Adaptation-promoting Effect of IP$_3$, Ca^{2+}, and Phorbol Ester on the Sugar Taste Receptor Cell of the Blowfly, *Phormia regina*

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ABSTRACT The fly has a receptor cell highly specialized for the taste of sugars. We introduced inositol 1,4,5-trisphosphate (IP$_3$), Ca^{2+}, or a phorbol ester, 12-deoxyphorbol 13-isobutylate 20-acetate (DPBA), into the cell and investigated their effects on the response to sucrose. The sugar receptor cell generates impulses during constant stimulation with sucrose, but the impulse frequency gradually declines as the cell adapts to the stimulus. Thus, this gradual reduction of the impulse frequency is a direct manifestation of adaptation of the cell. These reagents accelerated the gradual reduction of the impulse frequency, although the initial impulse frequency was little affected. In contrast to these reagents, glycolether-diamine-tetraacetate (EGTA) retarded the gradual reduction of the impulse frequency. Moreover, when IP$_3$ and DPBA were applied together, the gradual reduction of the impulse frequency was more accelerated than when either IP$_3$ or DPBA was applied. When IP$_3$ and EGTA were applied together, however, the accelerating effect of IP$_3$ tended to be canceled. Based on these results, we hypothesized that an intracellular cascade involving inositol phospholipid hydrolysis, intracellular Ca^{2+} mobilization, and protein kinase C–mediated phosphorylation promotes adaptation of the sugar receptor cell.

INTRODUCTION

In vertebrate taste cells, Avenet, Hofmann, and Lindemann (1988) and Tonosaki and Funakoshi (1988) independently reported with frog and mouse that cyclic nucleotide acts as an excitatory intracellular messenger as in visual (Fesenko, Kolesnikov, and Lyubarsky, 1985) and olfactory cells (Nakamura and Gold, 1987). At that time, Tonosaki and Funakoshi (1988) showed that the cell, which responded to sucrose, was also depolarized by a Ca^{2+} chelator EGTA, but concluded that Ca^{2+} is unlikely to act as an excitatory intracellular messenger. Contrary to them, Akabas, Dodd, and Al-Awqati (1988) showed that a bitter substance induced a rise in intracellular Ca^{2+} in a subpopulation of rat taste cells and concluded that Ca^{2+} may be involved in the

bitter taste transduction. However, it can be complicated to discuss diverse studies on the intracellular messenger in vertebrate taste systems because characterization, identification, and classification of the individual receptor cells within a taste bud have not been completely established.

The taste organ of the fly is a sensillum showing a much less complicated structure than the vertebrate taste bud. A sensillum contains four sensory processes elongated from four functionally differentiated taste cells. They respond with unique impulses to sugars, salts, water, and large anions like caprylate, respectively. Thus, it is easy to observe the electrophysiological response from a single taste cell by giving its adequate stimulus to a sensillum. Furthermore, it is also easy to examine pharmacological effects of reagents on a single taste cell by applying reagents to a sensillum and then checking the response.

Amakawa and Ozaki (1989) previously studied the effects of protein kinase inhibitors and activators on the sugar receptor cell of the fly. A cAMP- or cGMP-dependent protein kinase inhibitor, N-[2-(methylamino)ethyl]-5-isoquinolinesulphonamide dihydrochloride (H-8) depressed overall impulse discharge from the sugar receptor cell. As is the case with the frog taste cell (Avenet et al., 1988), a cyclic nucleotide might also act as an excitatory intracellular messenger via protein kinase in the sugar receptor cell of the fly. On the other hand, a protein kinase C inhibitor, 1-(5-isoquinolinylsulphonyl)-2-methylpiperazine dihydrochloride (H-7) retarded gradual reduction of the impulse frequency, and phorbol esters (which activate protein kinase C) accelerated it. Thus, the activity of protein kinase C is likely to be concerned with the cell adaptation. As an activating factor for protein kinase C, Ca^{2+} was then expected to be an intracellular messenger for the taste adaptation. Starting from these findings, we tried to confirm the role of Ca^{2+} and propose a hypothetical adaptation-promoting cascade in the sugar receptor cell of the fly.

MATERIALS AND METHODS

Materials

The adult blowfly, Phormia regina, reared in our laboratory, was decapitated 5–7 d after emergence. The isolated head was mounted and the largest type of sensilla on the labellum was used for the experiments.

Impulse Recording

The sugar receptor cell extends a sensory process into a cuticular sheath of the sensillum having a tiny pore at the tip. When the stimulus sugar coming through the tiny pore reaches the receptor membrane at the top of the sensory process of the sugar receptor cell, inward current occurs across the membrane and evokes the impulse generation near the cell body (Morita, 1959).

