Effect of Ca\textsuperscript{2+}, Cyclic GMP, and Cyclic AMP Added to Artificial Solution Perfusing Lingual Artery on Frog Gustatory Nerve Responses

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ABSTRACT The lingual artery of the bullfrog was perfused with artificial solution and the effects of Ca\textsuperscript{2+}, Ca-channel blockers (MnCl\textsubscript{2} and verapamil), cGMP, and cAMP added to the perfusing solution of the gustatory nerve responses were examined. The responses to chemical stimuli of group 1 (CaCl\textsubscript{2}, NaCl, distilled water, D-galactose, and L-threonine) applied to the tongue surface were greatly decreased by a decrease in Ca\textsuperscript{2+} concentration in the perfusing solution, suppressed by the Ca-channel blockers, enhanced by cGMP, and suppressed by CAMP. The responses to chemical stimuli of group 2 (quinine hydrochloride, theophylline, ethanol, and HCl) were practically not affected by a decrease in Ca\textsuperscript{2+} concentration, the Ca-channel blockers, cGMP, and cAMP. The responses to the stimuli of group 1 seem to be induced by Ca influx into a taste cell that is triggered by depolarization and modulated by the cyclic nucleotides in a taste cell. The responses to group 2 seem to be induced without accompanying Ca influx.

INTRODUCTION It is generally accepted that the initial event of taste reception is adsorption of chemical stimuli on the receptors of taste cell (Beidler, 1954). The adsorption depolarizes the taste cells, which leads to an increase of the taste nerve activities via chemical synapses between taste cells and taste nerve terminals (Murray, 1971; DeHan and Graziadei, 1971; Graziadei and DeHan, 1971). In general, an influx of Ca\textsuperscript{2+} into a presynaptic cell induces a release of a chemical transmitter, and hence Ca\textsuperscript{2+} seems to be involved in the taste transduction mechanism. However, any systematic studies on the role of Ca\textsuperscript{2+} in the transduction process have not been carried out.

There is increasing evidence that cyclic GMP (cGMP) may be involved in visual transduction mechanism (Weeler and Bitensky, 1977; Lipton et al., 1977; Yee and Liebman, 1978; Nicol and Miller, 1978; Woodruff and Bownds, 1978).
1979; Miller and Nicoll, 1979; Stryer et al., 1981). Within the field of chemosensory systems, a role of cyclic nucleotide was initially suggested by Kurihara and Koyama (1972); they showed that the homogenates of bovine taste buds bearing papillae and those of the rabbit olfactory epithelium exhibited high adenylate cyclase activities comparable to the activities found in the brain. Bovine taste papillae were also found to exhibit high activities of phosphodiesterase (Kurihara, 1972; Price, 1973). Recently, Nomura (1978) and Nomura and Asanuma (1980) examined the localization of adenylate cyclase, guanylate cyclase, and phosphodiesterase activities in the taste buds bearing papillae of various vertebrates by means of histochemistry and demonstrated that the activities of these enzymes were densely located only at the apex of taste buds. Since chemical stimuli are received at the apex of the taste buds, the above results suggest that the cyclic nucleotides play an important role in the transduction process in the gustatory reception.

There exist difficulties in elucidating the role of Ca$^{2+}$ and cyclic nucleotides in the taste transduction process; chemical reagents applied to the tongue surface are hardly permeable to the taste cell membranes and the epithelial cell membranes. In fact, we have observed that cyclic nucleotides or their dibutyryl derivatives applied to the tongue do not bring about any effect on the gustatory nerve responses. Similarly, a change in Ca$^{2+}$ concentration in a solution contiguous to the frog tongue surface does not bring about any specific effect on the gustatory nerve response, probably because intercellular fluid between taste cells cannot be replaced with the solution applied on the tongue surface.

