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# $G_q\alpha$ subunit mediates receptor site-specific adaptation in the sugar taste receptor cell of the blowfly, *Phormia regina*

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**Abstract**

The gustatory system is essential for almost all animals. The recent identification of G protein-coupled receptor proteins (GPCRs) has progressed molecular biological studies of gustatory systems, although the signal transduction mechanisms have not yet been fully elucidated. In vision and olfactory receptor cells, $G_q$ class G protein is known to be a major signal transducer. By functional blocking of intrinsic $G_q$ with an anti-$G_{q/11}\alpha$ antibody, we investigated the roles of $G_q$ in the sugar receptor cell of the blowfly, *Phormia regina*. Before and after introduction of the anti-$G_{q/11}\alpha$ antibody into the cell through the DOC-permeabilized cell membrane, we recorded the responses of the receptor cell to sucrose and D-fructose, which stimulate different receptor sites, respectively. The initial impulse frequency in response to either sucrose or D-fructose was not changed by antibody introduction, whereas the adaptation rate in sucrose stimulation, but not fructose stimulation, became slower after antibody introduction. These results indicate that: (1) $G_q$ is a regulator of adaptation in the sugar receptor cell of *Phormia*, rather than a transducer, and (2) different adaptation mechanisms are promoted by stimulations with sucrose and D-fructose.
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The molecular mechanisms of signal transduction in visual and olfactory receptor cells have been shown to be enzymatic cascades involving G protein-coupled receptors (GPCRs) [5,18], but those in the gustatory system are still ambiguous. In the sugar receptor cell of flies, three different hypotheses have been proposed that: (1) cyclic GMP (cGMP) acts as a second messenger [2]; (2) inositol 1,4,5-triphosphate ($IP_3$) acts as a second messenger [7]; and (3) the sugar receptor protein itself works as a channel [9]. Recently, Murata et al. reported that nitric oxide elicits a response in the sugar receptor cell in *Phormia* without taste stimulation [10]. This observation supports the hypothesis that the sugar receptor cell uses cGMP as a second messenger, since nitric oxide is known to be an activator of soluble guanylate cyclase.

On the other hand, $G_q$ class G protein expression was demonstrated in the taste organ of *Drosophila melanogaster* [7]. This protein is known to activate phospholipase C (PLC), leading to $IP_3$ synthesis [16]. However, it was not clear in which cell or how $G_q$ functions in the gustatory system. The contact chemosensillum of flies is in the form of a hair housing four chemoreceptor neurons and one mechanoreceptor neuron [14]. The chemoreceptor neurons are functionally differentiated and known as deterrent, water, salt and sugar receptor cells, respectively [4]. However, they are morphologically similar to each other and have a cell body, an axon and a dendritic process as the receptive region. The dendritic processes of the neurons extending into the sensillum possess taste receptor proteins and all the functional molecules necessary for signal transduction. In this study, we introduced the anti-$G_q$ antibody into the sugar receptor cell using the deoxycholate (DOC) method [1] and compared the electrophysiological responsiveness of the sugar receptor cell before and after antibody introduction.

Two sugar receptor sites have been identified on the sugar receptor cell. One is the P site for sucrose, D-glucose, and so

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on, and the other is the F site for D-fructose, D-galactose, and so on [15]. In this paper, we used sucrose and D-fructose as representative stimulants for the P and F sites, respectively.

The *Phormia regina* blowflies used in this study were originally donated by Professor H. Morita (Kyusyu University). They were then reared in our laboratory by feeding with 0.1 M sucrose at 22 ± 2 °C. Adult flies were used for electrophysiological experiments at 4–6 days after emergence.

The anti-$G_{q/11}\alpha$-subunit antibody, which was raised against an internal sequence (amino acids 283–300) of the human $G_q\alpha$ subunit, was purchased from Calbiochem. The ECL system for Western blotting detection was purchased from Amersham Biosciences.

We cut the proboscises of *Phormia* by hand and collected them in a dry plastic tube on ice. Using a previously reported freeze-vortex method [12], the labellar chemosensilla were isolated by repeated freezing and vortexing in the plastic tube, and the sensilla adhering to the inner wall of the tube were incubated in SDS–PAGE sample buffer. After SDS–PAGE, the separated proteins were blotted onto a nitrocellulose membrane. The membrane was subsequently treated with anti-$G_{q/11}\alpha$ antibody (1:1000 dilution) and a horseradish peroxidase-linked secondary antibody, followed by detection of the $G_{q/11}\alpha$-antibody-reactive proteins by a standard ECL method.

