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Our recent studies on sensory transduction: from vision to taste

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Abstract. The transduction in photoreceptors and chemoreceptors has common mechanisms. In photoreceptors, activation of rhodopsin by light triggers the enzymatic cascade mediated by a G-protein, transducin. In olfactory cells activation of the receptor molecule by odorant triggers the enzymatic cascade mediated by a G-protein, Golf. In taste receptor cells biochemical studies have also suggested a metabotropic transduction hypothesis, but we recently identified a cationic channel that was directly gated by a bitter taste substance. By reviewing these recent studies carried out in our laboratory in the last 10 years, the transduction machinery in these sensory receptor cells are summarized. (Keio J Med 50 (1): 13–19, March 2001)

Key words: phototransduction, retina, L-glutamate, chemoreception, olfaction, taste

In commemorating the 50th volume of the Keio Journal of Medicine, I would like to introduce the research activity of the Sensory Physiology Group of the Department of Physiology in the recent 10-year period. The history of the group goes back to 1957, when the late Professor Tsuneo Tomita started research on the physiology of the retina. In 1960s, Tomita and his group demonstrated, for the first time in the world, the existence in the vertebrate retina of three types of cone photoreceptors with different spectral sensitivity, and established the physiological basis of the trichromatic mechanism of color vision.[1] Thus, they gave the ultimate answer to the long-lasted battle between the trichromatic theory and the opponent color theory on the mechanism of color vision.

Following this discovery, they elucidated the mechanism on the generation of the hyperpolarizing light-evoked responses of photoreceptors.[2,3] The common knowledge at that time was that neural excitation is accompanied by depolarization of the cell. Our experiments presented evidence to interpret this contradictory observation. We found that light induced closing, not opening, of the cation-permeating channel. These observations stimulated, worldwide, the research on the mechanism of phototransduction and synaptic transmission of retinal neurons. Nowadays, the fundamental retinal circuit for processing the retinal image has been elucidated.[4]

The molecular mechanism of phototransduction is now understood in detail.[5] Figure 1 summarizes schematically the phototransduction process. When the incident light is absorbed by a rhodopsin molecule sitting in the disc membrane of the outer segment of a photoreceptor, the conformation of the rhodopsin molecule is changed and an enzymatic cascade is started. The first step of this cascade is the activation of transducin, a G-protein. Transducin will then activate the cyclic GMP-phosphodiesterase, which decomposes cyclic GMP and reduces its cytoplasmic concentration. The plasma membrane of the photoreceptor outer segment has cyclic GMP-gated cation channels, which are maintained in the open state in the dark when the concentration of the cytoplasmic cyclic GMP is high. Reduction of cyclic GMP concentration results in the closure of the channel and cell hyperpolarization. L-Glutamate is the transmitter of vertebrate photoreceptors.[6] It is released tonically in the dark when the photoreceptor is maintained in the depolarized state. Light-induced hyperpolarization results in the reduction of the amount of transmitter release.

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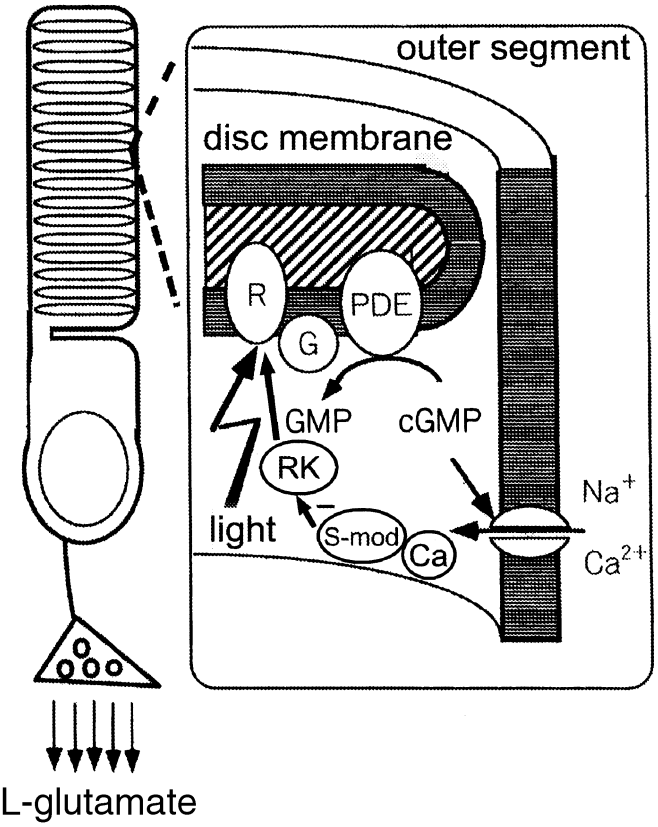