To supply certain chemical reagents in taste cells or intercellular fluid below the tight junction, the lingual artery of the bullfrog is perfused with an artificial solution containing various reagents in the present study. This method was first used by Rappuzzi (1964) and developed by Morimoto and Sato (1975, 1977), who tried to identify a chemical transmitter in frog taste cells. Morimoto (1978) also examined in part the role of Ca$^{2+}$ in the taste transduction mechanism and showed that the responses to some chemical stimuli such as CaCl$_2$ or distilled water were decreased by a decrease in Ca$^{2+}$ concentration in a perfusing solution. However, the detailed information has not been reported yet. In the present study, we have examined the effects of cGMP, cyclic AMP (cAMP), Ca$^{2+}$, and Ca-channel blockers added to the perfusing solution on the frog gustatory nerve responses. We have found that the nerve responses to chemical stimuli of one group (CaCl$_2$, NaCl, D-galactose, distilled water, and L-threonine) are highly dependent on Ca$^{2+}$ concentration in a perfusing solution, suppressed by the Ca-channel blockers, enhanced by cGMP, and suppressed by cAMP. On the other hand, chemical stimuli of another group (quinine, theophylline, ethanol, and HCl) are practically not affected by a decrease in Ca$^{2+}$ concentration, the Ca-channel blockers, cGMP, and cAMP.

**MATERIALS AND METHODS**

**Recording of the Gustatory Nerve Responses**

Adult bullfrogs, *Rana catesbeiana*, were used in the present experiments. The dissection of the glossopharyngeal nerve was carried out as described in a previous paper (Kamo...
et al., 1978). After a frog was anesthetized with an intraperitoneal injection of urethane solution (≈300 mg/100 g body wt), the glossopharyngeal nerve was dissected from the surrounding tissues and cut proximally. The tongue was pulled out and fixed with insect pins on a corkboard in an experimental chamber.

The nerve impulses were amplified with an AC-amplifier and integrated with an electronic integrator with a time constant of 0.3 s. The tongue of the bullfrog was bathed in frog Ringer's solution when not subjected to stimulation. The tongue was perfused with a stimulating solution at a flow rate of 1.7 ml/s. Stimulating solutions for CaCl₂, NaCl, HCl, and acetic acid were prepared by dissolving the chemicals in distilled water. To suppress the water response in the frog, quinine hydrochloride, theophylline, and ethanol were dissolved in 5 mM KCl solution. Since the presence of salts in stimulating solution leads to suppression of the responses to D-galactose (Miyake et al., 1976) and L-threonine (Yoshii et al., 1981), these chemicals were dissolved in distilled water and the stimulating solutions thus prepared were applied to the tongue after the water response was adapted to the spontaneous level.

**Perfusing of the Lingual Artery**

Perfusion of the lingual artery with artificial solution was carried out as described by Morimoto and Sato (1975, 1977). A polyethylene tube was cannulated into the lingual artery and Ringer solution (112 mM NaCl, 3.4 mM KCl, 3.6 mM MgSO₄, 2.5 mM NaHCO₃, pH 7.2) containing various concentrations of CaCl₂ and sodium heparine of 10 U/ml was perfused through the tube into the artery by using a peristaltic pump at a rate of 0.01 ml/s. The perfused solution was flowed out from the vein at the bottom of the tongue. It took 1–2 h, depending on body weight of the frog, until the blood in the artery was replaced completely with Ringer solution. During perfusion, the response to 1 mM CaCl₂ was measured as a reference response. After blood was completely eliminated and stimulation by 1 mM CaCl₂ came to give a constant response, a control response was recorded. Addition of the Ca-channel blockers, cGMP, or cAMP was perfused by switching the perfusing solution to the Ringer solution containing each reagent. All the experiments were carried out at 20°C.

**Chemicals**

cGMP, cAMP, and L-threonine were purchased from Nakarai Co., Kyoto, Japan. D-Galactose, quinine hydrochloride, and theophylline were purchased from Wako Pure Chemical Co., Osaka. Verapamil was kindly supplied by Eisai Co., Tokyo.

