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An Artificial Sweetener Stimulates the Sweet Taste in Insect: Dual Effects of Glycyrrhizin in *Phormia regina*

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Abstract

Glycyrrhizin, found in the root of licorice (*Glycyrrhizia glabra*), has been used extensively as a non-sugar sweetener for humans and also as a medicine. As far as we know, the present work is the first report describing that a non-sugar sweetener for humans induces a sweet taste in insects. In behavioural experiments, we found that glycyrrhizin induced the feeding response, including full proboscis extension in the blowfly, *Phormia regina*. Glycyrrhizin also induced impulses of the sugar receptor cell in the labellar chemosensillum, which is highly specialized for the tastes of sugars and nucleotides. The optimum concentration of glycyrrhizin was 3.0 mM, which is much lower than that of sucrose. It has been established that multiple receptor sites, the pyranose receptor site (P site) and the furanose receptor site (F site), are present in the sugar receptor cell of the blowfly and the fleshfly. The inhibitors specific to the P site, starch and PCMB (*p*-chloromercuribenzoate), partially inhibited glycyrrhizin-induced responses but not levan (an inhibitor to the F site), indicating that the P site on the sugar receptor cell is involved in the glycyrrhizin action but not the F site. When 30 s stimulation with 3.0 mM glycyrrhizin was repeated with an interval of 3–10 min, the impulse frequency to the second stimulus was higher than that to the first one and doubled within 6 min. The first stimulus lasting longer than 10 s potentiated the impulse generation and reduced the adaptation rate during the second stimulus. These results suggest that, in addition to the action via the P site, an additional mechanism, possibly in the signal transduction cascade of the sugar receptor cell, may be involved in the action of glycyrrhizin.

Introduction

Saponins are natural glycosides of steroids or triterpenoids, which have the typical property of forming a soapy lather when shaken with water. Several saponins taste sweet to humans (Nishizawa and Yamada, 1996), and some (strogin) can evoke a sweet taste sensation from pure water by pretreatment with the saponin (Sugita *et al*., 1998). Glycyrrhizin (GL), a triterpenoid saponin (Figure 1) found in the root of licorice (*Glycyrrhizia glabra*), has been used extensively as a sweetener and also as a medicine for humans. The relative sweetness of GL to sucrose is ~170-fold (Mizutani *et al*., 1994). GL has anti-ulcer (Doll *et al*., 1962) and antiviral (Pompei *et al*., 1979) activities, and is now used in Japan as an anti-allergic (Kuroyanagi and Saito, 1966) and for protection of the liver (Fujita *et al*., 1978). Moreover, GL selectively inhibits the activities of arachidonate cascade-related enzymes, such as lipo-oxygenase (Shimoyama *et al*., 1996) and phospholipase A_2 (Ohuchi *et al*., 1981; Okimasu *et al*., 1982; Ishikawa *et al*., 1990), *in vitro*.

Several studies have been performed to evaluate the taste profile of GL in vertebrates (Mizutani *et al*., 1994), but little is known about its effects in invertebrates. To our knowledge, this is the first work to show the effect of an artificial sweetener for human on the sensory cells of an invertebrate, i.e. *Phormia regina.*

It has been established that, because of its structure, the blowfly chemosensillum on the labellum is useful in proving the action of GL in the taste sensory system. The sugar receptor cell extends a sensory process into a cuticular sheath of the sensillum that has 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, an inward current occurs across the membrane and evokes the impulse generation near the cell body (Morita, 1959). Multiple receptor sites are present in the sugar receptor cell of the blowfly and the fleshfly (Dethier, 1955; Evans, 1963; Morita and Shiraishi, 1968; Omand and Dethier, 1969; Jakinovichi *et al*., 1971; Shimada *et al*., 1974; Shimada and Isono, 1978; Shimada and Tanimura, 1981; Amakawa *et al*., 1992). Shimada *et al*. (Shimada *et al*., 1974) showed the separation of two receptor

sites in a single labellar sugar receptor cell of the fleshfly by non-competitive inhibition with *p*-chloromercuribenzoate (PCMB) treatment. The P site (the pyranose receptor site) was completely inhibited by PCMB but the F site (the furanose receptor site) was not. The sugar receptor cell of blowfly, *P. regina*, possesses at least two types of receptor sites that are distinguished by selective competitive inhibition with polysaccharides: starch for the P site and levan for the F site (Hara, 1983). As a part of our electrophysiological studies on triterpenoid saponins, we have shown that chromosaponin I (CSI), a γ-pyronyl-triterpenoid saponin (Figure 1) isolated from the pea and other leguminous plants (Tsurumi *et al.*, 1992), stimulates the sugar receptor cells of the blowfly without affecting the sugar receptor sites on the membrane (Ahamed *et al.*, 2000). In contrast, Kennedy and Kolodny (Kennedy and Kolodny, 1989) reported that hodulcin, another triterpene glycoside, suppressed sugar responses by both competitive and non-competitive mechanisms in the blowfly. In the present paper, we show that GL, a structural analogue of CSI, induces full proboscis extension in the blowfly and exerts more intense signals of the sugar receptor cell than CSI. We also examine the effects of starch and levan, as well as PCMB, on the impulse frequency induced by GL, and show the dual functions of GL: activation of the P site on the sugar receptor cell and another pathway to stimulate the sweet response.