Fig. 1 A schematic illustration of phototransduction. The incident light activates rhodopsin (R) residing on the disc membrane. The activation of rhodopsin triggers a cascade chain reaction via transducin (G), a G-protein, followed by cyclic GMP phosphodiesterase (PDE), a hydrolyzing enzyme of cyclic GMP (cGMP). The plasma membrane of the outer segment has cationic channels that open when cGMP binds to them. Decomposition of cGMP results in the closure of this channel. The channel passes Na^+ and Ca^{2+}. Ca^{2+} entered into the cytoplasm binds to S-modulin (S-mod) that inhibits rhodopsin kinase (RK) which is the enzyme to phosphorylate the light-activated rhodopsin to terminate its activity. Thus, Ca-S-modulin complex prolongs the lifetime of light-activated rhodopsin and enhances the sensitivity of photoreceptors. Depolarization of photoreceptors accelerates the release of L-glutamate, the transmitter of photoreceptors from the terminal.

One of the recent contributions of our group to the understanding of phototransduction is the discovery of a Ca^{2+}-binding protein, S-modulin, that controls the sensitivity of photoreceptors during adaptation.[7] Under the high Ca^{2+} condition S-modulin slows down phosphorylation of the activated rhodopsin, which is the mechanism to stop the enzymatic cascade by inactivating the light-activated rhodopsin. In the dark, Ca^{2+} comes in through the cyclic GMP-gated cation channel, and binds to S-modulin. The target of Ca^{2+}-S-modulin complex is rhodopsin kinase. Rhodopsin kinase halts the activity of the light-activated rhodopsin by phosphorylation. Thus, the lifetime of rhodopsin is longer in the dark-adapted state than in the light-adapted state, and the light sensitivity of photoreceptors is controlled.

The signal from photoreceptors is transmitted to bipolar cells, the second-order neuron of the retina. There are two types of bipolar cells. The ON-type bipolar cells are depolarized by light, while the OFF-type bipolar cells are hyperpolarized by light. Both types of light responses are generated by L-glutamate released by the photoreceptors. Recently, the basis of the opposite polarity of light-evoked responses in bipolar cells was discovered.[8,9] The two types of bipolar cells have different L-glutamate receptors; the metabotropic type in the ON-type bipolar cell and the ionotropic type in the OFF-type bipolar cell. The signal transduction in the ON-type bipolar cell is very similar to that in the photoreceptors (Fig. 2A). The ON-type bipolar cells have cyclic GMP-gated cation channels in the plasma membrane and a high cytoplasmic cyclic GMP concentration. Consequently, cyclic GMP generates a "standing" inward current. These bipolar cells have a metabotropic glutamate receptor, mGluR6, in the dendritic tips.[10] Metabotropic glutamate receptors have 7 membrane-spanning helices, and their activation initiates an enzymatic cascade via a GTP-binding protein. The response of ON-type bipolar cells is interpreted as follows. L-glutamate binds to mGluR6, which in turn triggers a cascade of a GTP-binding protein and a cGMP phosphodiesterase (PDE). Activated PDE decomposes cytoplasmic cGMP and reduces its concentration, thus removing cGMP molecules from the cGMP-gated cation channels. Closure of the channel causes hyperpolarization. Light illumination reduces the amount of L-glutamate release, and stops the enzymatic cascade described above. The end result is the cell depolarization. Presence of the GTP-binding protein (Go) has been demonstrated in the dendrites of the ON-type bipolar cells.[11]