**RESULTS**

**Time Course of the Summated Responses to Various Stimuli**

Fig. 1A shows typical records of the summated responses of the frog glossopharyngeal nerve to various chemical stimuli applied to the tongue surface after the lingual artery was perfused with Ringer solution containing 0.2 mM CaCl₂. As seen from the records, the time course of the response is largely different, depending on the species of stimulants. Fig. 1B plots mean values of the responses of 10 preparations at different times after stimulation. Note that the responses at the bottom line are shown with expanded time scale. As seen from the figure, the responses to 1 mM CaCl₂, distilled water, 0.8 M D-galactose, and 0.05 M L-threonine have a relatively small phasic component and a large tonic component. On the other hand, the responses to 0.1 M HCl, 0.1 mM quinine hydrochloride, 2 mM theophylline, and 0.4 M ethanol
Figure 1. A. Typical records of the summated responses to various chemical stimuli applied to the tongue surface after the lingual artery was perfused with Ringer solution containing 0.2 mM CaCl₂. The bar at the bottom of each record represents duration of chemical stimulation. The 30-s scale in the figure repre-
have a large phasic component. The responses to 0.1 mM HCl and 0.1 mM quinine hydrochloride have a very small tonic component, whereas the responses to 2 mM theophylline and 0.4 M ethanol have a distinct tonic component.

**Effect of Ca^{2+} in a Perfusing Solution**

To examine the effect of Ca^{2+} concentration in the intercellular fluid below the tight junction between taste cells on the frog taste nerve responses, the summed responses of the glossopharyngeal nerve to various chemical stimuli applied to the tongue surface were recorded after the lingual artery had been perfused with Ringer solution containing CaCl\textsubscript{2} of various concentrations. Fig. 2 shows the magnitude of the gustatory responses to various stimuli as a function of Ca^{2+} concentration in a perfusing solution. The magnitude of response (R) in the figure represents a peak value of each summed response where the magnitude of the peak response to each stimulus at 0.2 mM CaCl\textsubscript{2} is taken as unit. As seen from the figure, the response to 1 mM CaCl\textsubscript{2}, distilled water, 0.8 M D-galactose, and 0.05 M L-threonine is greatly diminished by lowering Ca^{2+} concentration in a perfusing solution and is increased with increasing Ca^{2+} concentration in a perfusing solution. A similar curve was obtained with the response to 0.4 M NaCl (data not shown). On the other hand, the responses to 0.1 mM quinine hydrochloride, 0.1 mM HCl, and 0.4 M ethanol are practically independent of Ca^{2+} concentration below 0.2 mM and are decreased with a further increase of Ca^{2+} concentration. A similar curve was obtained with 0.5 mM acetic acid and 2 mM theophylline (data not shown). As shown in Fig. 1, the responses to CaCl\textsubscript{2}, distilled water, D-galactose, L-threonine, theophylline, and ethanol have a tonic component. Plots of the magnitude of these responses at 30 s after onset of stimulation also gave essentially similar curves to respective curves shown in Fig. 2.

The effect of Mg^{2+} and K^{+} in the perfusing solution on the responses was examined by perfusing the artery with Mg^{2+}-free solution (112 mM NaCl, 3.4 mM KCl, 2.5 mM NaHCO\textsubscript{3}, 2 mM CaCl\textsubscript{2}) or K^{+}-free solution (112 mM NaCl, 3.6 mM MgSO\textsubscript{4}, 2.5 mM NaHCO\textsubscript{3}, 2 mM CaCl\textsubscript{2}). Neither elimination of Mg^{2+} nor of K^{+} from the perfusing solution practically affected the responses to 1 mM CaCl\textsubscript{2} and 0.1 mM quinine hydrochloride. The effect of elimination of Na^{+} on the responses was not examined because perfusion with Na^{+}-free solution led to loss of the taste nerve activities.

