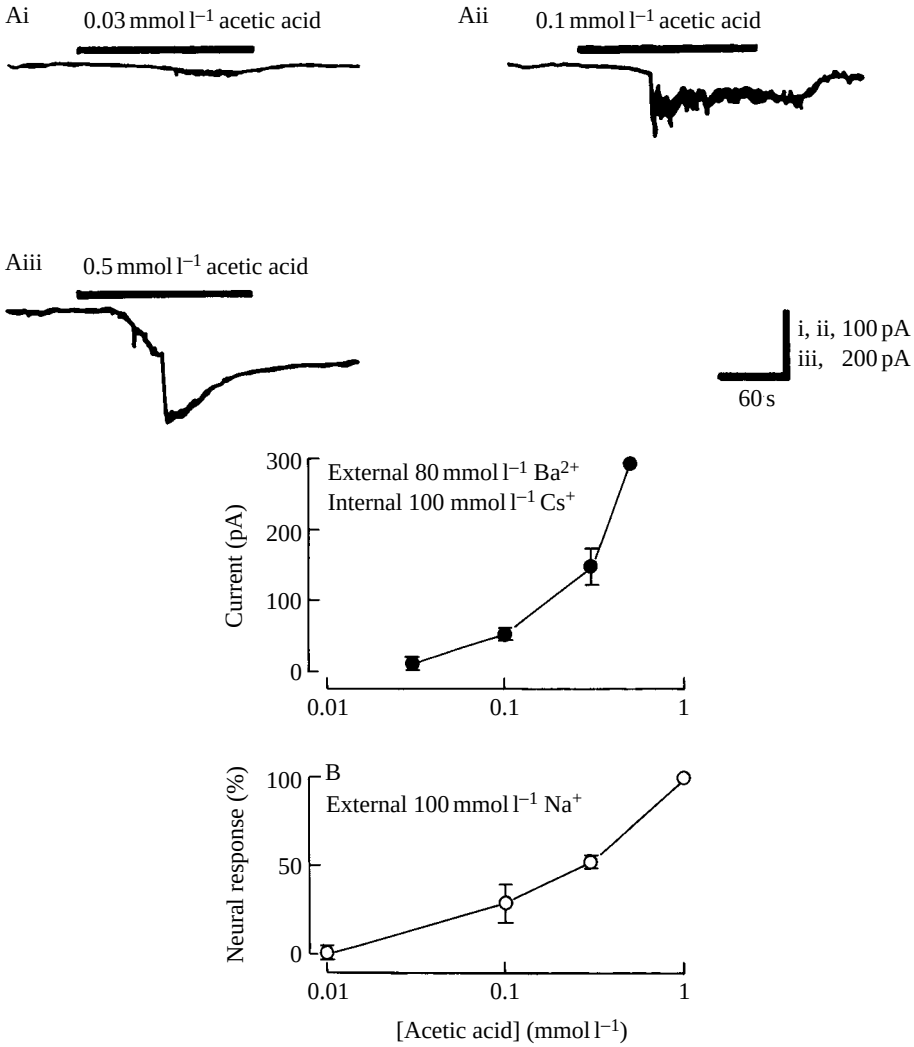

Fig. 1. Dose–response relationships for the acid-induced current (A) and the neural response (B). (A) The acid solutions (pH 3.9–4.7) contained 80 mmol l^{-1} Ba^{2+}. The recording pipette was filled with 100 mmol l^{-1} Cs^{+} internal solution. The membrane potential was held at −40 mV. The ordinate scale denotes the absolute value of the inward current. The actual current records are displayed above the graph. (B) The acid solutions (pH 3.8–5.1) contained 100 mmol l^{-1} Na^{+}. The magnitudes of the responses are expressed as the value relative to the response elicited by 1 mmol l^{-1} acetic acid. The points and error bars represent means ± S.E.M. of 2–4 cells or nerves.