Materials and methods

Fly

Adult blowflies (*Phormia regina*), 7–9 days after emergence, reared in our laboratory, were used for experimental purposes. They were fed with 0.1 M sucrose and water. The ambient temperature was kept at 24 ± 1°C.

Proboscis extension reflex (PER) test

For the behavioural test to observe the PER, flies were starved for 24–36 h (water was always supplied) prior to experiments. They were held by the wings with a small clothespin in order to restrict their movement. Prior to each test, the flies had been given sufficient water so that they would no longer show a PER to water. Test solutions containing various concentrations of GL were applied to the labellum with a wide-mouth plastic tip (a disposable tip for an automatic pipette, the tip opening was ~500 μm in diameter). The number of flies showing the feeding response of full proboscis extension within 5 s was counted. Flies that responded to 150 mM NaCl were eliminated from the batch.

The LL-type sensory hair is the largest chemosensory hair in the labellum (Wilczek, 1967). When a similar PER test was done by applying test solutions to a single labellar LL-type sensillum (Ahamed *et al.*, 2000), the sensillar tip was capped with a glass capillary containing test solutions up to 5 s under a binocular. During the experiment, round plastic rings were fixed around the thorax of the flies to restrain any head movement.

Electrophysiological procedure

The impulses were recorded by the tip recording method of Hodgson *et al.* (Hodgson *et al.*, 1955). A glass capillary containing a test solution was used as a recording electrode. Impulses, which were induced by capping the sensillar tip with the glass capillary, were recorded through a band-path filter (100–2000 Hz). The ambient temperature was 24 ± 1°C, with a relative humidity of 70–80%. The duration time of stimulus was 30 s. Intervals between stimuli were changed according to the purpose of the experiments. The effect of GL was examined by two methods. First, the time interval between GL stimuli was at least 15 min to avoid any remaining effect of GL. Second, to evaluate the remaining effect of GL, the second GL stimulus was given 6 min after the first one. Two types of electrophysiological responses were observed; normal response without a latency and response with a latency. To compare the magnitude of these responses, we counted the number of impulses during a period of 0.2 s starting 0.2 s after the beginning of stimulation (bars in Figure 2D,E).

Chemicals

GL (ammonium salt, purity ~75%), PCMB and levan were purchased from Sigma Chemical Co. (St Louis, MO). Sucrose, starch and other chemicals were purchased from Wako Pure Chemicals Industries, Ltd (Osaka, Japan).

We purified the Sigma GL by HPLC, using TSK gel ODS 120T column (300 × 7.8 mm) with a detection at 210 nm and a solvent including 65% methanol and 0.1% acetic acid. Since the purified GL showed fundamentally the same results as the Sigma GL on both behavioural and electrophysiological experiments, we used the Sigma GL without purification.

Test solutions

GL was dissolved in 20 mM MOPS buffer (pH 6.6). Sucrose solutions of 50 mM contained 10 mM NaCl for the electrical conductance. Soluble starch and levan were completely solubilized to adjust its concentration to 2% in MOPS buffer by heating in a water bath and stirring. GL was added when these solutions were completely cooled down. The viscosities of starch and levan were measured using an Ostowald Viscosimeter (Kusano, Japan). PCMB was prepared by the method of Shimada *et al.* (Shimada *et al.*, 1972). NaCl solutions of 150 and 10 mM were used to observe the responses of the salt and the water receptor cells, respectively.

Results

GL-induced PER test

The PER test was done with a population of 100 flies in two

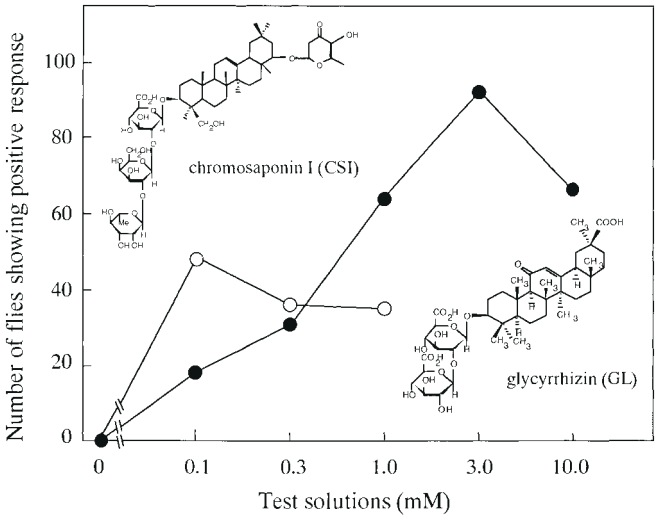

Figure 1 The PER to various concentrations of GL (●) and CSI (○). Test solutions were applied to the labellum up to 5 s. The number of flies showing a positive response was plotted against concentrations of triterpenoid saponins (n = 100). Molecular structures of GL and CSI are also indicated.