\[ \text{sent the time scale both for the records of the summed responses and the duration of chemical stimulation. The number at the top of each record represents species of chemical stimulus applied which is represented in Fig. 1B.} \]

\[ \text{Average time course of the summed responses of six preparations to various chemical stimuli. Each point at different times after stimulation is the mean value of the relative response at the time to the peak response. The magnitude of the peak response of each pattern is relative value to the peak response to 1 mM CaCl\textsubscript{2}. The expanded time scale is used for the responses at the bottom line. The leftmost pattern at the bottom is the record of the response to 0.1 mM quinine hydrochloride with the expanded time scale.} \]
Effects of Ca-Channel Blockers

Fig. 3 shows the effects of MnCl₂, which is known to block the Ca channel, on the responses to various stimuli when the blockers are added to the perfusing solution containing 0.2 mM CaCl₂. Each column in the figure represents mean values on the responses at the peak recorded from four preparations where the value of the response before application of the Ca-channel blocker is taken as unit for each column. The responses to 1 mM CaCl₂, 0.4 M NaCl (data not shown), distilled water, 0.8 M D-galactose, and 0.05 M L-threonine are greatly suppressed by addition of 0.1 mM MnCl₂. Removal of the blocker from the perfusing solution recovers reversibly the responses to the original level. On the other hand, the responses to 1 mM quinine hydrochloride, 2 mM theophylline, 0.1 mM HCl, 0.5 mM acetic acid (data not shown), and 0.4 M ethanol are unaffected by the addition of the Ca-channel blocker. The above results also indicate that 0.1 mM MnCl₂ does not directly affect the gustatory nerve. The effects of 0.1 mM verapamil on the responses to various stimuli were also examined by adding the Ca-channel blocker to the perfusing solution. The results obtained were essentially similar to those obtained with 0.1 mM MnCl₂.

**Figure 2.** The relative magnitude of the responses (R) to various stimuli as a function of logarithmic concentration of Ca²⁺ in a perfusing Ringer solution. The magnitude of the responses in the figure represents a peak value of each summed response where the magnitude of the peak response to each stimulus at 0.2 mM CaCl₂ is taken as unit. Each point in the figure shows mean value of data obtained from three preparations. Concentration of Ca²⁺ in the presence of 0.1 mM EGTA was calculated based on its binding constant to Ca²⁺.
The above results, together with results shown in Fig. 2, suggest that Ca\(^{2+}\) is involved in the taste transduction of the responses to chemical stimuli of group 1 (CaCl\(_2\), NaCl, distilled water, D-galactose, and L-threonine). On the other hand, the responses to chemical stimuli of group 2 (quinine hydrochloride, theophylline, ethanol, HCl, and acetic acid) seem to be produced without an influx of Ca\(^{2+}\) into taste cells.

**Effect of cGMP**

The effect of cGMP on the response to 1 mM CaCl\(_2\) was examined by adding 0.5 mM cGMP to the perfusing solutions containing various concentrations of CaCl\(_2\).

![Figure 3](image-url). Effect of 0.1 mM MnCl\(_2\) on the responses to various chemical stimuli. Each column represents mean value at the peak of the responses obtained from four preparations. Control (a) represents the response to each stimulus when the lingual artery was perfused with Ringer solution containing 0.2 mM CaCl\(_2\). Columns b and b* represent the responses 5 and 60 min after the addition of the Ca-channel blockers, respectively, and column c represents the responses 15 min after removal of the blocker from the perfusing solution, where the magnitude of the control response is taken as unit.

CaCl\(_2\). Fig. 4A shows typical records of the responses before and after addition of cGMP. Each column in Fig. 4B represents mean values at the peak of the responses obtained from at least five preparations where the value of the response to 1 mM CaCl\(_2\) after perfusion with Ringer solution containing 0.2 mM CaCl\(_2\) and no cGMP is taken as unit for each column. When the lingual artery is perfused with the solution containing 0.002 mM CaCl\(_2\), the addition of cGMP to the perfusing solution brings about only a small enhancement of the response \(\sim\)120 min after addition of cGMP. The response is greatly
enhanced within at most 5 min after addition of cGMP when the artery is perfused with the solutions containing CaCl\textsubscript{2} above 0.02 mM. It is noted that addition of cGMP to the perfusing solution does not practically affect appreciably at least the spontaneous activities of the gustatory nerve (see Fig. 4A).