Selective desensitization of the response to acid

Fig. 2 shows the glossopharyngeal nerve responses induced by 0.5 mmol l^{-1} acetic acid (pH 3.9) dissolved in 10 mmol l^{-1} $BaCl_2$ and by 500 mmol l^{-1} NaCl just before and 30 and 60 min after the onset of continuous adaptation of the tongue surface to 1 mmol l^{-1} HCl (pH 3.0) in 100 mmol l^{-1} NaCl. Addition of a solution of 10 mmol l^{-1} $BaCl_2$ usually

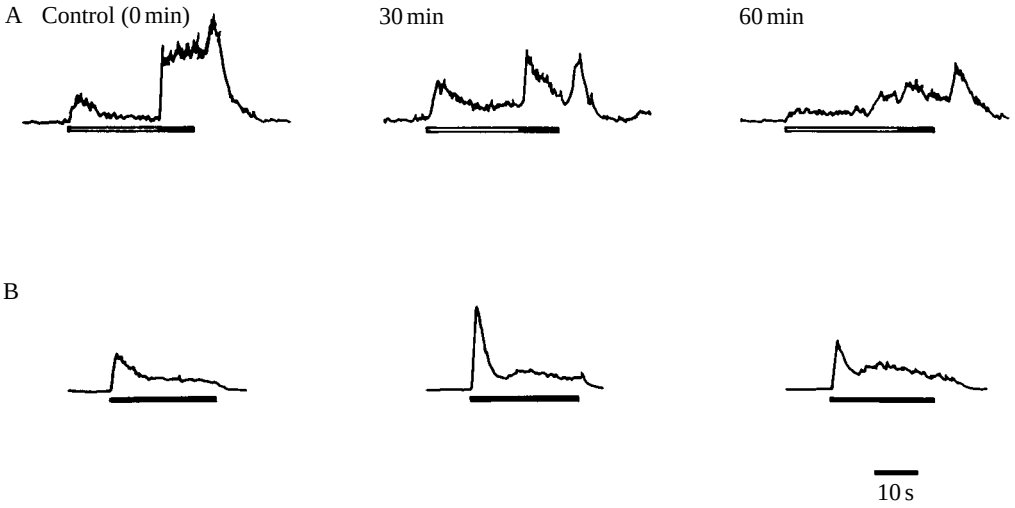

Fig. 2. Selective desensitization of the acid-induced neural response. The tongue surface was continuously adapted to 1 mmol l^{-1} HCl (pH 3.0) in 100 mmol l^{-1} NaCl except during the time of recording of the responses. (A) Responses elicited by 0.5 mmol l^{-1} acetic acid (pH 3.9) in 10 mmol l^{-1} $BaCl_2$ (filled bar) after 10 mmol l^{-1} $BaCl_2$ adaptation (open bar). (B) Responses elicited by 500 mmol l^{-1} NaCl (filled bar) after 10 mmol l^{-1} NaCl adaptation. The tongue surface was rinsed with 10 mmol l^{-1} NaCl after taste stimulation. Records A and B were obtained from the same preparation.

induced a small response. In contrast, 0.5 mmol l^{-1} acetic acid in 10 mmol l^{-1} $BaCl_2$ induced a large neural response (control). The acid response decreased to 40±6 % (*N*=3) of the control value 30 min after HCl adaptation and to 22±5 % (*N*=3) 60 min after adaptation. The prolonged adaptation might non-selectively damage the taste-receptive membrane. However, the response elicited by 500 mmol l^{-1} NaCl was not inhibited by HCl adaptation (107±10 % at 30 min, 95±8 % at 60 min, *N*=3). The difference in adaptation between acid- and NaCl-induced responses suggested that the two responses are mediated by different transduction mechanisms.

To elucidate the mechanism for HCl adaptation of the acetic acid response, bullfrog taste cells were isolated after 1 h of adaptation of the tongue surface to 3 mmol l^{-1} HCl (pH 2.6) in 100 mmol l^{-1} NaCl. HCl adaptation prior to cell isolation did not change the morphology of the taste cells, but it was expected that taste cells might lose their ability to respond to acid. At a holding potential of −40 mV, when external 80 mmol l^{-1} Ba^{2+} solution containing no Hepes buffer was replaced with 80 mmol l^{-1} Ba^{2+} containing 0.1 mmol l^{-1} acetic acid (pH 4.3), the HCl-treated cells did not display any response within 3 min. Thereafter, a small inward current accompanied by a small conductance increase developed (Fig. 3A). The current could not be discriminated from drift. A response profile like that shown in Fig. 1A was never observed after HCl adaptation. The magnitude of the slow response was about 20 % of the control value (Fig. 3Ci). The HCl treatment did not affect the voltage-activated Na^+ current (Fig. 3B,Cii).