batches. To investigate the effect of GL on the feeding behaviour of blowflies, we applied various concentrations of GL to the labellum of each fly and observed the PER. The PER induced by GL appeared without latency, as observed with sucrose, which is contrast to our previous observation that CSI-induced PER has a long latency (2–4 s) (Ahamed *et al.*, 2000). To compare the effects of GL and CSI, the concentration–PER relationships for both triterpenoid saponins are shown in Figure 1. We found that 3.0 mM GL is the most effective, inducing PER in 97% flies, indicating that GL is effective at concentrations much lower than sucrose. The optimum concentration of GL is 30-fold higher than that of CSI, but the number of flies showing PER at the optimum concentration was 2-fold greater in GL than in CSI. Since the PER observed after water satiation is ascribed to stimulation of the sugar receptor cells, this result suggests that GL stimulates the sugar receptor cell as CSI.

We also carried out the PER test by stimulating a single labellar LL-type sensillum (Ahamed *et al.*, 2000) and found that 73% of the 15 tested flies, all of which were PER-positive to 50 mM sucrose, showed the PER at 3.0 mM GL. This indicated that the sugar receptor cell in the LL-type sensillum is involved in the GL-induced PER as well as CSI-induced PER.

GL-induced electrophysiological response

Figure 2 shows the taste responses recorded in the same labellar LL-type sensillum with 50 mM sucrose, 150 mM NaCl, 10 mM NaCl and 3.0 mM GL. GL was solubilized into 20 mM MOPS buffer, which itself did not induce impulses of the sugar receptor cells. Impulses of the sugar, the salt and the water receptor cells of a labellar LL-type

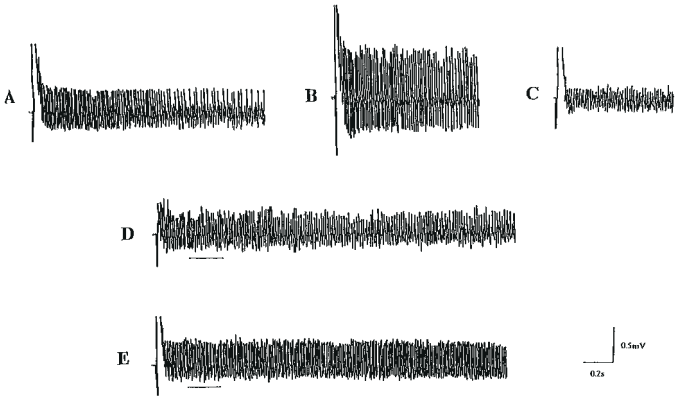

Figure 2 A typical set of recordings (from a total of 15 sets) on the responses from the same labellar LL-type sensillum to 50 mM sucrose **(A)**, 150 mM NaCl **(B)**, 10 mM NaCl **(C)**, the first stimulus with 3.0 mM GL **(D)** and the second stimulus with 3.0 mM GL **(E)** by the tip recording method. The second GL stimulus was given 6 min after the first.

Table 1 Impulse amplitude induced by various test solutions in the same LL-type sensillum

Test solutions	Average impulse amplitude ± SE (mV)	No. of impulses measured
Sucrose 50 mM	0.857 ± 0.050	45
NaCl 150 mM	1.682 ± 0.330	25
NaCl 10 mM	0.482 ± 0.080	25
GL 0.1 mM	0.832 ± 0.065	10
GL 0.3 mM	0.864 ± 0.078	15
GL 1.0 mM	0.849 ± 0.047	20
GL 3.0 mM	0.825 ± 0.097	25
GL 10.0 mM	0.839 ± 0.051	15

sensillum are easily distinguished from each other by comparing their amplitude. The amplitude of impulses to GL was same as that to sucrose and constant between 0.1 and 10 mM (Table 1), clearly indicating that GL stimulates the sugar receptor cells. The impulses induced by GL appeared without latency. This observation again contrasts with our previous finding that CSI-induced impulses appeared after a significant latency (0.1–1.0 s) (Ahamed *et al.*, 2000).