For electrophysiological experiments, an isolated *Phormia* head was mounted under an optical microscope on a platinum indifferent electrode surrounded by a moist pad. The electrophysiological responses were then recorded from the tip of an LL type chemosensillum located on the outer margin of the labellum, according to the tip-recording method of [6]. We stimulated the sensillar tip with 125 mM sucrose or 100 mM D-fructose in a recording glass capillary electrode for 20 s. These concentrations of sugar stimuli induce approximately 70% of the maximum responses of the sugar receptor cell. The evoked impulses were recorded through a computer-aided electrophysiological recording system (IDAC-USB; Syntech, Hilversum, The Netherlands). Upon stimulation of a sensillum, the four functionally different chemoreceptor cells within the sensillum all had the chance to respond, depending on the stimuli. The receptor cell producing the recorded impulses was determined from the amplitude, since all four receptor cells show characteristic amplitudes.

The ambient temperature was 20–24 °C throughout the experiments. Under these conditions, the isolated head preparations showed the stable responsiveness of the sugar receptor cell for about 100 min after decapitation.

The anti-$G_{q/11}\alpha$ antibody was diluted 10-fold in phosphate buffer (20 mM $KH_2PO_4$, 47 mM $Na_2HPO_4$, pH 7.17), and introduced into the receptor cells using the modified DOC method [1]. The examined sensillum, from which the impulses to sucrose or D-fructose had previously been recorded, was capped with a glass capillary containing 0.3% DOC in phosphate buffer for 2 min. Immediately (<1 min) after the end of the DOC incubation, a new capillary containing either the antibody solution or solvent buffer as a control was applied to the sensillum for 20 min, and the sensillar tip was maintained in air for 50 min until the impulses of the sugar receptor cell to sucrose or D-fructose were recorded again.

Impulse trains recorded from the sugar receptor cell in *Phormia* consist of an initial phasic and subsequent tonic parts. During the phasic period for 150 ms after beginning the stimulation, the impulse frequency shows a transient rapid increase that is not parallel with the receptor potential. On the other hand, the impulse frequency in the tonic part decreases in parallel with the receptor potential as cell adaptation gradually progresses [11]. Therefore, in our experiments, we only evaluated the tonic part of the impulses starting from 150 ms after beginning stimulation, such that we could investigate the transduction and/or adaptation mechanism in the generation of receptor potential. The impulse number was counted every 200 ms throughout the tonic part and the rates of impulse decrease were compared before and after antibody introduction. From the adaptation curves (see Figs. 3, 4E and F), the time until the impulse number decreased to half the initial level was determined both before ($t_{1/2\text{before}}$) and after ($t_{1/2\text{after}}$) antibody introduction [11]. Thus, we calculated the "relative adaptation rate" (RAR):

$$\text{RAR} = \frac{t_{1/2\text{after}}}{t_{1/2\text{before}}}$$

We performed partial cloning of the $G_q\alpha$ subunit cDNA from a labellum of *Phormia* by RT-PCR, and confirmed that the sequence of the epitope region (IMYSHLVDYFPEYDG-PQR) was completely conserved between *Drosophila* and *Phormia*, and fit to the used antibody. By Western blotting with the anti-$G_{q/11}\alpha$ antibody, a single band was detected in a chemosensillar extract of *Phormia* (Fig. 1). Fig. 1 also shows the blotting data for extracts of *Drosophila* head and appendages, which bear many contact chemosensilla, and the proboscis of *Phormia*. The apparent molecular mass of *Phormia* $G_q\alpha$ (44.5 kDa) was slightly larger than that of *Drosophila* $G_q\alpha$ (42.5 kDa). Since the isolated sensillum mainly contained the dendritic processes, rather than the cell bodies or axons, of the chemoreceptor neurons, $G_q$ might function in the receptive region and influence the electrophysiological responses of the receptor neurons in *Phormia*.

To check the applicability of membrane permeabilization by DOC incubation of each chemoreceptor cell in an examined sensillum, the impulse generations before and after DOC incubation were compared (Fig. 2). We stimulated a sensillum with 10 and 100 mM NaCl to investigate the impulses from water (ca. 0.5 mV amplitude) and salt (ca. 1.7 mV amplitude) receptor cells, respectively. The average numbers of impulses generated for the initial 200 ms of the tonic part from the water receptor cells were 16.5 ± 1.48 and 12.0 ± 1.61 before and 70 min (20 min in phosphate buffer plus 50 min in air) after DOC incubation, respectively (mean ± S.E.M., $n = 7$). The average numbers of impulses for the same 200 ms from the salt receptor cells were 13.5 ± 2.35 and 4.17 ± 1.56 before and after DOC incubation, respectively (mean ± S.E.M.,

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