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A Novel Mechanism of Bitter Taste Transduction

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Bitter taste is an important warning sign for animals that food is poisonous. The transduction mechanism of bitter taste is still a contradictory matter among researchers. The major current hypothesis, based mainly on biochemical data, involves metabotropic cascades, either via cytoplasmic cyclic nucleotide monophosphate (cNMP) or via IP_3. The former hypothesis involves cytoplasmic cNMP (Fig. 1). It has been shown that a taste receptor cell expresses a specific G protein (McLaughlin et al, 1992) that activates phosphodiesterase leading to the decomposition of cNMP (Price, 1973; Ruiz-Avila et al, 1995). The frog taste receptor cell has a cationic channel, which is kept closed at a high cNMP concentration. Bitter stimuli reduce the cNMP concentration and release the cationic channel from the closed state (Kolesnikov & Margolskee, 1995).

The latter hypothesis involves the cytoplasmic inositol 1,4,5-trisphosphate (IP_3) as a second messenger (Fig. 2). This hypothesis proposes that a bitter substance increases the cytoplasmic IP_3 concentration by activating a G protein and phospholipase C. IP_3 triggers Ca^{2+} release from the endoplasmic reticulum, which in turn directly or indirectly induces transmitter release from the taste receptor cells (Akabas et al, 1988; Hwang et al, 1990; Spielman et al, 1994; Spielman et al, 1996).

We found recently an entirely different mechanism for the bitter taste transduction. We excised a patch membrane from an isolated taste receptor cell of the fungiform papillae of the bullfrog (Tsunenari et al, 1996, 1999) and recorded in the outside-out configuration. The patch pipette was filled with a solution containing 120 mM CsCl, 2 mM Na_2-EGTA and 10 mM Na-HEPES. The outside of the patch membrane was exposed to the 120 mM NaCl solution containing 120 mM NaCl and 2 mM Na-HEPES. Thus, on the cytoplasmic face of the patch membrane none of the second messenger candidates or their precursors (eg. cyclic nucleotide, IP_3, Ca^{2+}, ATP and GTP) was present.

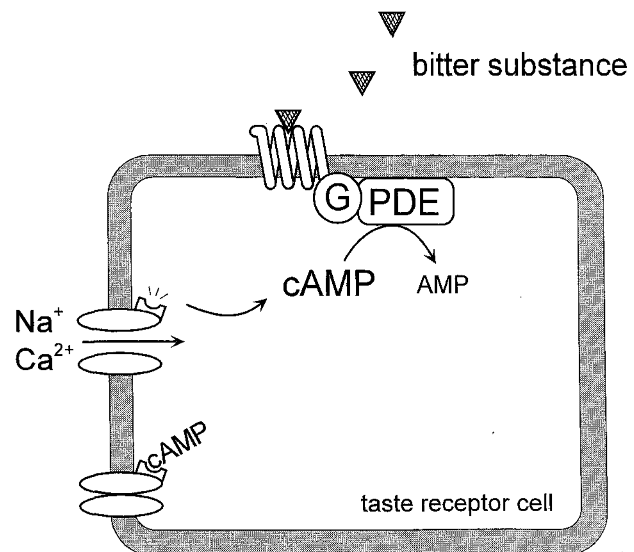

Fig. 1. A model of bitter taste transduction based on the G protein−cNMP cascade hypothesis. A bitter tastant binds to the receptor that is coupled to a specific G protein, gastoducin. Active gastoducin triggers phosphodiesterase activation leading to the decomposition of cNMP. The cationic channel, kept closed at a high cNMP concentration is now released from the closed state. Through this channel cation flows in and depolarize the cell.

Application of a bitter taste substance, 1 mM quinine quinine, to the external surface of the patch membrane induced channel openings (Fig. 3). The

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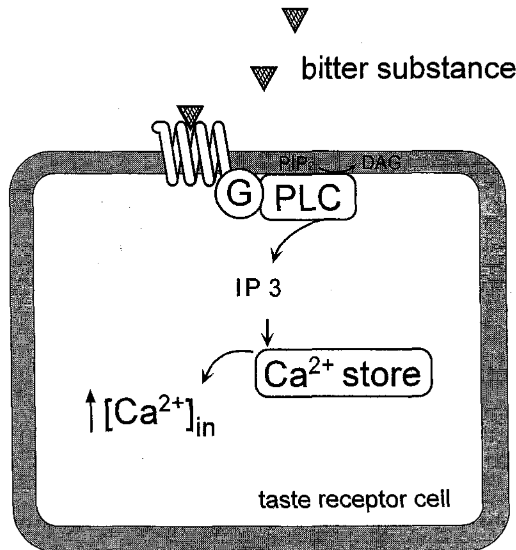