Response generation in OFF-type bipolar cells is more straightforward (Fig. 2B). Ionotropic glutamate receptor channels in the dendrites of OFF-type bipolar cells have been proposed to be of the AMPA type.[9] These channels have cation permeabilities of low selectivity, passing Na^+, K^+ and Ca^{2+}. The reversal potential of the L-glutamate-induced inward current is near 0 mV. In the dark, L-glutamate released from photoreceptors opens this receptor channel, maintaining the cell in a depolarized state. Light illumination reduces the amount of L-glutamate release, and hyperpolarizes the OFF-type bipolar cell.

The metabotropic mechanism of sensory transduction is also found in the olfactory receptor cell (Fig. 3).[12] The receptor cell in the olfactory epithelium is equipped with cilia emanating from the free surface of the receptor cell into the nasal cavity. On the plasma membrane of the olfactory cilia, the receptor protein

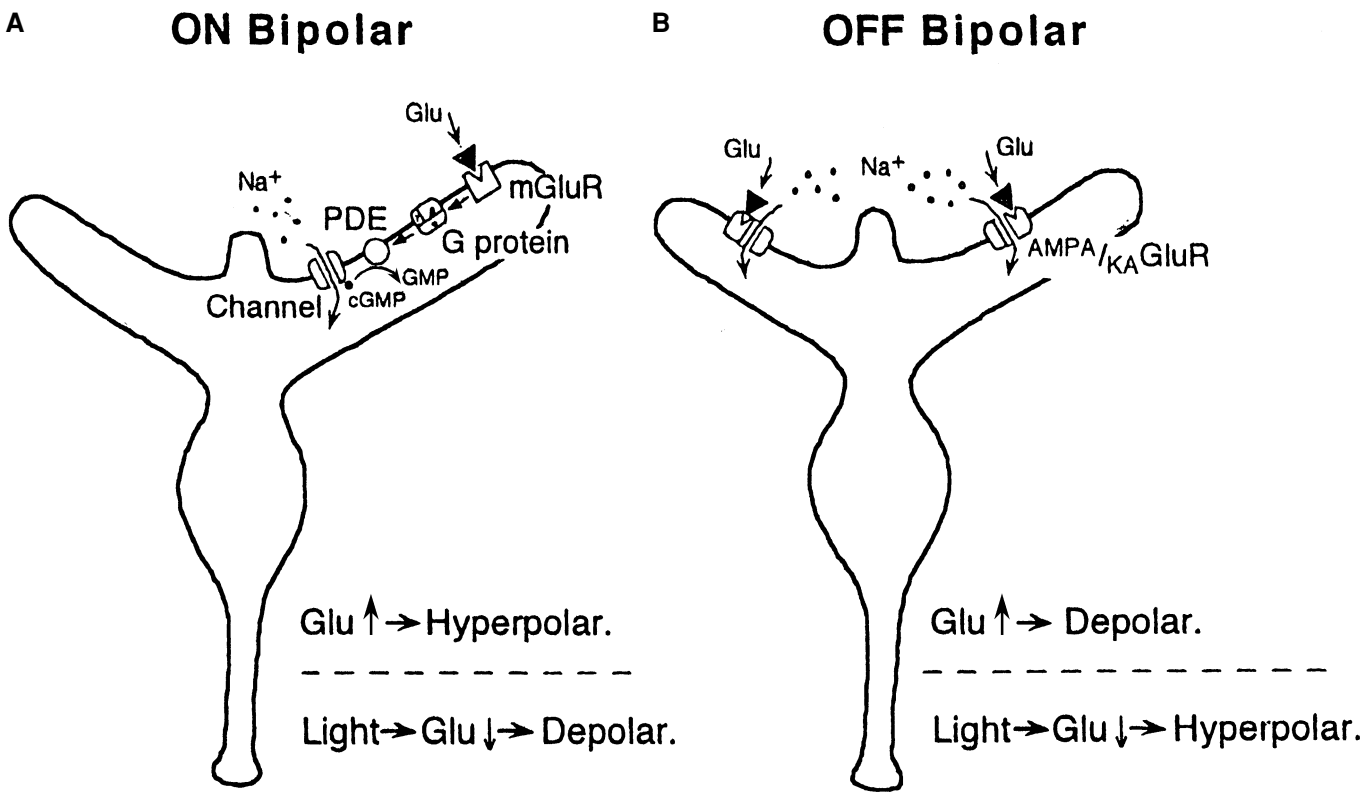

Fig. 2 Schematic diagram illustrating the mechanism of response generation in ON- and OFF-type bipolar cells. A) The ON-type bipolar cell has a metabotropic glutamate receptor (mGluR) which, when activated by glutamate, triggers an enzymatic cascade consisting of GTP binding protein and cGMP phosphodiesterase (PDE). The activated PDE decomposes cytoplasmic cGMP and reduces its concentration. The plasma membrane has cGMP-gated channels through which cations flow continuously. Reduction of cytoplasmic cGMP results in closure of the channels and causes hyperpolarization. Because light illumination reduces the amount of glutamate release, an inverse cascade operates, and depolarizes the cell. B) The OFF-type bipolar cell has an ionotropic glutamate receptor. At this receptor, glutamate opens a cation channel with low ionic selectivity. In the dark, glutamate is tonically released from photoreceptors and the channels remain open, keeping the cell depolarized. Illumination reduces glutamate release and results in the closure of channels and hyperpolarization of the cell.

