Goα Is Involved in Sugar Perception in Drosophila

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Abstract
Detection of chemical compounds in food sources is based on the activation of 7 transmembrane gustatory receptors (GRs) in mammals and in insects such as Drosophila, although the receptors are not conserved between the classes. Different combinations of Drosophila GRs are involved in the detection of sugars, but the activated signaling cascades are largely unknown. Because 7 transmembrane receptors usually couple to G-proteins, we tried to unravel the intracellular signaling cascade in taste neurons by screening heterotrimeric G-protein mutant flies for gustatory deficits. We found the subunit Goα to be involved in feeding behavior and cell excitability by different transgenic and pharmacological approaches. Goα is involved in the detection of sucrose, glucose, and fructose, but not with trehalose and maltose. Our studies reveal that Goα plays an important role in the perception of some sweet tastants. Because the perception of other sweet stimuli was not affected by mutations in Goα, we also found strong indication for the existence of multiple signaling pathways in the insect gustatory system.

Key words: Drosophila, G-protein, Go, gustatory receptor, signaling

Introduction
The sense of taste enables the fruit fly, Drosophila melanogaster, to detect sugars in the environment, but our knowledge of the molecular mechanisms involved in detection of chemical signals is still fragmentary. The main gustatory organs of Drosophila are the 2 labial palps at the tip of the proboscis (Shanbhag et al. 2001). Taste chemosensation is mediated via sensory sensilla that are distributed in the proboscis, legs, and wings, as well as in the ovipositor (Stocker 1994; Singh 1997; Vosshall and Stocker 2007). These sensilla contain the dendrites of gustatory receptor neurons (GRNs), in which defined subsets of 60 gustatory receptor (GR) genes are expressed (Clyne et al. 2000; Dunipace et al. 2001; Scott et al. 2001; Robertson et al. 2003). The GR Gr5a is expressed in most sugar-responsive GRNs and was found to be necessary for the response to a small subset of sugars, particularly trehalose (Dahanukar et al. 2001, 2007; Ueno et al. 2001). Activation of Gr5a-positive neurons was found to be sufficient to elicit acceptance behavior (Wang et al. 2004; Marella et al. 2006). Gr64a is essential for the detection of multiple other sugars, including sucrose, glucose, and maltose (Dahanukar et al. 2007; Jiao et al. 2007; Slone et al. 2007). Strikingly, deletion mutants lacking both Gr5a and Gr64a do not show electrophysiological or behavioral responses to common sugars, indicating that these receptors are essential for the detection of sugars in general (Dahanukar et al. 2007). More recent studies demonstrate that Gr64f is a coreceptor, which is required for the detection of most sugars in concert with Gr5a and Gr64a, suggesting that GRs function as heteromultimers (Jiao et al. 2008).

GRs are 7 transmembrane proteins (Clyne et al. 2000; Robertson et al. 2003), which usually couple to guanine nucleotide-binding protein (G-protein)-linked signaling cascades. In mammals, the G-protein α-gustducin was shown to be involved in response to sweet tastants (McLaughlin et al. 1992; Wong et al. 1996). There was evidence presented that different G-protein subunits, Gγ1, Gisα, and Gqα, are involved in sugar reception in Drosophila (Ishimoto et al. 2005; Ueno et al. 2006; Kain et al. 2010). Further downstream in the signaling cascades, the phospholipase Cβ21C, the Inositol triphosphate (IP3) receptor, as well as the adenyl cyclase 78C were shown to play a role in the detection of sugars (Usui-Aoki et al. 2005; Ueno and Kidokoro 2008; Kain 2007).
et al. 2010). These findings imply the existence of more than one sugar-evoked signaling pathway in GRNs of Drosophila.

Our study was intended to unravel the intracellular signaling cascade in the gustatory neurons, with special emphasis to the role of the Gα protein subunit. Behavioral screening assays provided evidence that Goz is required for the behavioral response to sucrose. The electrophysiological responses to certain sugar stimuli were impaired in Goz-deficient flies, whereas responses to other sugars remained unaffected. Our results demonstrate that Goz plays an important role in the perception of certain sugars, and we propose that distinct G-protein–related pathways are involved in the recognition of sugar taste information in Drosophila.