Fig. 2. A model of bitter taste transduction based on the G protein $-$ IP_3 cascade hypothesis. A bitter substance increases the cytoplasmic IP_3 concentration by activating a G protein and phospholipase C. IP_3 triggers Ca^{2+} release from the endoplasmic reticulum, which in turn directly or indirectly induces transmitter release from the taste receptor cells.

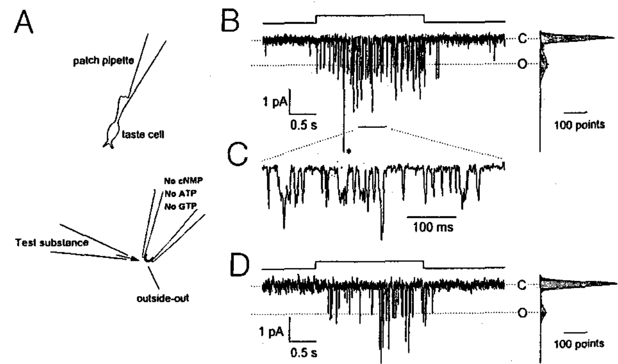

Fig. 3. Currents induced by bitter substances in the outside-out membrane patches. *A*, Schematic diagram showing the experimental procedure. After the whole-cell configuration (upper panel) was established, a patch membrane was excised from an isolated taste receptor cell by a patch pipette filled with a solution containing no cNMP, Ca^{2+}, ATP, or GTP. A bitter substance was applied to the outer surface of the patch membrane by pressure (lower panel). *B*, Quinine-induced current response recorded from an outside-out membrane patch held at -79 mV. Upward step of the horizontal line on the top indicates the timing of 1 mM quinine application from the puffer pipette with a pressure of 20 kPa. The outside of the membrane was bathed in the 120 mM NaCl (no Ca^{2+} added) solution and the patch pipette contained 120 mM CsCl. The amplitude histogram on the right side of the current trace was made from 2000 points during the 2-s quinine application. The histogram was fitted by a sum of two Gaussian distributions represented by continuous curves. The higher peak represents the closed state (C) and the lower peak represents the open state of one channel (O). The event labeled with an asterisk has a quadruple amplitude to that of the unitary current. *C*, A part of the trace in *B* (indicated by a short bar below the current trace) reproduced in an expanded time scale. *D*, Denatonium-induced current response in another outside-out patch. Denatonium (5 mM) was puff-applied with a pressure of 50 kPa. The bath solution contained 115 mM NaCl and 2.5 mM KCl (no Ca^{2+} added). Other conditions were identical to those in *B* (Reproduced from Tsunenari et al, J. Physiol. 519, 1999).

channel events can be seen more clearly in an expanded time scale (Fig. 3B). The mean amplitude of the unitary current was approximately -1.0 pA. Quinine applied to the cytoplasmic side of the membrane was ineffective.

The channel was activated not only by quinine but also by other bitter taste substances. We tested different bitter substances in the excised patch preparation. As Fig. 3C illustrates, denatonium generated the response very similar to that evoked by quinine. Also in the whole-cell preparation, an inward current was induced by either 5 mM quinine, 5 mM denatonium or 10 mM strychnine (Fig. 4), and these responses showed identical reversal potentials.

The response could be recorded more than 10 min after the excision of the patch membrane (Fig. 5). This observation strongly indicate that during this period any soluble substances are expected to be washed away if it remained on the cytoplasmic side of the patch membrane. Furthermore, we tested whether there is any sign indicating the contribution of G protein cascade to the response generation. If G protein activation precedes the channel opening, addition of GDPβS, a hydrolysis-resistant GTP analog that binds to G protein without activating it, would block the cascade, and thus block the channel opening (Fig. 6A). On the other hand, it is expected that addition of GTPγS, a hydrolysis-resistant activator of G protein, would activate G protein, if some remained on the patch membrane, and would activate the channel continuously (Fig. 6B). We added either GDPβS (1 mM) or GTPγS (1 mM) to the cytoplasmic side of the membrane, but the response was unaffected (Fig. 6). These data suggest that the bitter taste substances directly gated the channel.