with 7 membrane-spanning helices is incorporated. Binding of an odorant to this receptor protein activates the receptor molecule, and triggers the enzymatic cascade mediated by a G-protein, G_{olf}, followed by the synthesis of cyclic AMP from the cytoplasmic ATP by an adenylate cyclase. The ciliary membrane has cyclic AMP-gated cation channels. Cytoplasmic cyclic AMP binds to this channel and opens it, and consequently the inward current through the channel depolarizes the olfactory receptor cell.

As has been stated, the metabotropic enzymatic cascade is widely used in the sensory transduction systems: photoreceptors, ON-type retinal bipolar cells and olfactory receptors. These observations drove us to expand our research field into the chemosensory system. We found the taste transduction as one of the research fields that needs to be studied with modern technology. The transduction mechanism of bitter taste is still a contradictory matter among researchers. The major current hypothesis, based mainly on the biochemical data, involves metabotropic cascades, either *via* cytoplasmic cyclic nucleotide monophosphate (cNMP) (Fig. 4) or *via* IP_3. It has been shown that a taste receptor cell expresses a specific G protein, gustoducin,[13] which activates phosphodiesterase leading to the decomposition of cNMP.[14,15] 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.[16]

The IP_3 hypothesis (Fig. 5) 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.[17–20]

We found recently an entirely different mechanism for the bitter taste transduction,[21,22] which will be described in detail. We excised an outside-out patch membrane from an isolated taste receptor cell of the