Materials and methods

Fly stocks

Flies were raised at room temperature (23 ± 2 °C) on semi-defined medium (http://flybase.org) and were kept under a 12 h light:dark cycle. All UAS-Gα RNAi strains were purchased from VDRC (Dietzl et al. 2007). Gr5a-GAL4 and Gr66a-GAL4 were kindly provided by H. Amrein (Duke University Medical Center) and J. R. Carlson (Yale University); UAS-Go, UAS-GoGDP, and UAS-ptx were a kind gift from A. Tomlinson (Columbia University). The NP3200/+ line was from the Drosophila Genomics Resource Center collection, Kyoto (number 104-410 (y[+] w[+]; P{C(1)GawB}NP3200/SM1)). We confirmed the integration site of P{C(1)GawB}NP3200 in the Goz gene locus via polymerase chain reaction (PCR) using primers as follows: Go-fw 5’-TGC GAGTT GCT TCT C-3’, Go-rv 5’-AGG TAG TAC AGC ACG TGA TAA GG-3’, and GAL4-rv 5’-TTG TTA AGG AGT CCA TTC AAT GCA AAA TTG TTAT CC-3’. We amplified the different expression levels by applying the delta-delta Ct method (Livak and Schmittgen 2001).

Quantitative real-time PCR

Quantitative real-time PCR (Q-PCR) experiments were performed using the iQ SYBR Green Supermix reagent and the iQ5 thermal cycler apparatus from BIO-RAD, with regard to the manufacturer’s recommendations. cDNA samples were prepared following the RT-PCR method. Primer sequences were as follows: pair 1 (according to Figure 3A), dGo1-fw 5’-GCA GCC GCC AGG AGG CT C-3’, dGo1-rv 5’-GAC AAC CGG TCG ATA TTG TT A-3’; pair 2, dGo2-fw 5’-GCC ATC CAG CGA TCC AAA CA G-3’, dGo2-rv 5’-GAC AAC CGG TCG ATA TTG TT A-3’; pair 3, Go-fw 5’-GTA GGT GTC GAG TAG TAC GC-3’; and pair 4, Go-rv 5’-CTC AGC CCT ACG ATT CC TGG TG-3’. We amplified the PCR products for 40 cycles. The Q-PCR procedure was repeated for at least 3 times in independent runs. We calculated the different expression levels by applying the delta-delta Ct method (Livak and Schmittgen 2001).

Fluorescence microscopy

NP3200/UAS-mCD8::GFP flies were mounted in 2% agarose, such that the labellum and the legs were uncovered. The GFP fluorescence was observed with a Leica DM6000 confocal fixed stage. We developed Z sections of approximately 0.4 μm, which were processed to construct maximum projections.

Immunohistochemistry

For the immunohistochemical localization of Goz in the labellum, we fixed heads from Gr5a-GAL4/UAS-mCD8::GFP flies in phosphate-buffered saline (PBS) containing 4%
paraformaldehyde and 0.2% Triton X-100 for 30 min on wet ice. After rinsing the heads 3 times for 10 min in PBS and 0.2% Triton X-100 (PBST), they were incubated with PBST containing primary antibodies rabbit anti-Goα (Thambi et al. 1989) in a dilution of 1:100 at 4 °C overnight. After washing for 3 times with PBST, we exposed the heads for 2 h at room temperature to secondary antibodies goat anti-rabbit immunoglobulin G coupled to Alexa-633 (Invitrogen). The heads were mounted with Mowiol/DABCO and analyzed by confocal microscopy with a Zeiss LSM 510 META.

Electrophysiology

Extracellular recordings of action potentials on single taste sensilla were performed with modifications of previously described methods (Hodgson et al. 1955; Wieczorek and Wolff 1989). Briefly, newly enclosed flies were transferred to vials with semidefined medium. Female flies, which were aged 4–12 days, were introduced to 2- to 100-μl pipette tips, such that the anterior portion of the head protrudes from the end of the tip. An indifferent electrode was inserted into the thorax of the flies. The labellar sensilla (L1, L2, and L3) were stimulated with a recording electrode that was filled with the respective test solution. Signals were remitted to an EPC7 patch clamp amplifier (List Medical) in the current clamp mode. We counted the number of impulses in a period of 500 ms, starting 200 ms after onset of stimulation. As electrolyte, we used 50 mM tricholine citrate in all test solutions, except for KCl which was dissolved in distilled water. Tricholine citrate (50 mM) was chosen as electrolyte in the different sugar solutions, as this component was shown to inhibit exclusively the responses from water receptors (Wieczorek and Wolff 1989).