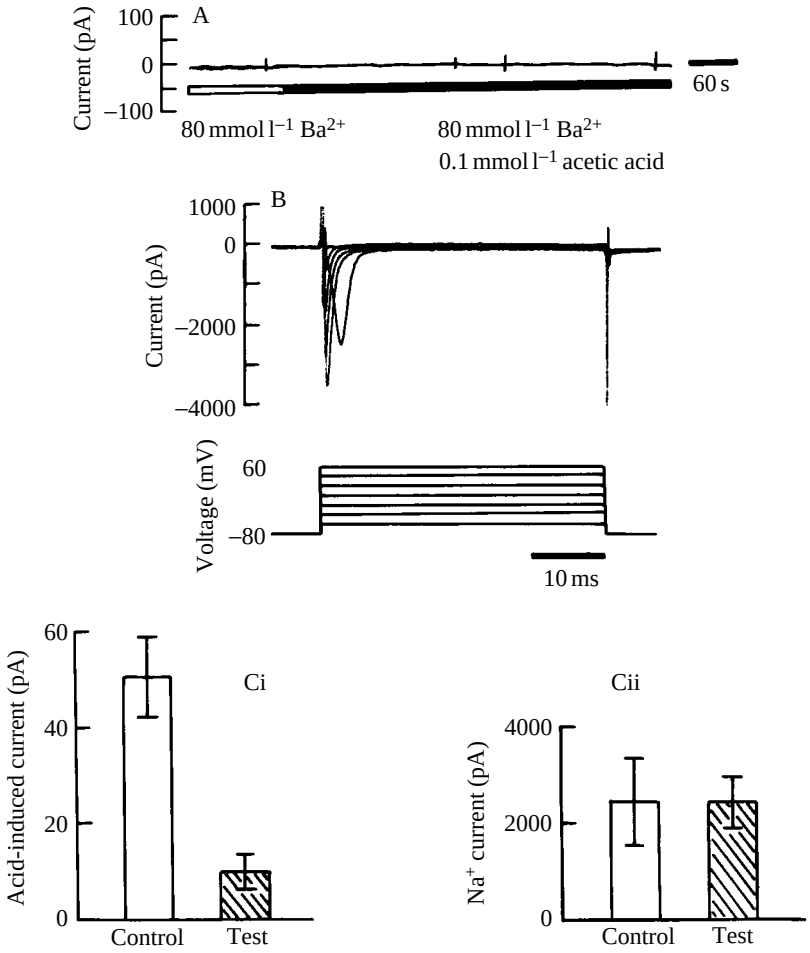

Fig. 3. Selective desensitization of the acid-induced current. The tongue surface was exposed to 3 mmol l^{-1} HCl (pH 2.6) in 100 mmol l^{-1} NaCl for 1 h prior to isolating taste cells. (A) Chart trace of the current at a holding potential of −40 mV. A transient current deflection was induced by the ramp voltage (ramp rate, 94 mV s^{-1}) between −80 and 80 mV. The recording pipette was filled with 100 mmol l^{-1} Cs^+ internal solution. (B) Voltage-activated Na^+ current after HCl treatment. The current was induced by a 30 ms depolarizing voltage step from −80 mV. (C) The mean values of acid-induced current and voltage-activated Na^+ current after HCl treatment. The ordinate scale denotes the absolute value of the inward current. The acid-induced current at a holding potential of −40 mV was obtained by subtracting the resting current level (in 80 mmol l^{-1} Ba^{2+}) from the acid-induced current level [in 0.1 mmol l^{-1} acetic acid (pH 4.3) and 80 mmol l^{-1} Ba^{2+}]. The voltage-activated Na^+ current was induced by a depolarizing voltage step from −80 to −20 mV. The columns and error bars represent means ± S.E.M. of four cells.