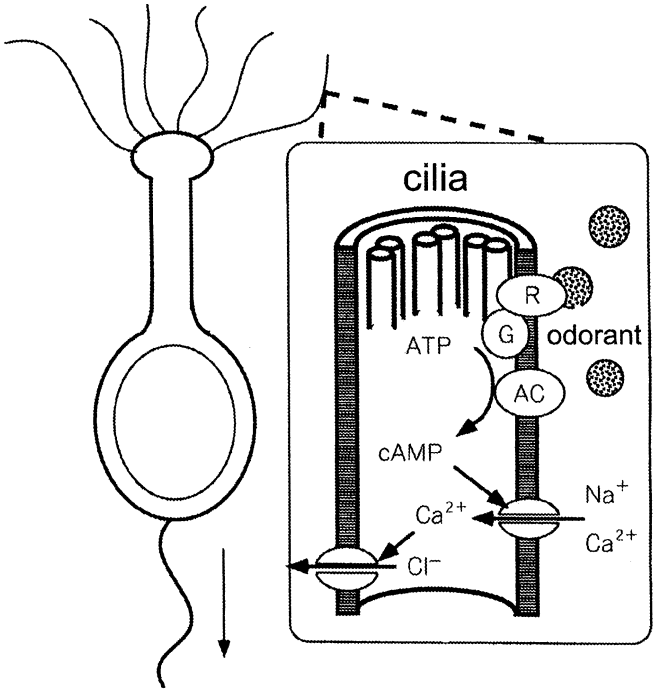

Fig. 3 Schematic diagram illustrating the mechanism of response generation in olfactory receptor cells. Odorant binds to the olfactory receptor molecule (R) contained in the plasma membrane of the olfactory cilia and activates the molecule. The activated receptor molecule triggers an enzymatic cascade consisting of GTP binding protein and adenylate cyclase (AC). AC synthesize cyclic AMP (cAMP) which binds the cationic channel on the plasma membrane. Na^+ and Ca^{2+} flow in through the opened channel and depolarize the receptor cell. Ca^{2+} in the cytoplasm opens Ca-dependent Cl channel and the outward movement of Cl^- results in an enhanced depolarization of the receptor cell.

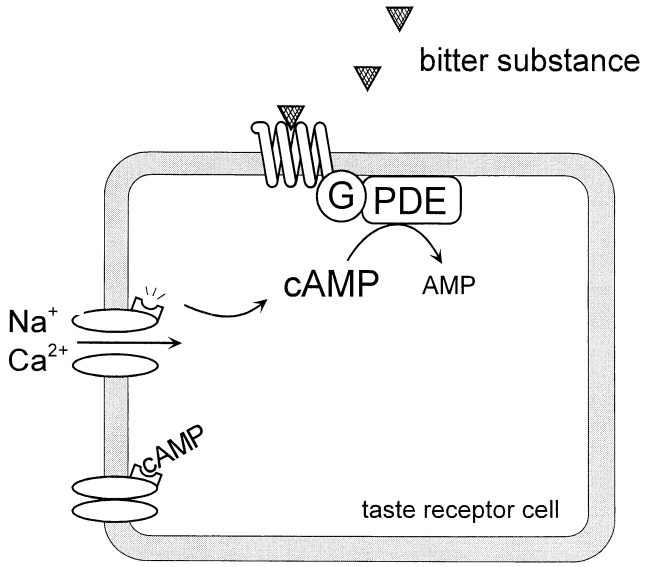

Fig. 4 A model of bitter taste transduction based on the G protein – cNMP cascade hypothesis. A bitter tastant binds to the receptor that is coupled to a specific G protein, gastoducin. Active gastoducin triggers phosphodiesterase activation leading to the decomposition of cNMP. The cationic channel, kept closed at a high cNMP concentration is now released from the closed state. Through this channel cation flows in and depolarizes the cell.

fungiform papillae of the bullfrog. Under our recording condition, none of the second messenger candidates or their precursors (e.g. cyclic nucleotide, IP_3, Ca^{2+}, ATP and GTP) was present on the cytoplasmic face of the patch membrane. Application of a bitter taste substance, 1 mM quinine, to the external surface of the patch membrane induced channel openings (Fig. 6). The channel events can be seen more clearly in an expanded time scale (Fig. 6B). 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, denatonium or strychnine (Fig. 7).

The response could be recorded more than 10 min after the excision of the patch membrane (Fig. 8). This observation strongly indicates 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