The impulses were recorded by the tip recording method (Hodgson, Lettvin, and Roeder, 1955). The sensillum was capped with a recording electrode of glass capillary containing 0.1 M sucrose and 10 mM NaCl so that stimulation and impulse recording were carried out at the same time.

The side-wall recording method, by which the receptor potential as well as the impulses are recorded, was not applied here because it can be used for simple experiments of response recording but not for complex experiments combined with reagent application.

When the stimulus sugar is given to a sensillum tip as 10 mM NaCl solution, not only the sugar but also the water receptor cells discharge their impulses, although the salt receptor cell discharges few impulses. However, these impulses are easily distinguished from each other so that we could selectively count the impulses of the sugar receptor cell (see Discussion and Fig. 8).

Reagent Application

Reagents were also applied to the sensillum tip with the glass capillary. The applied reagent first reaches the receptor membranes at the top of the sensory process and then diffuses along the 400-μm-long sensory processes to the cell bodies. Therefore, the reagent most strongly affects the receptor membranes but affects the cell bodies or the impulse-generating region less. Effects on the axons may be negligible.

By this method, four sensory processes within a sensillum were exposed to the same reagent at the same time, but its effects on the sugar receptor cell can be selectively reflected in the response to sucrose.

To investigate the effect of extracellular Ca^{2+}, we simply added $CaCl_2$ in the recording electrode besides 0.1 M sucrose and 10 mM NaCl. To introduce the Ca^{2+} into the cell, the sensillum tip was preincubated with a Ca^{2+} ionophore, A23187, for 2 min. When we state "without Ca^{2+}" or "0 mM Ca^{2+}," it means nominal 0 mM Ca^{2+}. We did not prepare a 0 mM Ca^{2+} solution by manipulating with chelators.

Since the sensory processes are very thin and enclosed with a rigid cuticle sheath, microinjection was not possible. Thus, the sodium deoxycholate (DOC) method (Amakawa and Ozaki, 1989) was applied to introduce inositol 1,4,5-trisphosphate (IP₃), glycolether-diamine-tetraacetate (EGTA), or 12-deoxyphorbol 13-isobutylate 20-acetate (DPBA) into the cell. The sensillum tip was incubated in a capillary containing 0.03% DOC plus reagent dissolved in 67 mM sodium phosphate buffer (pH 7.2) for 2 min. This concentration of DOC is three times lower than the critical micellar concentration, so that it hardly induces membrane lysis but temporarily makes the receptor membrane permeable to water-soluble reagents. Immediately after the incubation, the impulse frequency of response to 0.1 M sucrose is depressed to 70% of that before the incubation but recovers completely within 5 min. Thus, to examine the effects of these reagents on the sugar receptor cell, the responses to sucrose recorded 5 min after incubations with plain DOC and with DOC plus reagent were compared.

Reliability of the DOC Method

If the incubating capillary containing 0.03% DOC was connected to the recording electrode, noisy negative pulses were observed during the incubation. These pulses were first observed by Kashihara, Y., M. Ninomiya, M. Ozaki, and H. Morita (manuscript in preparation). They are apparently different from the injury discharge of impulses accompanied by membrane lysis. Fig. 1 shows the noisy negative pulses (*A*) and the injury discharge of impulses (*B*) recorded during incubation of the sensillum with 0.03% DOC. At this concentration the injury discharge occurred very seldom, and if it did occur, only one type of impulses was observed. Kashihara et al. (manuscript in preparation) thought that the noisy negative pulses represent inward currents across the receptor membrane, resulting from the repetition of the momentary tiny breakdowns of the receptor membrane. When the concentration of DOC was <0.03%, such electric signals were not always observed and the experiments were not successful. If the concentration of DOC was higher than the critical micellar concentration, membrane lysis frequently occurred at the top of the sensory process and the injury discharge of three or four types of impulses was usually observed instead of the noisy negative pulses. Once the membrane lysis occurred, it took 30 min–1 h for the sugar receptor cell to recover normal

responsiveness (Ninomiya, Ozaki, Kashihara, and Morita, 1986). Thus, either no electric signal or the injury discharge of impulses was a bad sign for the experiments, whereas the noisy negative pulses indicated a moderate looseness of the receptor membrane becoming permeable to the water-soluble reagents.