As shown in Figure 2E, when the second 3.0 mM GL stimulus was given 6 min after the first one, the impulse frequency to the former was always greater than that to the latter. Moreover, the adaptation of the sugar receptor cell to GL during the second stimulus was slower than that during the first one. To analyse the effects of repeated stimuli on the sugar receptor cell, we investigated the effects of time intervals between the two GL stimuli at 3.0 mM using the same LL-type sensillum (Figure 3). When the second GL stimulus was given 3–10 min after the first one, the impulse frequency was higher than that to the first one. The impulse

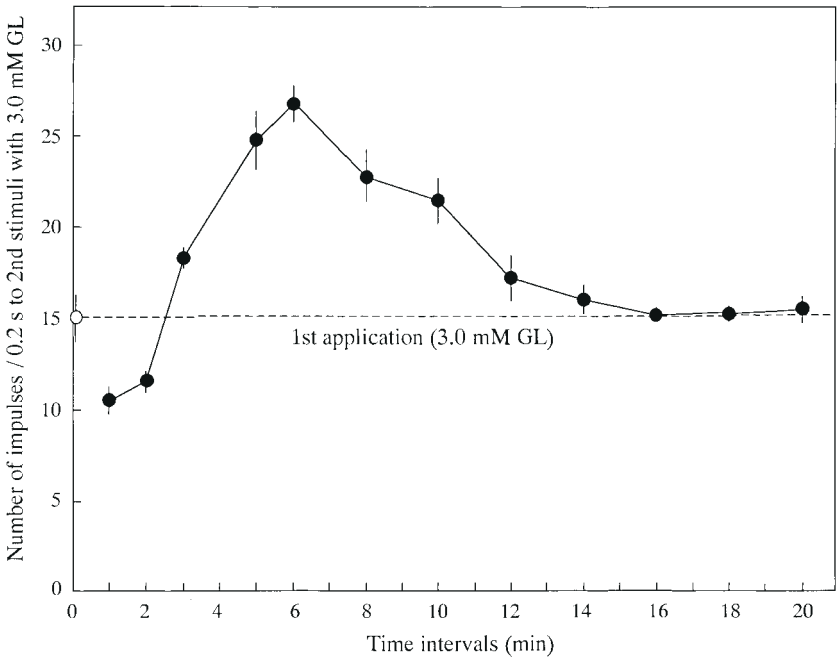

Figure 3 Effect of time intervals between the first and second 3.0 mM GL stimuli on the impulse frequency to the second one (●), compared with the impulse frequency to the first one (○). The time interval between each set of two stimuli was 15 min and the duration time for all stimuli was 30 s. Data are the average (±SE) of the number of impulses generated during a period of 0.2 s, starting 0.2 s after the beginning of stimulation ($n = 10$).

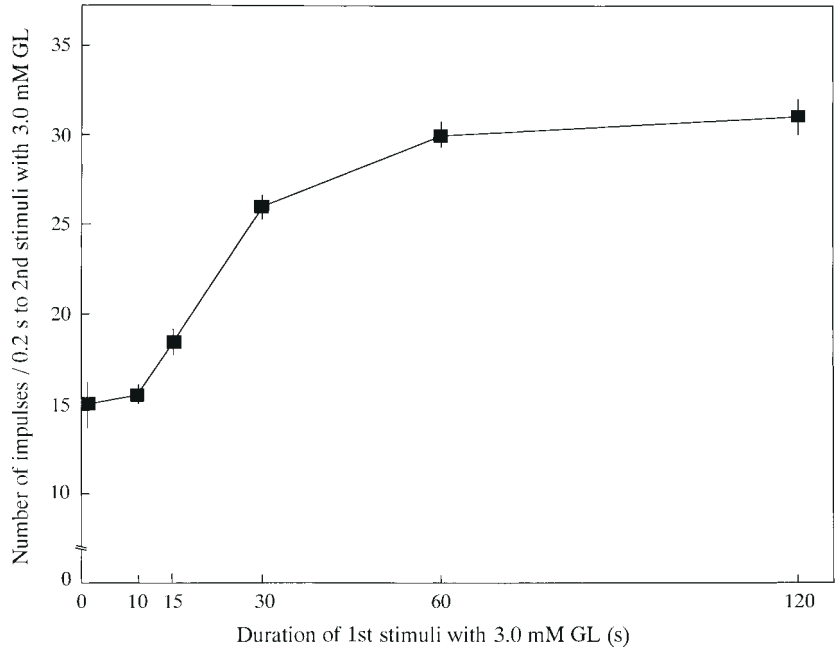

Figure 4 Effect of duration time of the first 3.0 mM GL stimulus on the impulse frequency to the second 3.0 mM GL stimulus (■). The time interval between the two stimuli was 6 min and the interval between each set of stimuli was 15 min. The duration time of the second stimulus was 30 s. Data are the average (±SE) of the number of impulses generated during a period of 0.2 s, starting 0.2 s after the beginning of stimulation ($n = 10$).

frequency to the second stimulus is highest with a 6 min time interval, and an interval of 15 min or longer is required to nullify the remaining effect of the first stimulus on the response to the second one.