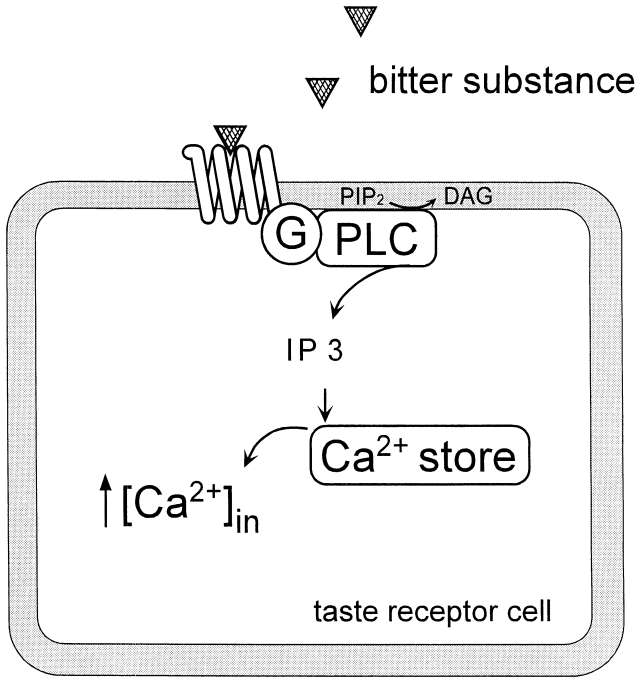

Fig. 5 A model of bitter taste transduction based on the G protein – IP_3 cascade hypothesis. A bitter substance increases the cytoplasmic IP_3 concentration by activating a G protein and phospholipase C. IP_3 triggers Ca^{2+} release from the endoplasmic reticulum, which in turn directly or indirectly induces transmitter release from the taste receptor cells.

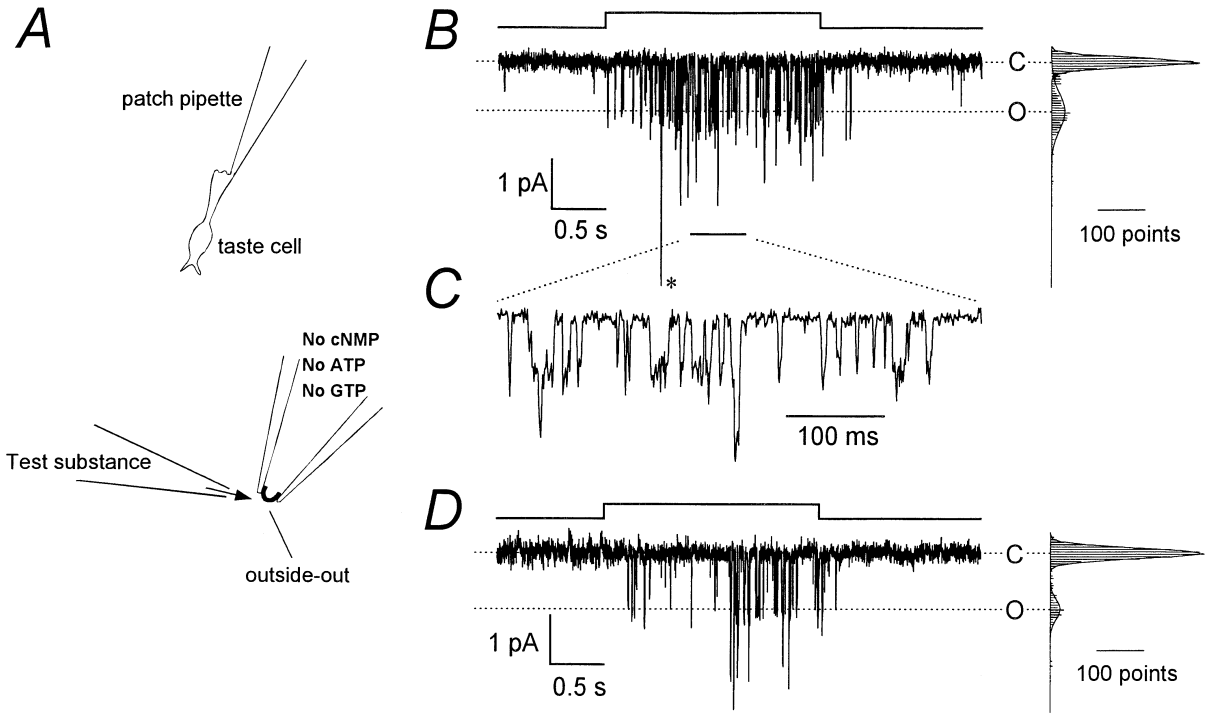

Fig. 6 Currents induced by bitter substances in the outside-out membrane patches. A) Schematic diagram showing the experimental procedure. After the whole-cell configuration (upper panel) was established, a patch membrane was excised from an isolated taste receptor cell by a patch pipette filled with 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).