By electron microscopic investigations, a number of membrane vesicles or fragments were found within several micrometers from the tip of a sensillum fixed within 1 min after the incubation with 0.1% DOC (nearly equal to the critical micellar concentration) for 2 min (Ozaki, Ninomiya, Kashihara, and Morita, 1986). These irregular membranes resulted from the membrane lysis of the sensory processes. In a sensillum fixed within 1 min after the incubation with 0.03% DOC treatment, however, the four sensory processes were observed from the top to the bottom of the sensillum and the membranes of these sensory processes looked as tight as those in the intact sensilla. Receptor membranes that become loose during the incubation may become tight again within 5 min. Thus, the water-soluble reagents that penetrate into the cell during the incubation may be able to remain in the cell.

It is difficult to determine the concentrations of the reagents remaining in the cell. The receptor membrane can be a significant barrier to the water-soluble reagents even if DOC helps them to penetrate. The 2-min incubation might be too short for the reagents to equilibrate on both sides of the membrane. Moreover, the reagents that succeed in penetrating into the cell may immediately start to diffuse along the sensory process to the cell body. At any rate, the
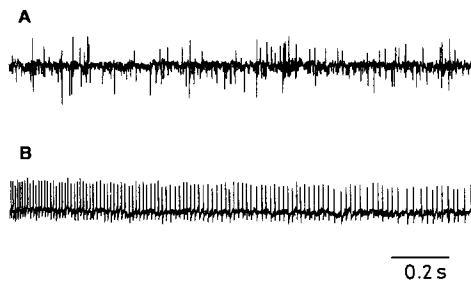

FIGURE 1. Electrical records of the noisy negative pulses (A) and the injury discharge of impulses (B). These electrical signals were recorded during the incubation of sensilla with 0.03% DOC in 67 mM phosphate buffer (pH 7.2).

concentrations of the reagents remaining inside of the cell at 5 min after the treatment should be much less than those given in the incubating capillaries. Thus, we reserved an absolute evaluation of the efficacies of the reagents.

Declining Sensitivity Curve

Based on impulse recordings lasting ~4 s, declining sensitivity curves were plotted, showing the number of impulses generated every 0.2 s versus time after the beginning of stimulation. However, the impulses during the initial period (to 0.15 s after the beginning of stimulation) were ignored because the impulse frequency was not proportional to the receptor potential during the initial period (Morita, 1969, 1972). Impulses were recorded from the oscilloscope with a pen recorder and the number of impulses was manually counted. As a convenient way to evaluate the declining sensitivity rate, $t_{1/2}$ was defined as the time it took the impulse number to decrease by 50% of the first count for each declining sensitivity curve.

In a series of experiments, data were obtained from a single batch of flies; for some unknown reason $t_{1/2}$ differed between batches. Such differences in $t_{1/2}$ can also be found between normal declining sensitivity curves for the response to sucrose in Fig. 3 ($t_{1/2}$ = 1.63 s) and Figs. 4–7 ($t_{1/2}$ = 1.27–1.34 s).

Chemicals

IP₃ and dibutyryl cyclic GMP (dbcGMP) were purchased from Sigma Chemical Co. (St. Louis, MO). A23187 and DPBA were from Funakoshi Chemicals Ltd. (Tokyo, Japan).

RESULTS

In response to continuous sucrose stimulation of the sugar receptor cell, the impulse frequency rises to an initial burst and then gradually decreases to zero level. However, an adequate interval after the stimulation can completely restore the responsiveness of the cell. The cell that is conditioned by a constant stimulation with sucrose has a concentration–response curve of the same shape as that before conditioning, but simply shifted to the right (Ozaki, M., and T. Amakawa, manuscript in preparation). Thus, the gradual reduction of the impulse frequency is regarded as a direct manifestation of a cell adaptation involving a decline of sensitivity rather than a general diminution in responsiveness.

Fig. 2 shows three impulse trains recorded with the same stimulus of 0.1 M sucrose from the same sensillum. They are obviously different from each other. In comparison with Fig. 2 *A*, which is an impulse discharge of ordinary response from the sugar
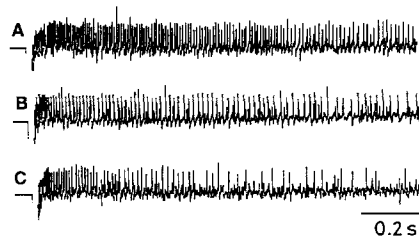

FIGURE 2. Electrical records of impulses in response to 0.1 M sucrose (*A*), 0.1 M sucrose plus 6.4 mM CaCl₂ (*B*), and 0.1 M sucrose plus 1.6 mM CaCl₂ after A23187 incubation (*C*).

receptor cell, Fig. 2 *B* shows an overall depressed impulse frequency. Impulse frequency in Fig. 2 *C* is hardly depressed in the initial period but rapidly decreases after that. In other words, *B* shows a decline of responsiveness of the cell, whereas in *C* responsiveness does not simply decline, but rather adaptation of the cell is accelerated. Actually, before stimulation and impulse recording, we treated the sensillum tip with nothing in *A*, with Ca²⁺ extracellularly in *B*, and with Ca²⁺ intracellularly through a Ca²⁺ ionophore, A23187, in *C*. At this point we start to examine the effects of Ca²⁺, IP₃, or DPBA on the cell adaptation by establishing alterations in impulse frequency. All data were plotted in declining sensitivity curves, so that the alterations in impulse frequency were easily evaluated.