The pharmacological application of PTX was carried out as follows: Responses of L-type sensilla from wild-type flies were monitored at 20 and 100 mM sucrose, respectively. The same sensilla were incubated for 20 min with 10 μg/ml PTX diluted in 50 mM tricholine citrate followed by stimulation with 20 and 100 mM sucrose again. As a control, the described procedure was performed apart from delivery of PTX to the solution.

Behavioral assays

Two-choice preference tests were performed using modifications of previously described work (Tanimura et al. 1988). Newly enclosed flies were collected in vials with fresh fly food. Three- to six-day-old flies were starved for 20–24 h in vials containing 1% agarose allowing them the access to water. Forty to sixty of those flies were introduced to 6-well cell culture dishes that contained 2 alternating concentrations of sugar dissolved in 1% agarose. These sugar solutions additionally included either 2 mg/ml sulfurhodamine B monosodium salt (Fluka) or 0.5 mg/ml erioglaucine (Sigma-Aldrich). After flies were fed for 2 h in the dark at room temperature, they were anesthetized and were then classified with respect to their abdominal color: blue (#b), red (#r), purple (#p), and colorless (#c). The preference index (PI) was calculated as follows: (#b + ½ #p)/(#b + #r + #p) or (#r + ½ #p)/(#b + #r + #p). The closer the PI is to one the higher is the preference for the blue and accordingly the red substrate. A PI of 0.5 indicates no preference for any substrate. The percentage of #c flies was lower than 10% in each test. At least 120 flies per strain were tested in 3–6 independent tests. The food coloring did not affect the behavioral response of the flies to the diverse sugar concentrations, as we switched the tastant–dye combination and saw no preference differences in comparison to those that were observed before. For the avoidance tests, flies were treated as described above. The 6-well cell culture dishes were alternatively filled with 2 mM maltose and 8 mM maltose alone or 8 mM maltose blended with 8 mM caffeine or 1 mM quinine. The PI was calculated as described above.

For the proboscis extension reflex (PER), 3- to 6-day-old flies were starved for 20–24 h in vials with 1% agarose. After anesthetization, the dorsal abdominal side of flies was fixed on a microscope slide by employing a solvent-free contact adhesive (Peho). Half an hour later, we used glass pipettes to saturate the flies with pure water until they did not extend their proboscis to the waterdrop. Then, the test solution, 100 mM sucrose, was applied to the first legs of the fly for 3 s, meanwhile monitoring the extensions of the proboscis. After a period of 1–2 min, the procedure was repeated for overall 5 times. In between, we checked the water saturation of the flies. Responses were scored as follows: a fly that extended its proboscis for at least 3 times after stimulation was counted as 1, a fly that responded less than 3 times to the stimulus was counted as 0.

Results

Expression of RNAi against Goα in Gr5a neurons affects the behavioral response to sucrose

To define an expression profile of Gα protein subunits in the labellum of the fly, we carried out RT-PCR analysis and detected mRNAs of all Gα subunits in the main gustatory organ except for CG30054 (Supplementary Figure S1). In order to identify Gα protein subunits that participate in sugar perception, we performed 2-choice preference tests (Tanimura et al. 1988) with fly lines expressing RNAi directed against different G-protein subunits (UAS-Gα RNAi) (Dietzl et al. 2007) in sugar sensory neurons. The behavioral screening tests were performed with, if available, 2 independent UAS-Gα RNAi strains, in which the P{UAS-Gα RNAi} inserted in different integration sites of the chromosomes, whereas the expressed RNAi molecules were directed against the same G-protein mRNA. Given that Gr5a serves as a marker for the sugar neuron in each sensillum of the labellum (Marella et al. 2006), we expressed the interfering RNA against the different Gα subunits under control of the Gr5a.
promoter (Gr5a-GAL4). As no fly line was available that expresses RNAi against mRNA of the G-protein subunit concertina, this subunit was not examined in the 2-choice screening assay. We discovered that flies expressing Goα RNAi in Gr5a cells (Gr5a-GAL4; UAS-Goα RNAi) exhibit a strongly decreased ability to distinguish between 5 mM sucrose and 2 mM sucrose (Figure 1). The control strains, wild type (CantonS), homozygous Gr5a-GAL4, and homozygous UAS-Goα RNAi, displayed a strong bias to consume the higher concentrated sucrose solution (Figure 1). Because mutants expressing RNAi against other Goα protein subunits in Gr5a cells retained a preference for 5 mM sucrose in the 2-choice assay, we focused our studies on Goα transgenic flies.