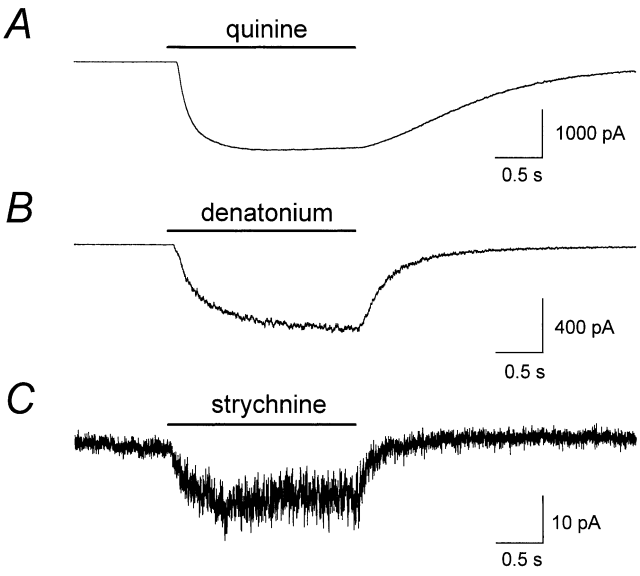

Fig. 7 Response of a taste receptor cell isolated from the fungiform papilla of the bullfrog recorded under the whole cell configuration voltage clamped at –54 mV. Records from three different cells. Bitter substances (5 mM quinine, 5 mM denatonium, 10 mM strychnine) were applied by pressure ejection. 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, and 2 mM Na_2ATP (pH 7.2). The bath solution contained 115 mM NaCl, 2.5 mM KCl, 2 mM Na-HEPES, and 2 mM glucose (pH 7.2).

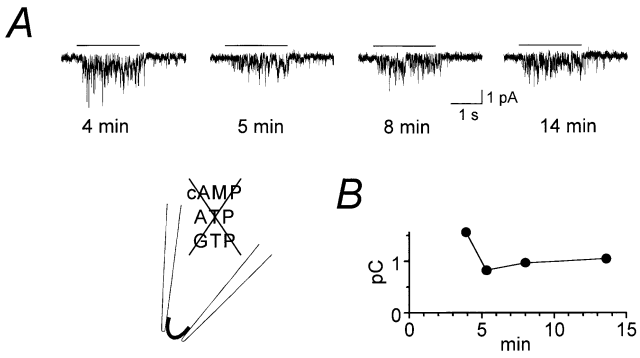

Fig. 8 Evaluation of whether the cytoplasmic soluble elements contribute to the response generation. A) Response of a membrane patch to quinine recorded 4, 5, 8 and 14 min after membrane excision. Quinine (5 mM) was ejected from a puffer pipette in the timing indicated by the bar over each current trace. 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.

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. 9B). 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. 9C). We added either GDPβS (1 mM) or GTPγS (1 mM) to the cytoplasmic side of the membrane, but the response was unaffected (Fig. 9). These data suggest that the bitter taste substances directly gated the channel.

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 in 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. The permeability ratio to Na^+, K^+, and Cs^+ was