The effect of cGMP on the responses to various stimuli was examined by adding 0.5 mM cGMP to the perfusing solution containing 0.2 mM CaCl\textsubscript{2}. Fig. 5 represents mean values at the peak of the responses obtained from five preparations before and at different times after addition of cGMP. The responses to 0.4 M NaCl, distilled water, 0.8 M D-galactose, and 0.05 M L-threonine are greatly enhanced by addition of cGMP. On the other hand, the responses to 0.1 mM quinine hydrochloride, 2 mM theophylline (data not shown), 0.4 M ethanol, and 0.1 mM HCl are practically unaffected by addition of cGMP.

As described before, the responses to chemical stimuli of group 1 were greatly suppressed by the Ca-channel blockers added to the perfusing solutions. When cGMP was added to the perfusing solution in the presence of the blockers (MnCl\textsubscript{2} and verapamil), the response to 1 mM CaCl\textsubscript{2} was still greatly suppressed.

**Effect of cAMP**

The effect of cAMP on the responses to 1 mM CaCl\textsubscript{2} was examined by adding 0.5 mM cAMP to the perfusing solution containing 0.2 mM CaCl\textsubscript{2}. Fig. 6A shows the mean values at the peak of the responses obtained from six preparations. As seen from the figure, the responses to chemical stimuli of group 1 (1 mM CaCl\textsubscript{2}, 0.4 M NaCl, distilled water, 0.8 M D-galactose, and 0.05 M L-threonine) are decreased by addition of cAMP. This suppressive effect appears more gradually than the effect of cGMP. Contrary to chemical stimuli of group 1, the responses to group 2 (0.1 mM quinine hydrochloride, 2 mM theophylline [data not shown], 0.4 M ethanol, and 0.1 mM HCl) are not affected by addition of cAMP.

It is known that intracellular concentration of cAMP is 4-60 times higher than that of cGMP (Steiner et al., 1972). This suggests that effective concentration of cAMP in cells is much higher than that of cGMP. Hence the effects of 5 mM cAMP added to the perfusing solution on the responses to various stimuli were examined with four preparations. Fig. 6B shows that the responses to 1 mM CaCl\textsubscript{2} and distilled water are suppressed at most within 5 min after addition of 5 mM cAMP. This is, the time course of the effect of 5 mM cAMP is similar to that of 0.5 mM cGMP. Fig. 6B also shows that addition of 5 mM cAMP to the perfusing solution does not affect the responses to 0.1 mM quinine hydrochloride, 2 mM theophylline, and 0.1 mM HCl.

**DISCUSSION**

The results obtained in the present study indicate that chemical stimuli are classified into two groups. The responses to chemical stimuli of group 1 (CaCl\textsubscript{2}, NaCl, D-galactose, distilled water, and L-threonine) are highly dependent on Ca\textsuperscript{2+} concentration in the perfusing solution, suppressed by the Ca-channel blockers, enhanced by cGMP, and suppressed by cAMP. The responses to
Effect of 0.5 mM cGMP added to the perfusing solution containing various concentrations of CaCl₂ on the summated response to 1 mM CaCl₂. The arrow in the figure means the addition of cGMP. A. Typical records of the summated responses before and at different times after addition of cGMP to the perfusing solution containing 0.2 mM CaCl₂. B. The magnitude of mean values at the peak of the responses obtained from at least five preparations where the value of the peak response to 1 mM CaCl₂ after perfusion with Ringer solution containing 0.2 mM CaCl₂ and no cGMP is taken as unit for each column. Time indicated at the bottom of each column shows time after addition of cGMP.
chemical stimuli of group 2 (quinine hydrochloride, theophylline, ethanol, and HCl) are practically not affected by a decrease in Ca\(^{2+}\) concentration in a perfusing solution, the Ca-channel blockers, cGMP, and cAMP. The responses to chemical stimuli of group 1 have large tonic components and those
Figure 6. Effect of 0.5 mM cAMP (A) and 5 mM cAMP (B) added to the perfusing Ringer solution containing 0.2 mM CaCl₂ on the responses to various stimuli. Each column represents mean value at the peak of the response obtained from six preparations (A) and four preparations (B) before and at different times after addition of cGMP indicated by the arrow. Time indicated at the bottom of each column represents time after addition of cGMP. The control response to each stimulus before addition of cGMP is taken as unit. Typical records of the summed responses illustrating the effect of cAMP are shown at the top of panel B.
to group 2 have large phasic components (see Fig. 1). However, the above classification does not depend only on type of phasic and tonic components; the responses to group 1, which have phasic and tonic components, are greatly affected by various reagents but the responses to ethanol and theophylline, which also have both components, are not affected by the reagents.