Effects of Ca²⁺

We first treated the sensillum tip extracellularly with Ca²⁺ (Fig. 3). As long as the Ca²⁺ concentration was lower than 1.6 mM, overall impulse frequency tended to decrease slightly but hardly any changes were seen in the declining sensitivity rate (●, ▲). The time for the impulse frequency to be reduced by half, $t_{1/2}$, was ~1.6 s. When the Ca²⁺ concentration was increased to 6.4 mM, overall impulse frequency
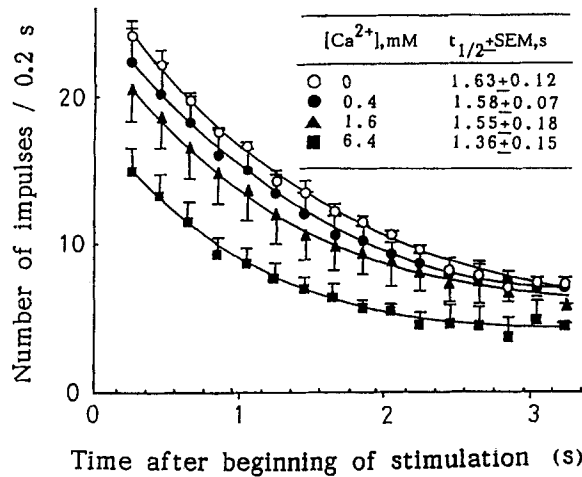
[Ca^{2+}],mM	t$_{1/2}$±SEM,s
O 0	1.63±0.12
● 0.4	1.58±0.07
▲ 1.6	1.55±0.18
■ 6.4	1.36±0.15

FIGURE 3. Effect of Ca^{2+} on the declining sensitivity curve for the sugar receptor. The receptor cell was stimulated with 0.1 M sucrose plus 0 (O), 0.4 (●), 1.6 (▲), and 6.4 mM CaCl$_2$ (■). Declining sensitivity curves are shown with the mean number of impulses ± SEM for five sensilla tested in Figs. 3 and 4. Insets summarize $t_{1/2}$ for each curve in Figs. 3–8.

was apparently depressed (■). However, the declining sensitivity rate was still not significantly affected. $t_{1/2}$ was then altered from 1.63 to 1.36 s, but this difference was not statistically significant by Student's t test with a 95% confidence level. The overall depressed impulse frequency induced by the extracellularly added Ca^{2+} might result from nonspecific adsorption of Ca^{2+} into the receptor membrane. Excess Ca^{2+} might block or modulate receptor proteins or ion channels on the receptor membrane from outside.

Next, we preincubated the sensillum tip with a Ca^{2+} ionophore, A23187, and carried out the same experiment (Fig. 4). With this treatment, the receptor membrane of the sugar receptor cell became permeable to Ca^{2+} and the extracellularly added Ca^{2+} could permeate into the cell and act from inside. Under these conditions, the decline of sensitivity was accelerated in a Ca^{2+}-dependent manner (●, ▲). As the Ca^{2+} concentration increased from 0 to 1.6 mM, $t_{1/2}$ decreased from 1.15 to 0.58 s. In

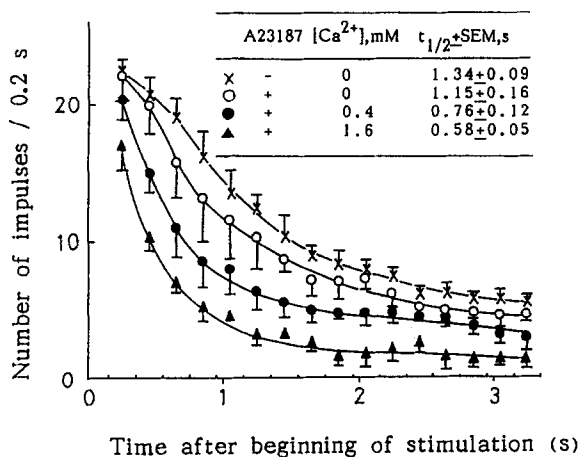| A23187	[Ca^{2+}],mM	t$_{1/2}$±SEM,s
X −	0	1.34±0.09
O +	0	1.15±0.16
● +	0.4	0.76±0.12
▲ +	1.6	0.58±0.05

FIGURE 4. Effect of Ca^{2+} introduced into the cell through ionophore on the declining sensitivity curve for the sugar receptor. The receptor cell was incubated with 10 μg/ml A23187 for 2 min and stimulated with 0.1 M sucrose plus 0 (O), 0.4 (●), or 1.6 mM CaCl$_2$ (▲). The declining sensitivity curve before A23187 incubation is plotted with X's.