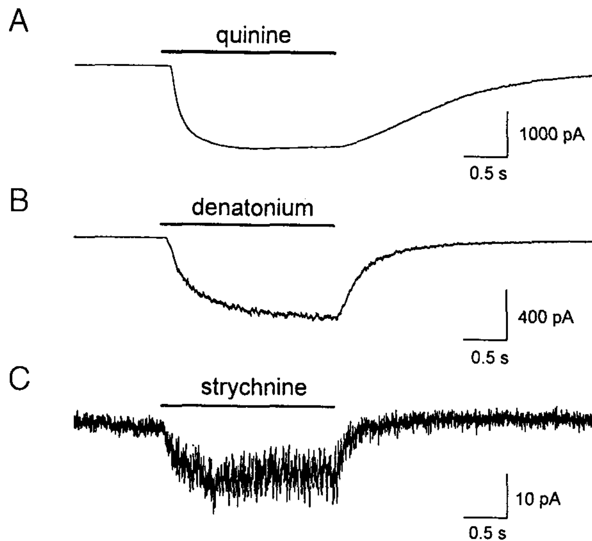

Fig. 4. Response of a taste receptor cell isolated from the fungiform papilla of the bullfrog recorded under the whole cell configuration voltage clamped at −54 mV. Records from three different cells. Bitter substances (5 mM quinine, 5 mM denatonium, 10 mM strychnine) were applied by pressure ejection. The patch pipette was filled with a solution containing 115 mM CsCl, 2 mM $MgCl_2$, 0.5 mM $CaCl_2$, 2 mM Na-EGTA, 10 mM Na-HEPES, 2 mM Na_2ATP (pH 7.2). The bath solution contained 115 mM NaCl, 2.5 mM KCl, 2 mM Na-HEPES, 2 mM glucose (pH 7.2).

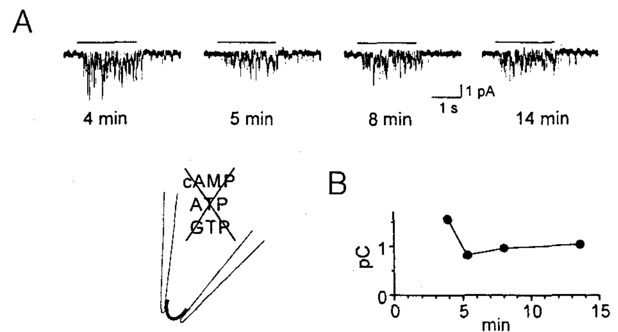

Fig. 5. Evaluation of whether the cytoplasmic soluble elements contribute to the response generation. *A*, Response of a membrane patch to quinine recorded 4, 5, 8 and 14 min after membrane excision. Quinine (5 mM) was ejected from a puffer pipette in the timing indicated by the bar over each current trace. The holding voltage was −54 mV. *B*, Magnitude of the integrated response of *A* as expressed by the amount of charge generated during quinine application.

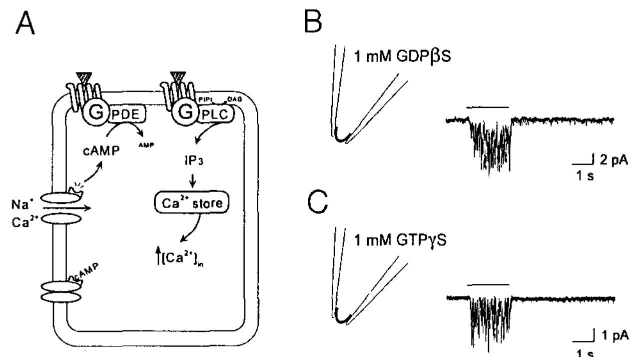

Fig. 6. *A,* A model illustrating the bitter taste transduction based on the G protein−cNMP cascade hypothesis and the G protein−IP_3 cascade hypothesis. *B,* A current response to quinine (1 mM, ejected from a puffer pipette with pressure) recorded 3.6 min after the membrane excision with a patch pipette containing 1 mM GDPS. The holding voltage was −79 mV. *C,* A current response (1 mM, ejected from a puffer pipette with pressure) recorded 4.0 min after the membrane excision with a patch pipette containing 1 mM GTP γ S. The holding voltage was −54 mV. The bath solution (*B*−*C*) contained 115 mM NaCl, 2.5 mM KCl (no Ca^{2+} added) and the patch pipette solution contained 120 mM CsCl (Reproduced from Tsunenari et al, J. Physiol. 519, 1999).