We also changed the duration of the first GL stimulus, while the time interval between the two stimuli was 6 min and the duration of the second GL stimulus was 30 s. The impulse frequency to the second GL stimulus increased dependent on the duration of the first stimulus beyond 10 s, becoming saturated over 60 s (Figure 4).

In Figure 5, two GL stimuli at the same concentration with an interval of 6 min were given to an LL-type sensillum. In these experiments the interval times between each set of stimuli were at least 15 min to avoid remaining effects of GL (Figure 3). The frequencies of impulses to the first and second GL stimuli were highest at 3.0 mM, which is the same as the optimum concentration for the PER test (Figure 1). The frequency of impulses to the second stimulus was always higher than that to the first (Figure 5) and the high frequency of impulses continued for a longer period during the second stimulus compared with the first (Figure 2D, E). We noticed 10–12 impulses/0.2 s even 2 min after the beginning of the second stimulus with 3.0 mM GL. Figure 6 shows the time course of the response of sugar receptor cells to 50 mM sucrose and 3.0 mM GL (second stimulus). It can be observed that compared with 50 mM sucrose solution, adaptation is very low in the response to GL. The highest frequency of impulses to the second stimulus was 27 impulses/0.2 s, corresponding to the saturation level induced by 100 mM sucrose (Amakawa, 1978). These results prompted us to examine the interaction between GL and the sugar receptor sites.

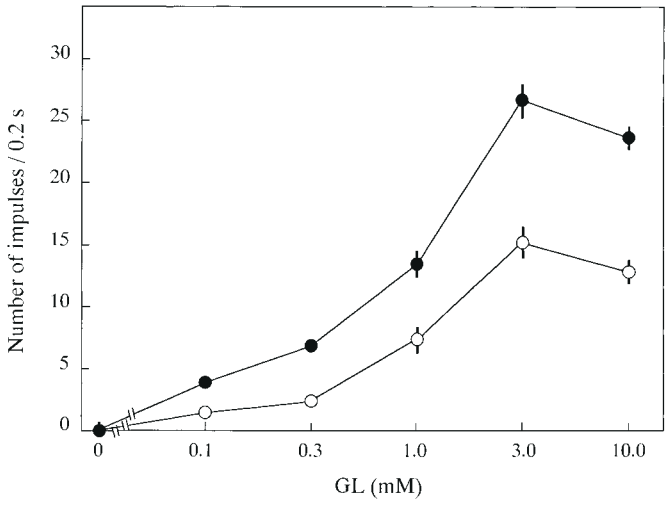

Figure 5 Electrophysiological responses from the LL-type sensillum to various concentrations (0.1, 0.3, 1.0, 3.0 and 10.0 mM) of GL during the first (○) and second (●) stimuli. Five sets of two GL stimuli with concentrations ranging from 0.1 to 10 mM were given to a single LL-type sensillum in a series from lower to higher concentration. The time interval between the two stimuli was 6 min and the interval between each set of two stimuli was 15 min. The duration time for all stimuli was 30 s. Data are the average (±SE) of the number of impulses generated during a period of 0.2 s, starting 0.2 s after the beginning of stimulation (n = 10).

Involvement of sugar receptor sites to sense GL

Starch is an inhibitor of the P site and levan inhibits the F site of the sugar receptor cell of the blowfly (Hara, 1983). To elucidate the relationship between GL and the sugar receptor sites (the P and F sites) we performed the PER test of GL in the presence of starch or levan. Prior to the experiment, we measured the relative viscosity of both 2% starch and levan, and found it to be 1:1.07:3.63 (water: starch:levan). To see the effect of inhibitors more clearly, we selected the GL concentration at the half-maximum value, i.e. 1.0 mM. Figure 7 shows the PER to GL with or without inhibitors of sugar receptor sites. This experiment was repeated three times. The buffer itself did not induce any