Time after beginning of stimulation (s)

comparison with the intact cell ($\times$), the cell preincubated with A23187 showed a slightly accelerated decline of sensitivity even when the cell was stimulated without Ca^{2+} ($\bigcirc$).

Recently, Amakawa, Ozaki, and Kawata (1990) found that a membrane-permeable cGMP analogue, dbcGMP, evokes impulse discharge from the sugar receptor cell. However, the impulse discharge showed remarkably less decline of sensitivity than the impulse discharge from sucrose, and was not influenced by Ca^{2+}, which was introduced into the cell through A23187.

Involvement of the Inositol Phospholipid Pathway

As IP_3, one of the products of phosphatidylinositol bisphosphate (PIP_2) hydrolysis, is well known to release Ca^{2+} from intracellular Ca^{2+} sources, we introduced IP_3 into
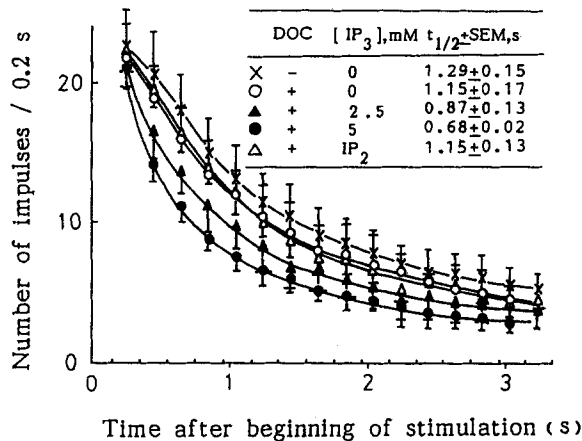

FIGURE 5. Effect of IP_3 on the declining sensitivity curve for the sugar receptor. The receptor cell was stimulated with 0.1 M sucrose ($\times$), incubated with various incubation solutions for 2 min, and stimulated with 0.1 M sucrose 5 min after that. The incubation solution contains 0.03% DOC in 67 mM phosphate buffer (pH 7.2) ($\bigcirc$), plus 5 mM IP_2 ($\triangle$), 2.5 mM ($\blacktriangle$), or 5 mM ($\bullet$) IP_3. Declining sensitivity curves are shown with mean number of impulses $\pm$ SEM for 25 tested sensilla before incubation and 5 sensilla tested after each incubation.

the cell (Fig. 5). Since we adopted the DOC method to introduce IP_3, the declining sensitivity curve for the response at 5 min after the incubation with DOC alone ($\bigcirc$) should be a control. This control curve ($\bigcirc$) tended to be a little steeper than the normal declining sensitivity curve ($\times$), presumably because of some penetration of Ca^{2+} from the sensillum lymph into the cell (Amakawa and Ozaki, 1989). When the sensillum tip was incubated with DOC plus IP_3, decline of sensitivity was obviously accelerated in a IP_3-dependent manner ($\bullet$, $\blacktriangle$). Inositol bisphosphate (IP_2), when applied instead of IP_3 for a stricter control, showed no effect on the decline of sensitivity ($\triangle$).

In contrast to Ca^{2+} or IP_3, EGTA retarded the decline of sensitivity ($\blacktriangle$). When IP_3 was applied with EGTA, the accelerating effect of IP_3 on decline of sensitivity appeared to be cancelled ($\triangle$) (Fig. 6). The $t_{1/2}$ decreased from 1.16 to 0.67 s by 5 mM IP_3 and increased to 1.82 s by 2 mM EGTA, but was 1.19 s when IP_3 and EGTA were

DOC	IP$_3$	EGTA	$t_{1/2}\pm$SEM,s
X	-	-	-	1.29±0.17
O	+	-	-	1.16±0.13
▲	+	-	+	1.82±0.18
●	+	+	-	0.67±0.03
△	+	+	+	1.19±0.05