Effects of ions on the acid-induced current

To identify the ionic selectivity of the acid-induced current, *I–V* relationships were examined under various ionic conditions (Fig. 4). The cytoplasm was dialyzed with 100 mmol l^{-1} Cs^+ internal solution. The *I–V* relationships of the respnse induced by

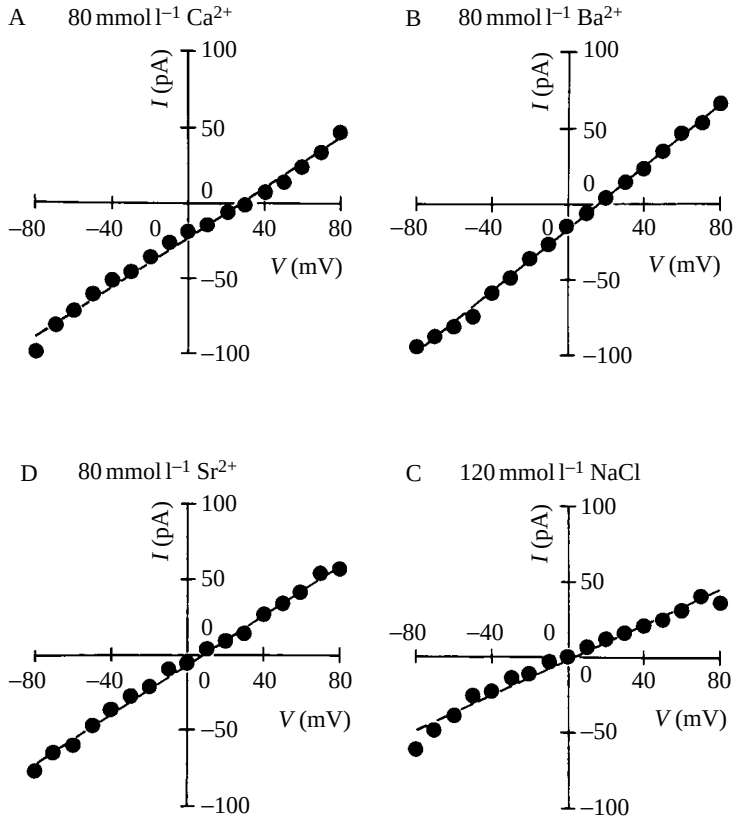

Fig. 4. *I–V* relationships of the current induced by 0.1 mmol l^{-1} acetic acid recorded under four different external ionic conditions: (A) 80 mmol l^{-1} Ca^{2+} (pH 4.5); (B) 80 mmol l^{-1} Ba^{2+} (pH 4.3); (C) 80 mmol l^{-1} Sr^{2+} (pH 4.3); and (D) 120 mmol l^{-1} Na^{+} (pH 4.3). The *I–V* curves were obtained by applying a voltage ramp (ramp rate, 94 mV s^{-1}) between −80 and 80 mV and were corrected for the resting current component. The recording pipette was filled with 100 mmol l^{-1} Cs^{+} internal solution.

0.1 mmol l^{-1} acetic acid were nearly linear at membrane potentials between −80 and 80 mV. The reversal potentials obtained under each of the conditions were 17.5±3.3 mV for Ca^{2+} (N=7), 10.2±2.2 mV for Ba^{2+} (N=7), 2.7±3.2 mV for Sr^{2+} (N=4) and 4.3±5.3 mV for Na^{+} (N=3).

Permeability ratios relative to Cs^{+} were calculated from the reversal potentials by using the Goldman–Hodgkin–Katz equation (Goldman, 1943; Hodgkin and Katz, 1949):

$$\sum P_X Z_X^2 \frac{E_{rev}F^2}{\boldsymbol{R}T} \frac{[X]_o - [X]_i \exp(Z_X F E_{rev}/\boldsymbol{R}T)}{1 - \exp(Z_X F E_{rev}/\boldsymbol{R}T)} = 0, \quad (1)$$

where F, $\boldsymbol{R}$ and T are physical constants, Z_X is the valence and P_X is the permeability coefficient of the ion X, $[X]_o$ and $[X]_i$ are extracellular and intracellular concentrations of X and E_{rev} denotes the reversal potential. The results for intracellular receptor potentials elicited by acid suggested that anion permeability does not contribute to the acid response

(Miyamoto *et al.* 1988*a*). The present experiments on ion exchange also indicate that anions may not pass through acid-activated channels. When the anion permeability is ignored, the relative permeabilities of the various cations through the acid-activated conductance were P_{Ca}:P_{Ba}:P_{Sr}:P_{Na}:P_{Cs}=1.87:1.17:0.73:0.99:1.00.