In the present study, various reagents were added to a solution perfusing the lingual artery. Application of the reagents (0.5 mM cGMP, 5 mM cAMP, 0.5 mM their dibutyryl derivatives, 0.1 mM EGTA, and 0.1 mM verapamil) on the tongue surface brought about practically no effect on the gustatory responses. Application of 0.1 mM MnCl₂ on the tongue led to enhancement of the responses to salt stimuli (Kashiwagura et al., 1978), whereas addition of MnCl₂ to the perfusing solution suppressed the responses to salt stimuli. It was difficult to know how fast reagents added to the perfusing solution reached taste cells. The effect of 0.1 mM MnCl₂, 0.1 mM verapamil, 0.5 mM cGMP, and 5 mM cAMP added to the perfusing solution appeared within 5 min after addition of these reagents. It took most of this time until the reagents added to the artery via a polyethylene tube reached taste cells, which was inferred by observing diffusion of methylene blue added to the perfusing solution into tongue tissue.

The present results indicated that a transduction mechanism of the responses to chemical stimuli of group 1 differs from that of the responses to group 2. A possible transduction mechanism for chemical stimuli of group 1 is as follows. Application of chemical stimuli to the tongue depolarizes taste cells. Two mechanisms on depolarization of taste cells have been proposed. According to one mechanism, adsorption of chemical stimuli on the microvilli membrane induces the surface pressure changes at the membrane, which propagates along the cell membranes and induces ion permeability changes at the taste cell membrane below the tight junction (Beidler, 1971; DeSimone et al., 1977). According to another mechanism, adsorption of chemical stimuli on the microvilli membrane induces the surface potential changes as well as the ion permeability changes at the microvilli membranes, which causes depolarization at the membrane (Kamo et al., 1974; Kurihara et al., 1978). The depolarization induces electric current from the microvilli to the cell membranes below the tight junction and depolarizes the synaptic area of the cells (Kashiwayanagi et al., 1981). Regardless of the depolarization mechanisms, the depolarization at the synaptic area of the taste cells opens voltage-dependent Ca channel at the synaptic area and induces Ca influx from the intercellular medium into the taste cells. The Ca influx will lead to a release of a chemical transmitter. Application of chemicals to the tongue also seems to activate adenylate cyclase, guanylate cyclase, or phosphodiesterase located at the microvilli membrane and change cyclic nucleotides level in taste cells. However, the cyclic nucleotides themselves seem not to contribute to the changes in the membrane potential of the taste cell since the nucleotides did not practically affect the spontaneous activities of the gustatory nerve. The nucleotide (cGMP) did not exhibit any effect on the gustatory nerve responses under the conditions that Ca²⁺ concentration in the perfusing solution is low
(0.002 mM) or the Ca-channel blockers are present in the solution. These results suggest that the cyclic nucleotide acts in the presynaptic area. The cyclic nucleotides probably modulate the Ca influx triggered by depolarization. The following data in other papers also support the idea that the cyclic nucleotides operate in the taste cell. Adenylate cyclase, guanylate cyclase, and phosphodiesterase exist only at the apex of the taste buds (Nomura, 1978; Nomura and Asanuma, 1980). The external application of dibutyryl cyclic GMP to the blowfly chemoreceptor with stimulant sucrose increased the firing of the sugar-sensitive receptor and that of cAMP decreased the firing (Daley and vande Berg, 1976), although the extent of the enhancement and the suppression by the nucleotides was much less than that in the present study.