Number of impulses / 0.2 s
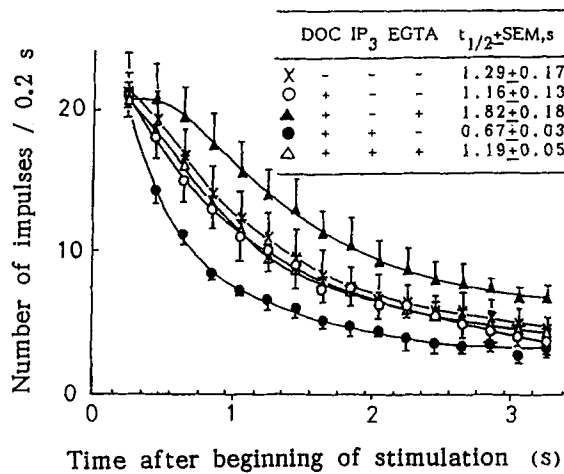
Time after beginning of stimulation (s)

FIGURE 6. Effect of IP$_3$ and/or EGTA on the declining sensitivity curve for the sugar receptor. The experimental procedure was the same as in Fig. 4 but the incubation solution contained 0.03% DOC in the same buffer (O), plus 5 mM IP$_3$ (●), 2 mM EGTA (▲), or 5 mM IP$_3$ and 2 mM EGTA (△). Declining sensitivity curves are shown with mean number of impulses ± SEM for 20 tested sensilla before incubation and 5 sensilla tested after each incubation.

applied together. These results suggested that EGTA chelates the Ca^{2+} that is mobilized by IP$_3$, resulting in cancellation of the effect of IP$_3$.

Moreover, a phorbol ester is known to accelerate the decline of sensitivity (Amakawa and Ozaki, 1989). When IP$_3$ was applied with a phorbol ester, DPBA (△), the decline of sensitivity tended to be more accelerated than when either IP$_3$ (●) or DPBA was applied (▲) (Fig. 7). Under these conditions, $t_{1/2}$ was altered from 1.16 to 0.67 and 0.79 s by 5 mM IP$_3$ and 1 mM DPBA, respectively, but was 0.61 s when both were applied together. The decrease in $t_{1/2}$ from 0.67 s with IP$_3$ to 0.61 s with IP$_3$ plus DPBA was statistically significant by Student's t test with a 95% confidence level.

We usually detected the effects of reagents applied by the DOC method 5 min after the treatments. However, when we tried to detect them later, all the effects gradually diminished and completely reversed 15–30 min after the treatments.
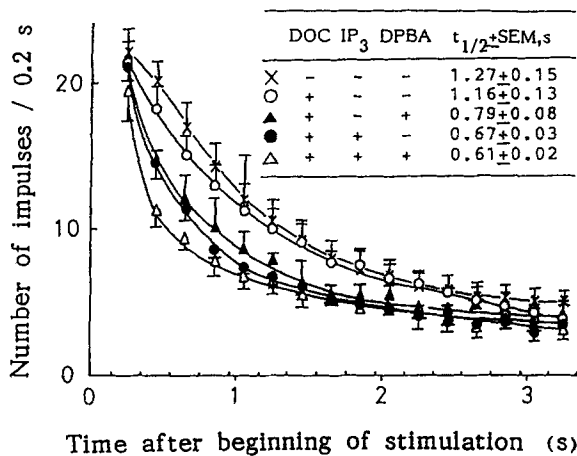
DOC	IP$_3$	DPBA	$t_{1/2}\pm$SEM,s
X	-	-	-	1.27±0.15
O	+	-	-	1.16±0.13
▲	+	-	+	0.79±0.08
●	+	+	-	0.67±0.03
△	+	+	+	0.61±0.02

Number of impulses / 0.2 s

Time after beginning of stimulation (s)

FIGURE 7. Effect of IP$_3$ and/or DPBA on the declining sensitivity curve for the sugar receptor. The experimental procedure was the same as in Fig. 4 but the incubation solution contained 0.03% DOC in the same buffer (O), plus 5 mM IP$_3$ (●), 1 mM DPBA (▲), or 5 mM IP$_3$ and 1 mM DPBA (△). Declining sensitivity curves are shown with mean number of impulses ± SEM for 20 tested sensilla before incubation and 5 sensilla tested after each incubation.

DISCUSSION

Impulse Frequency and Receptor Potential

In the sugar receptor cell of the fly, the receptor potential is generated by the inward currents across the receptor membrane at the top of the sensory process. The inward currents hardly leak across the lateral membrane of the sensory process because of an insulator of cuticle wall (dendritic sheath), but flow out near the cell body and evoke the impulses there (Morita, 1969, 1972).