In external 120 mmol l^{-1} choline chloride and 120 mmol l^{-1} TEA^+, 0.1 mmol l^{-1} acetic acid (pH 4.3) did not elicit a response in taste cells (data not shown), but 0.3 mmol l^{-1} acetic acid (pH 4.0) in 120 mmol l^{-1} choline chloride elicited an inward current (Fig. 5A). The magnitude was smaller than that in Ba^{2+} solution. The mean reversal potential of the current in the presence of 0.3 mmol l^{-1} acetic acid in 120 mmol l^{-1} choline chloride was 15.8±3.6 mV (*N*=4) (Fig. 5B).

Discussion

The present study demonstrates that acetic acid induces an inward current in isolated taste cells from bullfrog. The inward current is mediated by an increased cationic conductance. The cationic conductance induced by acetic acid is probably located on the apical receptive membrane because the current was greatly decreased by treatment with mucosal HCl prior to isolating the taste cell. The acid-induced conductance in isolated bullfrog taste cells was permeable to alkali metal and alkali earth metal ions. The results were consistent with glossopharyngeal neural responses and intracellular receptor potentials elicited by acid stimuli (Okada *et al.* 1987; Miyamoto *et al.* 1988*a*). Similar ionic selectivity has been observed in other sensory transduction channels (Ohmori, 1985; Restrepo *et al.* 1992; Yau and Baylor, 1989). When external cations were replaced with choline, 0.1 mmol l^{-1} acetic acid (pH 4.3) did not induce any response in the taste cells, but 0.3 mmol l^{-1} acetic acid (pH 4.0) in the presence of choline elicited a small inward current. This result suggests the possibility of proton permeation through the amiloride-insensitive acid-induced channel. In the present experiments, the patch pipette for intracellular dialysis did not contain ATP and GTP and the acid-induced current was recorded 15–20 min after attainment of the whole-cell configuration. Since water-soluble intracellular transmitters might be washed out under these conditions, the acid stimuli could activate the cation channels directly. However, we could not dismiss the possibility of G-protein-mediated direct activation of the acid-induced conductance (Brown and Birnbaumer, 1990).

Recently, it was suggested that proton permeation through amiloride-sensitive Na^+ channels might work as a receptor for sour taste (Gilbertson *et al.* 1992, 1993). The present experiments in bullfrog taste cells showed that desensitization treatment with HCl did not decrease the NaCl-induced response (Fig. 2). Furthermore, acid-induced cellular and neural responses were not inhibited by amiloride (Herness, 1987; Miyamoto *et al.* 1988*a*). Similarly, we reported that salt-induced responses in bullfrog were not inhibited by amiloride (Miyamoto *et al.* 1989). These results indicate that an amiloride-sensitive pathway is not involved in acid and salt receptor responses in bullfrogs. In contrast, acid-induced responses in hamster taste cells were greatly inhibited by micromolar levels of amiloride and were independent of extracellular cation concentration (Gilbertson *et al.* 1992, 1993). This suggests that the transduction mechanism of the acid response in the