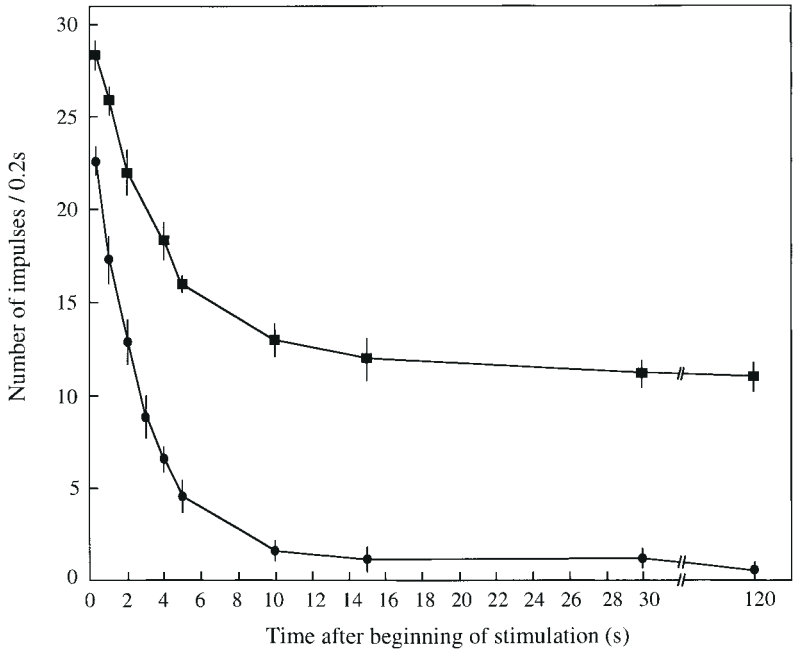

Figure 6 Time course of the response of sugar receptor cell to 50 mM sucrose (●) and the second 3.0 mM GL stimulus (■). Data are the average (±SE) of the number of impulses generated during a period of 0.2 s (n = 5).

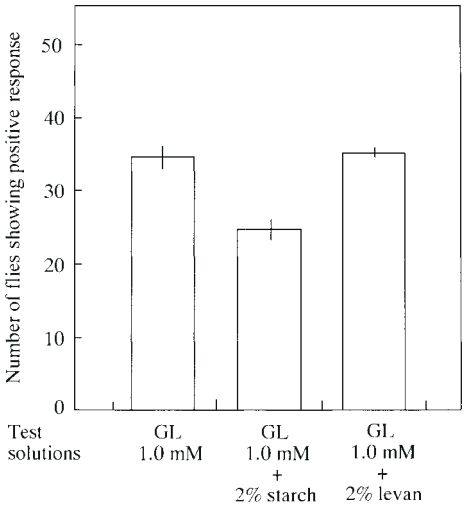

Figure 7 The PER to 1.0 mM of GL with or without 2% starch and 2% levan. Test solutions were applied to the labellum up to 5 s. The histogram indicates the number of flies showing positive response out of 50 flies and the average (±SE) of three experiments.

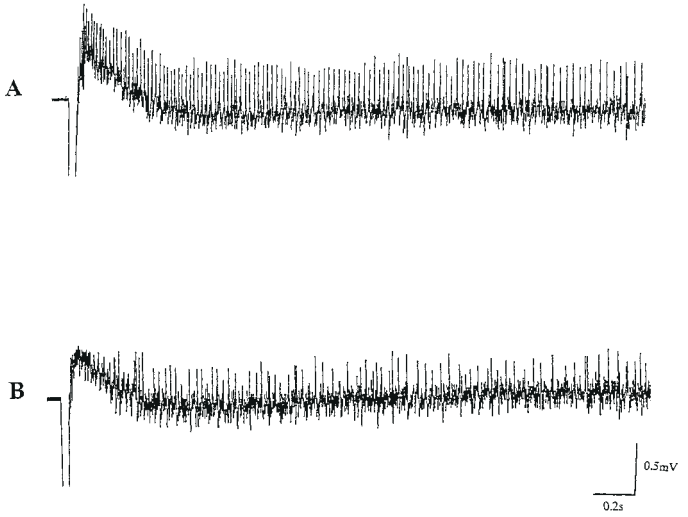

Figure 8 A typical set of recordings (from a total of 10 sets) on the responses from the same labellar LL-type sensillum to 3.0 mM GL **(A)** and 3.0 mM GL plus 2% starch **(B)** by the tip recording method.

PER. Application of 2% starch reduced the number of flies showing PER, whereas no significant change was observed in the case of 2% levan.

To clarify our findings on behavioural tests, we observed the electrophysiological response of flies to GL solutions containing starch and levan separately. Figure 8 shows typical recordings on the responses from the same labellar LL-type sensillum to 3.0 mM GL (A) and 3.0 mM GL plus 2% starch (B). Surprisingly, when we used 2% starch with GL, the response showed a clear latency (0.1–0.5 s) and the frequency of impulses was reduced compared with the control. The impulse frequency to GL in the presence of starch was less than that in its absence in the GL concentration range of 1.0–10 mM (Figure 9). In contrast, the presence of levan did not change the response to GL (data not shown). These results suggest that the P site on the sugar receptor cell is involved in the GL-induced stimulation of this cell. To confirm the involvement of the P site for the GL action, we also examined the effect of PCMB, which is a non-competitive inhibitor for the P site on the sugar receptor cell of fleshflies (Shimada *et al.* 1972). Since 0.3 mM PCMB was the most effective concentration at inhibiting impulses induced by glucose in blowflies (data not shown), we used this concentration in our experiment. The impulse frequency to GL in the presence of 0.3 mM PCMB was reduced compared with control (Figure 10). During every experiment, only sugar and water impulses were observed when stimulated by GL.