We also studied the properties of the bitter taste substance-gated channel. The response was dose-dependent. The lowest effective concentration of quinine was 0.1 mM, and the response to quinine saturated at 1 mM. The dose response curve was fitted to a Hill equation with a $K_{1/2}$ of 0.52 mM and a coefficient of 3.8.

The channel had non-selective permeability to cation. As shown in Fig. 7, current through the bitter taste substance-gated channel reversed its polarity at approximately +20 mV under 120 mM NaCl (outside) /120 mM CsCl (inside) condition, and the reversal potential was identical under the Cl^--free condition. Under symmetrical ionic conditions (120 mM NaCl) the reversal potential was −1.4 mV. The single channel conductance measured in 120 mM NaCl (outside) /120 mM CsCl (inside) solution was 10 pS. The permeability ratio was $P_{Na} : P_K : P_{Cs}$ = 1 : 0.48 : 0.39. The permeability ratio and the single channel conductance were almost identical to those obtained in our previous study on the whole-cell current response ($P_{Na} : P_K : P_{Cs}$ = 1 : 0.5 : 0.42, Tsunenari et al, 1996) and by noise analysis (12 pS; Tsunenari & Kaneko, 1997, 1998).

In earlier studies, blocking of K^+ channels by quinine has been attributed to the underlying mechanism

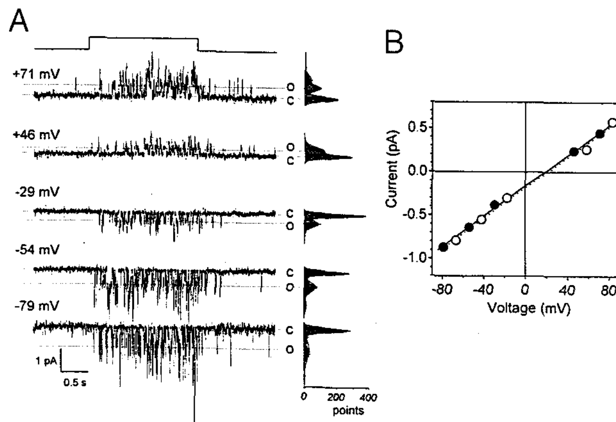

Fig. 7. Voltage dependence of the quinine-evoked unitary events. *A*, Quinine-induced currents recorded from an outside-out membrane patch held at various holding voltages. Upward shift of the horizontal line on the top indicates the timing of quinine application. Quinine (1 mM) was ejected from a puffer pipette with a pressure of 20 kPa. The outside of the membrane was bathed in a solution containing 120 mM NaCl and 2 mM Na-HEPES. The patch pipette solution contained 120 mM CsCl, 2 mM Na_2-EGTA and 10 mM Na-HEPES. Amplitude histograms on the right side of each current trace were made from 2000 points during the 2-s quinine application and was fitted by a sum of Gaussian distributions represented by continuous curves. "C" indicates the close state and "O", the open state of a single channel. *B*, *I-V* relation of the unitary current amplitude. The unitary amplitude was measured from the peaks of the Gaussian curves as shown in *A*. Filled circles (connected by a continuous line) represent the data of *A* recorded in the 120 mM NaCl solution. Open circles (connected by a dotted line) represent the data of the same patch membrane recorded in the Cl^--free solution (NaCl was replaced by equimolar Na-gluconate). Lines were fitted by the least-square method (Reproduced from Tsunenari et al, J. Physiol. 519, 1999).

of bitter taste reception (Avenet & Lindemann, 1987; Kinnamon & Roper, 1988; Sugimoto & Teeter, 1991; Cummings & Kinnamon, 1992). Blockade of the voltage-dependent K^+ channel, which is presumably coexistent with the ligand-gated channels, increases the input resistance of a cell, making the inward current through the ligand-gated channel more effective. Thus, the two mechanisms can work synergistically. The present findings give new evidence to suggest that bitter taste transduction involves direct channel opening by the bitter taste substance, but does not necessarily rule out the possibility of the metabotropic process involving the second-messenger system. In fact, it has been suggested that the taste transduction contains parallel pathways of different mechanisms (Lindeman, 1996). It will now be of interest to ask whether the ionotropic and the metabotropic processes co-exist in bitter taste transduction, and if so how they interact.

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