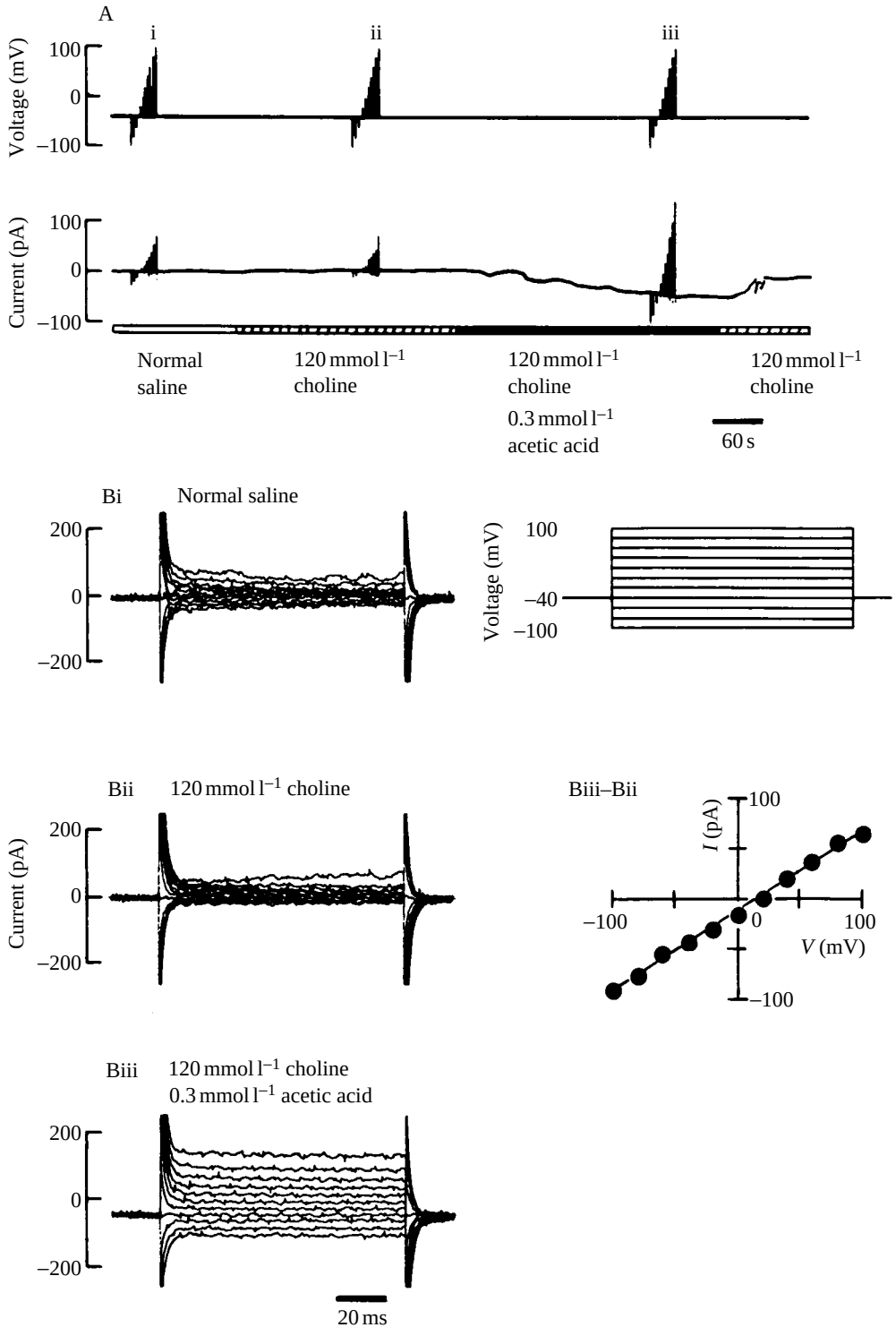
A
i
ii
iii
Voltage (mV)
100
0
−100
Current (pA)
100
0
−100
Normal saline
120 mmol l−1 choline
120 mmol l−1 choline
0.3 mmol l−1 acetic acid
120 mmol l−1 choline
60 s
Bi
Normal saline
200
0
−200
Voltage (mV)
100
−40
−100
Bii
120 mmol l−1 choline
Current (pA)
200
0
−200
Biii–Bii
I (pA)
100
−100
0
100
V (mV)
−100
Biii
120 mmol l−1 choline
0.3 mmol l−1 acetic acid
200
0
−200
20 ms

Fig. 5

hamster may be different from that in the frog. However, in other mammals it has been reported that acid-induced neural responses are not inhibited by amiloride (Hellekant *et al.* 1988; Nakamura and Kurihara, 1990; Scott and Giza, 1990). Furthermore, amiloride did not inhibit the acid-induced short-circuit currents of dog lingual epithelia (Simon and Garvin, 1985) and the perception of sour taste in humans (Schiffman *et al.* 1983). In contrast, Ninomiya and Funakoshi (1988) reported that amiloride inhibited NaCl- and HCl-induced responses in Na^+-sensitive single fibres from rat chorda tympani (80 % and 40 % inhibition, respectively), although it did not inhibit the responses of proton-sensitive fibres. Since information about salty taste may be sent to the brain by the amiloride-sensitive specific pathway (Scott and Giza, 1990), proton permeation through amiloride-sensitive Na^+ channels may result in a lack of discrimination between salty and sour tastes. Therefore, it is not clear whether the amiloride-sensitive Na^+ channels work as sour receptors even if protons can pass through the channels. However, the frog gustatory neural responses elicited by NaCl and by acid were equally enhanced by interstitial application of arginine vasopressin (Okada *et al.* 1991), suggesting that transduction mechanisms other than ionic channels for both responses might contain a common component.