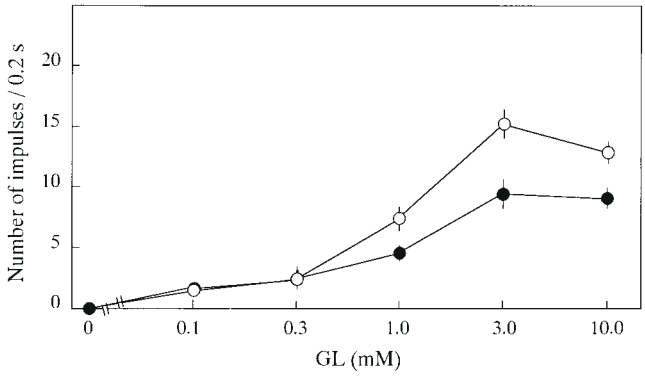

Figure 9 Electrophysiological responses from the LL-type sensillum to various concentrations (0.1, 0.3, 1.0, 3.0 and 10.0 mM) of GL in the absence (○) and presence (●) of 2% starch. The time interval between each stimulus was 15 min and the duration time for each stimulus was 30 s. Data are the average (±SE) of the number of impulses generated during a period of 0.2 s, starting 0.2 s after the beginning of stimulation (n = 15).

Discussion

GL activates the P site of sugar receptor cells

GL was found to induce sugar response in blowflies. This has been confirmed by both electrophysiological and behavioural experiments. In the optimum response at 3.0 mM GL, the impulse frequency was ~15 impulses/0.2 s during the first stimulus and 27 impulses/0.2 s during the second stimulus (Figure 5). The latter frequency corresponds to the saturation level induced by 100 mM sucrose (Amakawa, 1978). This strengthens the idea that GL is much more stimulatory than sugar molecules. The glycosidic moiety of GL is composed of two molecules of glucuronic acid (Figure 1). Since the glucuronic acid itself is tasteless for the blowfly (Ahamed *et al.*, 2000), we suspect that the whole GL molecule is involved in the induction of the sugar response.

In all the experiments we found that GL interacts with the P site on the membrane of the sugar receptor cell (Figures 7–10). As starch is a competitive inhibitor that specifically

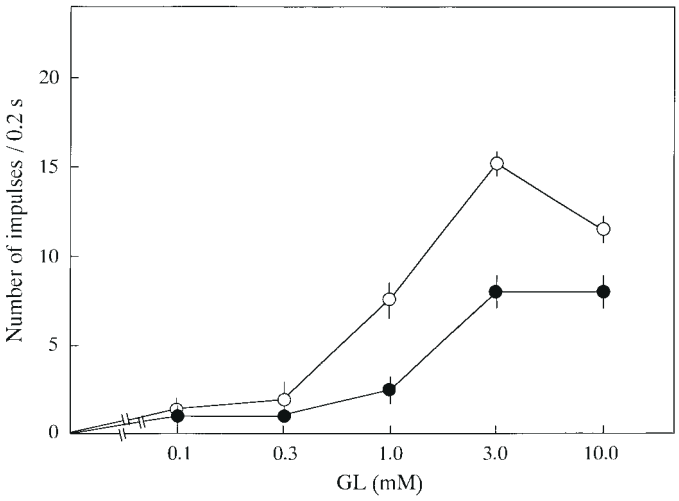

Figure 10 Electrophysiological responses from the LL-type sensillum to various concentrations (0.1, 0.3, 1.0, 3.0 and 10.0 mM) of GL with (●) and without (○) 0.3 mM PCMB treatment. The time interval between each stimulus was 15 min and the duration time for each stimulus was 30 s. Data are the average (±SE) of the number of impulses generated during a period of 0.2 s, starting 0.2 s after the beginning of stimulation (n = 5–10).

inhibits the P site of sugar receptor cells in the blowfly (Hara, 1983), the marked difference in responses to GL in the presence and absence of starch indicates that the sugar response induced by GL involves the P site of sugar receptor cells. This is in contrast to CSI, since the PER induced by CSI was not inhibited by starch (data not shown). We also used a non-competitive inhibitor of the P site, PCMB, and found a marked decrease in response to GL. However, we did not find any change in response by using an F site inhibitor, levan (Figure 7). Similarly, there was no change in response to CSI when using levan. These results suggest that GL has no interaction with the F site but interacts specifically with the P site.