The lower trace in Fig. 8 A is a side-wall record of the response to 0.1 M sucrose. (The side-wall recording method is an extracellular recording method, but the negative-going potential recorded during stimulation is derived from the receptor potential of the exciting receptor cell within the sensillum. Although the recorded
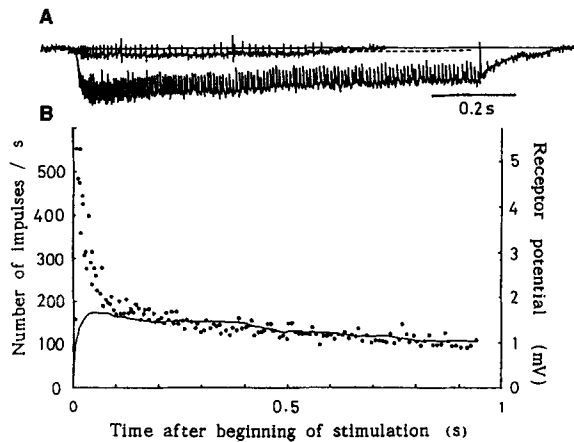

FIGURE 8. Proportionality of the impulse frequency to the receptor potential of the sugar receptor cell. A fine time course of the impulse frequency (● in B) was constructed by plotting the reciprocals of the intervals between impulses of the sugar receptor cell responding to 0.1 M sucrose versus time after the beginning of stimulation. Time course of the receptor potential (solid line in B) was traced after a correction for the contribution of the water receptor cell by subtracting the DC component of a side-wall record of the response to distilled water (*upper trace* in A) from that of the response to 0.1 M sucrose (*lower trace* in A). The upper trace to be subtracted from the lower trace was partially extrapolated as indicated by a broken line, because the stimulation duration was not long enough.

potential was a reduced receptor potential, we simply call it receptor potential, according to Morita [1969, 1972].) The baseline starts to decline immediately after the beginning of stimulation, showing the receptor potential generation. Besides the impulses of the sugar receptor cell (large spikes), the impulses of the water receptor cell (small spikes) are seen on the trace. Although some are hidden, the impulses of the water receptor cell show almost the same height and frequency as the impulses in the upper trace, which is the response to distilled water.

Fig. 8 B shows the time courses of the impulse frequency and receptor potential of the sugar receptor cell. The time course of the impulse frequency (●) was drawn by plotting the reciprocals of the intervals between impulses of the sugar receptor cell. The time course of the receptor potential (solid line) was constructed by subtracting the DC component of the upper trace from that of the lower trace in Fig. 8 A. The

polarity of the receptor potential is reversed and the ordinates are chosen so that these two time courses overlap each other as much as possible. They do not overlap during the initial 0.15 s but completely overlap after that. This suggests that the impulse generation is well controlled by the receptor potential. Thus, investigation of the gradual decrease in the impulse frequency is a useful way to evaluate the cell adaptation as a feedback regulation of the transduction process to the receptor potential generation. However, we should not discuss the change in the impulse frequency as a reflection of the cell adaptation during the initial 0.15 s because the impulse frequency is not in proportion to the receptor potential in this period. Amakawa (1991) once observed the acceleration of the decline of sensitivity by phorbol esters at the receptor potential level as well as at the impulse frequency level in a preliminary experiment. This suggests that the reagents promote the feedback regulation for the receptor potential generation process before the impulse generation.

Ca^{2+} and Taste Responses

Previously, Akabas et al. (1988) reported that in some rat taste cells a bitter substance induced a rise in intracellular Ca^{2+} concentration and the Ca^{2+} source was intracellular. They examined saccharine, a sweet substance, and saw no intracellular Ca^{2+} concentration change. These results implied that taste cells that respond to bitter substances have an intracellular Ca^{2+}-regulating mechanism involving inositol phospholipid hydrolysis coupled with the receptor protein. However, the cells that respond to saccharin do not have such a Ca^{2+}-regulating mechanism, or the mechanism is uncoupled with the "saccharin receptor protein." They concluded that Ca^{2+} is an excitatory intracellular messenger for bitter taste transduction.

We have not succeeded in visualizing a rise in intracellular Ca^{2+} with a Ca^{2+}-sensitive fluorescent dye because the cuticle sheath of the sensillum shows significant autofluorescence. We also found no subcellular structures that could serve as intracellular Ca^{2+} sources in the sensory processes, except microtubules and fuzzy strings. However, our present data imply that the sugar receptor cell of the fly also has a stimulus-dependent, intracellular Ca^{2+}-regulating mechanism involving inositol phospholipid hydrolysis.