Sodium acetate is incompletely dissociated in aqueous solution so, even at neutral pH, a small part of the salt will be in the form of uncharged molecules. Cell membranes are generally much more permeable to such molecules than to charged ones (Thomas, 1984). Therefore, external undissociated sodium acetate (about $0.2\,mmol\,l^{-1}$ at pH 7.5) may enter the cell and the acid may subsequently dissociate to give protons and acetate ions in the cell, resulting in a decrease in intracellular pH. However, $120\,mmol\,l^{-1}$ sodium acetate (pH 7.5) did not induce any response in frog taste cells. This suggests that the effect of undissociated sodium acetate on intracellular pH could be eliminated by internal Tris–Hepes buffer supplied from the patch pipette. Similarly, in hamster taste cells, intracellular acidification neither elicited any response nor affected the acid-induced response (Gilbertson *et al.* 1992). It is well known that acetic acid has a stronger sour taste than HCl at the same pH in humans (Kurihara and Beidler, 1969). In frogs, the magnitudes of the gustatory neural response and the intracellular receptor potential elicited by acetic acid were also larger than those induced by HCl at the same pH (Y. Okada, unpublished data). This indicates that sour taste cannot simply be due to the decreased pH resulting from acid stimuli.

In mudpuppy and larval tiger salamander, which live in water while breathing through their gills, it has been suggested that the voltage-gated and TEA^+-sensitive outward K^+ channels function as receptors for sour and bitter tastes (Kinnamon and Roper, 1988*a*,*b*;

Fig. 5. Effects of external choline chloride on the acid-induced current. (A) Chart traces of voltage and current at a holding potential of −40 mV. A train of voltage steps from −40 mV and voltage-induced current deflections were superimposed. The recording pipette was filled with $100\,mmol\,l^{-1}$ Cs^+ internal solution. (B) Current recordings between −100 and 100 mV induced by a 100 ms voltage step from a holding potential of −40 mV. Recordings i, ii and iii correspond with i, ii and iii in A. In this cell, the reversal potential of the acid-induced current was 12.5 mV, which was calculated by subtracting record ii from record iii. The calculation was made at steady state.

Sugimoto and Teeter, 1991). Since the K^+ channels in these animals are localized to the apical membrane and maintain the resting potential (Kinnamon *et al.* 1988), protons and quinine may directly block the channels, inducing the depolarization of the taste cells. This mechanism may result in a lack of discrimination between sour and bitter tastes in these animals (Bowerman and Kinnamon, 1992). In contrast, in bullfrog taste cells, acid-induced currents occurred in external 80 mmol l^{-1} Ba^{2+} and internal 100 mmol l^{-1} Cs^+, which completely abolished voltage-activated K^+ currents. When the patch pipette was filled with K^+ internal solution, the taste cell showed a voltage-gated K^+ current that could be inhibited by Ba^{2+}. Protons may also block the current. However, acid stimuli could elicit a conductance increase even after block of the K^+ conductance by Ba^{2+} (Y. Okada, unpublished data). This suggests that proton block of K^+ channels may not play an important role in the acid response of bullfrogs and that K^+ can permeate through the proton-gated channels. The ability of bullfrogs to discriminate among the four basic taste stimuli has been confirmed by cross-adaptation analysis of the glossopharyngeal nerve (Sugimoto and Sato, 1981, 1982). Since the neural response elicited by quinine (bitter stimulus) in bullfrogs was not inhibited by 10 mmol l^{-1} Ba^{2+}, which blocked the delayed outward K^+ currents completely (Y. Okada, unpublished data), direct blocking of K^+ channels may not have a part to play in the perception of bitter taste in the frog. We proposed that quinine-induced Cl^- secretion through the apical taste membrane is responsible for bitter taste transduction (Okada *et al.* 1988).

In conclusion, the bullfrogs may be able to discriminate a sour taste from other tastes as a result of the apical distribution of acid-gated cation channels.

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