Dual effects of GL in the sugar receptor cells

Although GL is likely to interact with the P site, the pattern of impulses generated by GL is different from the typical response to sucrose. We observed two phenomena. First, the impulse frequency to GL was greater during the second stimulus than during the first (Figures 2D,E and 5). Secondly, the impulse generation by the second GL stimulus showed a lower reduction in frequency compared with that generated by the first stimulus. These characteristics of the pattern of impulse generation have never been observed for sugar-induced responses through the P site. Therefore, we suggest that another mechanism can explain both the enhanced frequency of impulses to the second GL stimulus and the less adaptation during this stimulus. We hypothesize that the GL molecule penetrates the cell membrane of sugar receptor cells and interacts with a part of the sugar signal transduction pathway inside the cell. This idea is consistent with the finding that the impulses induced by GL appeared after a relatively long latency (0.1–0.5 s) when applied with starch (Figure 8). In the case of CSI, such a latency was observed in the absence of starch, and CSI has also been suggested to penetrate into the sugar receptor cell before stimulating the cell (Ahamed *et al.*, 2000). When two GL stimuli were given to the same labellar LL-type sensillum, the first stimulus displayed some remaining effects in potentiating the impulse frequency to the second one (Figures 3–5). The frequencies induced by the second stimulus changed dependent on the time intervals between the two GL stimuli (Figure 3). The remaining effect of the first stimulus reached the optimum 6 min after the first stimulus, then decreased gradually and disappeared. We suspect that some component responsible for the accelerated response may accumulate in this period. This component could be the P site on the cell membrane or some signaling compound involved in sugar taste sensation inside the cell. The remaining effect of the first stimulus also depends on the duration of the stimulus, with a duration >10 s being required to potentiate the second stimulus (Figure 4). The requirement of relatively long duration of the GL stimulus is in accordance with our idea including the penetration of the GL molecule into the sugar receptor cells. Moreover, though we found a moderate decrease in response to PCMB treatment, we could not find complete inhibition of the sugar response to GL with the inhibitor (Figure 10). In the fleshfly PCMB is known to completely inhibit the P site on the sugar receptor cell (Shimada *et al.*, 1974). From all these results we affirm that GL has two functions in inducing sugar signals: first, by binding to the P site on the sugar receptor cells as a stimulant; secondly, by penetrating the cell membrane and interacting with a part of the sugar signal transduction pathway inside the sugar receptor cell.

GL may interact with a part of the signal transduction cascade for sweet sensation in addition to the P site

Recently, Murakami and Kijima (Murakami and Kijima, 2000) used the patch-clamp technique to study the responses of the labellar taste cells of the pupa of the fleshfly (12–24 h before emergence) and proposed that a receptor–channel complex be activated directly by sucrose without mediation by second messenger or G-protein. On the other hand, Amakawa *et al.* (Amakawa *et al.*, 1990, 1992) found that membrane-permeable cyclic GMP analogues, dibutyryl cyclic GMP and 8-bromo-cyclic GMP, induced impulses only of the sugar receptor cells in adult blowflies. The authors hypothesized that these cyclic GMP analogues, which penetrated into the sugar receptor cells, opened the ion channels from the inside of the cell, mimicking the intracellular messenger. Koganezawa and Shimada (Koganezawa and Shimada, 1997) provided evidence for the involvement of G-protein in the sugar signal cascade of adult fleshflies by inhibiting the cascade with an inhibitor of G-protein, GDPβS, and also by stimulating the cascade with a stimulant, GTPγS. These experimental findings suggest the

existence of the signal transduction cascades in the receptor membrane. Naim *et al.* (Naim *et al.*, 1994) reported very interesting evidence regarding some amphipathic taste substances (saccharin, sodium cyclamate, aspartame, neohesperidin dihydrochalcone, naringin, etc.) that are direct activators of G-protein in vertebrates. It is interesting to note that another triterpenoid saponin, dehydrosoyasaponin I, reversibly increases the open probability of calcium-activated potassium (maxi-K) channels from bovine tracheal smooth muscle by binding the channel protein when applied to the intracellular, but not the extracellular, side of the membrane (McManus *et al.*, 1993; Giangiacomo *et al.*, 1998). Although the first target site for GL is likely to be the P site on the sugar receptor cells, the second target site is not yet known, but it could be the signaling cascade including G-protein or the ion channel in the sugar receptor cell.

In the present paper we have shown that GL, an artificial sweetener for humans, induced a sweet taste in blowflies, suggesting that GL binds to the sweet receptor site in humans as well as the P site of sugar receptor cells in blowflies. It is intriguing to note that another triterpenoid saponin, strogin, binds to the sweet receptor site in humans (Sugita *et al.*, 1998). Earlier reports concerning biological effects have demonstrated that GL has various activities in vertebrates. However, to date, little comparative information is available on the sensory cells in both vertebrates and invertebrates. Further studies will clarify the biological significance of the GL-induced sweetness in blowflies and its relationship to the human system.

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