As for other taste cells in the sensillum, we did not find any notable effects of 1.6 mM Ca^{2+} on responses of the salt and water receptor cells. The sugar, salt, and water receptor cells may have different transduction mechanisms and different feedback regulation mechanisms. Unfortunately, the fly has no specialized taste cell for bitter substances, but it is possible that different types of taste cells have similar intracellular mechanisms in different animals. When our results are compared with those of Akabas et al. (1988), however, it is remarkable that similar mechanisms are likely to work for transduction in bitter taste cells of the rat and for adaptation of sugar receptor cells of the fly. As both transduction and adaptation mechanisms occur nearly simultaneously in the same region, it is frequently difficult to identify whether a stimulus-dependent factor contributes to transduction or adaptation. In the sugar receptor cell of the fly, Ca^{2+} is more likely to be an intracellular messenger for adaptation than for transduction.

An Adaptation-promotive Cascade Model

Our electrophysiological results are summarized as follows: (*a*) The extracellularly applied Ca^{2+} depressed the response of the sugar receptor cell, but did not accelerate the decline of sensitivity. (*b*) When the cell was pretreated with A23187, the Ca^{2+} accelerated the decline of sensitivity even at such a low concentration that the response of the intact cell was hardly affected. (*c*) IP_3 applied by the DOC method accelerated the decline of sensitivity. (*d*) EGTA applied by the DOC method retarded the decline of sensitivity. (*e*) EGTA applied with IP_3 by the DOC method canceled the effect of IP_3. (*f*) A phorbol ester, DPBA, applied by the DOC method accelerated the decline of sensitivity. (*g*) The DPBA applied with IP_3 by the DOC method further accelerated the decline of sensitivity.

Result *b* suggests that Ca^{2+} is involved in the adaptation mechanism of the sugar receptor cell. Considering *a* and *b*, the Ca^{2+} is likely to act on the adaptation mechanism from inside. Results *c* and *e* show that IP_3 also accelerates the adaptation and that the effect of IP_3 may be mediated by Ca^{2+}. Because of result *a*, the Ca^{2+} that affects adaptation is unlikely to come from outside of the cell through channels on the receptor membrane. It is most probable that the intracellular Ca^{2+} mobilized by IP_3 affects the adaptation. Furthermore, result *f* is consistent with our previous results suggesting that the adaptation mechanism involves protein kinase C (Amakawa and Ozaki, 1989), and result *g* raises a possibility that the Ca^{2+} mobilized by IP_3 affects the adaptation via protein kinase C.

To interpret these results consistently, we speculated a cascade-promoting cell adaptation. The first step of the cascade may be activation of phospholipase C, which might couple with the receptor protein via a GTP-binding protein as reported in the squid photoreceptor cell (Baer and Saibil, 1988). Then, the inositol phospholipid pathway starts mobilization of Ca^{2+} and activation of protein kinase C. One possible scheme for the feedback regulation is that Ca^{2+} promotes the adaptation via protein kinase C. What could be the target of the protein kinase C? The target may be a key protein in the transduction process. The sugar receptor protein might be inactivated by phosphorylation as is rhodopsin, where inactivation may contribute to light adaptation of visual cells (Shichi and Somers, 1978; Kelleher and Johnson, 1986). Alternatively, Ca^{2+} may regulate the concentration of cGMP via inhibition of guanylate cyclase (Koch and Stryer, 1988; Dizhoor, Ray, Kumar, Niemi, Spencer, Brolley, Walsh, Philipov, Hurley, and Stryer, 1991) or activation of phosphodiesterase (PDE) (Robinson, Kawamura, Abramson, and Bownds, 1980; Kawamura and Bownds; 1981, Kawamura and Murakami, 1991) as is also argued to occur in the visual system. Previously, Amakawa et al. (1990) showed that extracellularly applied dbcGMP evokes less adapting impulse discharge from the sugar receptor cell, suggesting that dbcGMP could act as an excitatory intracellular messenger in place of cGMP. Even if, as argued by Kijima, Nagata, Nishiyama, and Morita (1988), the channel opening contributing to the receptor potential is primarily triggered by stimulus sugars without any cascadal reactions, cGMP could be involved in the transduction mechanism for sustaining the ion channels in the open state. At any rate, the adaptation cascade would link with the transduction process at an earlier step than the opening or sustaining of the ion channel by cGMP.

However, Nicol and Bownds (1989) mentioned that Ca^{2+} regulates some, but not all, aspects of light adaptation in rod photoreceptor cells. For the taste adaptation in the sugar receptor cell of the fly, other mechanisms than the adaptation cascade proposed here might exist.

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