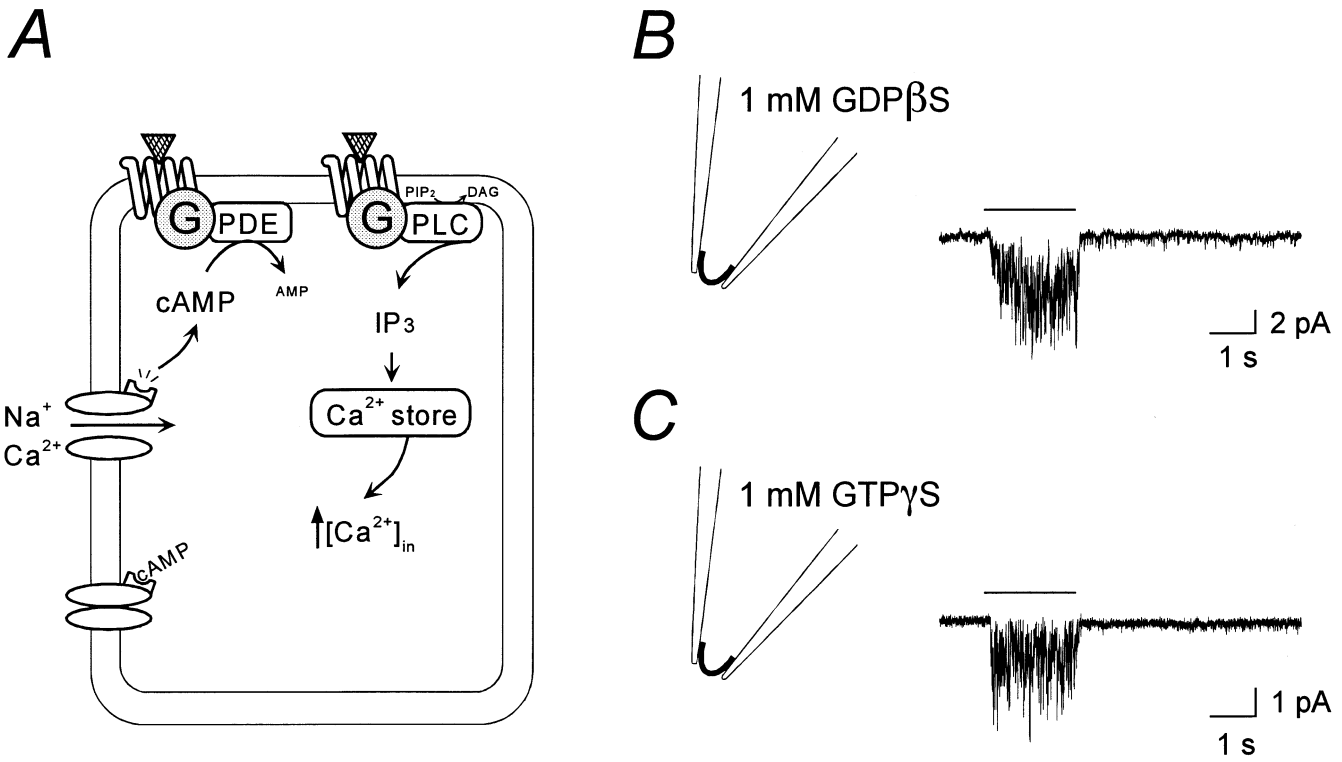

Fig. 9 A) A model illustrating the bitter taste transduction based on the G protein – cNMP cascade hypothesis and the G protein – IP_3 cascade hypothesis. B) A current response to quinine (1 mM, ejected from a puffer pipette with pressure) recorded 3.6 min after the membrane excision with a patch pipette containing 1 mM GDPβS. 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 GTPγS. 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 T, *et al.*, J. Physiol. 519, 1999)

$P_{Na} : P_K : P_{Cs} = 1 : 0.48 : 0.39$, and the single channel conductance was 10 pS. Similar values were obtained in our previous study on the whole-cell current response[21] ($P_{Na} : P_K : P_{Cs} = 1 : 0.5 : 0.42$) and by noise analysis (12 pS).[23]

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.[24] 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.

In this review article I have made a brief summary of the research achievement of our laboratory in the past 10 years. The sensory organ detects the information of our surrounding environment. Study of the sensory transduction mechanism makes a bridge between the psychological phenomena and the physiological performance of our body structure. It also elucidates the mechanisms of various sensors sensitive to various parameters within the body: body temperature, pH and other chemical components of the body fluid, and the stretch. Studies on these biological sensors are indispensable not only to understand our body function, but also to help designing sensitive and reliable sensors in the industrial fields.

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