Recently, a large number of papers on the role of cGMP in the visual transduction mechanism has been published (see references cited before). The kinetic studies of light-activated phosphodiesterase in rod outer segment (Yee and Liebman, 1978; Stryer et al., 1981) suggested that high specific activity of the enzyme could provide gain comparable to that required of a mechanism mediating visual excitation. The present results suggested that cyclic nucleotides modulate the gustatory responses rather than mediate them. The actual time course of the effect of the cyclic nucleotides could not be determined in the present study and hence it is not known whether the nucleotides modulate both the phasic response and the tonic response or only the tonic response. It is interesting to note that cGMP is involved in intracellular signaling in the chemotactic response of Escherichia coli (Black et al., 1980).

The present results showed that cGMP and cAMP exhibit opposite effects on the responses to chemical stimuli. The examples that the two cyclic nucleotides produce opposite effects are known in various systems (Goldberg et al., 1973). In addition, it is often observed that the agent increasing cGMP level in cells leads to a decrease in cAMP level (Goldberg et al., 1973). There are two concepts to explain these effects of the two cyclic nucleotides: the "dualism concept" of the physiological regulation through opposite actions of cGMP and cAMP, and the "unitary concept" of the regulation imposed through one species of the cyclic nucleotides. It is unknown at present which concept is applicable to the regulation of taste responses under the physiological conditions.

The transduction mechanism for chemical stimuli of group 2 is unknown at present. The results obtained in the present study suggested that these stimuli produce neural responses without accompanying Ca influx into taste cells. In this connection, it is interesting to note that HCl and quinine induce depolarization in a frog taste cell with no or only a small decrease in the membrane resistance (Akaike et al., 1976). One may consider the possibility that taste nerve responses to chemical stimuli of group 2 were brought about not by stimulation of taste receptors but by direct stimulation of the nerve endings with chemical stimuli since the responses to these stimuli are considered to be induced without Ca influx into taste cells. However, a large number of data have been reported that indicate that the taste receptor potentials are induced by HCl and quinine in concentration regions similar to those where the neural
response to respective stimuli are induced (Kimura and Beidler, 1961; Sato, 1973, 1980). Furthermore, Akaike et al. (1976) directly demonstrated that in the frog the relations between the neural responses to various chemical stimuli including HCl and quinine and their concentrations are similar to relations for respective stimuli between the receptor potentials and their concentrations. Therefore, it is more likely that the taste nerve responses to chemical stimuli of group 2 were also brought about by stimulation of the taste cells. Recently, we found that responses to chemical stimuli of group 2 as well as group 1 are greatly increased after the frog lingual artery was perfused with Ringer solution containing noradrenaline (Nagahama and Kurihara, unpublished data). This suggested that the uptake of noradrenaline into taste cells results in the enhancement of the responses and that noradrenaline is released from taste cells in response to chemical stimuli of both group 1 and group 2. Examples that secretion of transmitters and hormones is induced by both mechanisms via Ca influx and not via Ca influx are known; e.g., verapamil, which is a Ca-channel blocker, did not inhibit the first phase of glucose-induced insulin release from islet β cell but inhibited the second phase (Wollheim and Sharp, 1981). Considering together the discussions in these secretion systems, the stimulation by the chemicals of group 2 may lead to a release of the chemical transmitter from taste cells via an increase in Ca\(^{2+}\) concentration in the cytosol by inhibition of Ca efflux or by a release of Ca\(^{2+}\) from the intracellular stores. However, further study will be needed to explore the true mechanism by which the taste responses are induced by chemical stimuli of group 2.

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