Goα RNAi–expressing gustatory neurons show reduced responses to sucrose

In order to validate the effect of RNAi against Goα, we performed Goα expression analysis in Gr5a-GAL4 and Gr5a-GAL4; UAS-Goα RNAi flies and compared the expression levels with those from wild-type flies. Whereas the amount of Goα transcripts in wild-type and Gr5a-GAL4 flies was equal, the Gr5a-GAL4; UAS-Goα RNAi mutants displayed a reduced amount of Goα transcripts (Figure 2A). For a further characterization of cells expressing RNAi against Goα (Gr5a-GAL4; UAS-Goα RNAi), we used an electrophysiological approach to test the neuronal activity of these cells. We generated sugar-induced action potentials in GRNs performing tip recordings from the L-type sensilla, L1, L2, and L3, as described (Hodgson et al. 1955) (Figure 2B). Sucrose was chosen as stimulus because this disaccharide is known to elicit strong physiological responses (Dahanukar et al. 2007). The control strains, Gr5a-GAL4 and UAS-Goα RNAi, showed similar firing rates at sucrose concentrations ranging from 20 to 500 mM. In contrast, Gr5a-GAL4; UAS-Goα RNAi flies exhibited a significantly reduced spike rate (Figure 2C,D). A 100 mM sucrose solution elicited neural responses of 43 spikes per second in these flies, whereas the neurons showed 82 and 80 spikes per second in Gr5a-GAL4 and UAS-Goα RNAi flies. To exclude the possibility that the impaired electrophysiological responses to sucrose in Gr5a-GAL4; UAS-Goα RNAi flies were due to developmental defects of Gr5a neurons, we utilized the heat shock–inducible GAL4 expression (hsGAL4) during the adult stage in the UAS-Goα RNAi background. hsGAL4; UAS-Goα RNAi flies were reared at 19 °C to minimize expression of RNAi against Goα during pupal development. To induce RNAi expression, 6-day-old adults were heat shocked at 37 °C for 30 min and were allowed to recover for 16 h at 25 °C. Adults of the genotype hsGAL4; UAS-Goα RNAi that were continuously kept at 25 °C served as control flies. Tip recordings from the L-type sensilla revealed that the heat shock–induced expression of RNAi against Goα results in decreased neuronal responses at various sucrose concentrations (Figure 2E,F). A heat shock at 37 °C for 30 min and subsequent recovery for 16 h at 25 °C had no effect on the sucrose-induced responses in wild-type flies (data not shown). We therefore conclude that Goα contributes to the process of gustatory transduction and the decreased responses to sucrose were most likely not caused by defects in neural development. Because the observed neuronal responses of Gr5a-GAL4; UAS-Goα RNAi and hsGAL4; UAS-Goα RNAi flies were consistent with the observed phenotype of Gr5a-GAL4; UAS-Goα RNAi flies in the 2-choice preference tests, these experiments strongly suggest an involvement of Goα in sucrose perception in Drosophila.

Similar phenotypes were observed in another transgenic approach, where we overexpressed a constitutively inactive form of Goα (GoαGDP) in Gr5a-positive cells (UAS-GoαGDP; Gr5a-GAL4). GoαGDP has a lower affinity for GTP and could be expected to have a dominant-negative effect, as the mutant form of the G-protein subunit competes with endogenous Goα (Katanaev et al. 2005). Dose–response curves of neuronal electrophysiological activities display that UAS-GoαGDP; Gr5a-GAL4 flies showed reduced electrical responses to various concentrations of sucrose (Supplementary Figure S2). Moreover, flies expressing GoαGDP in sugar neurons yielded significantly lowered PIs than the control lines in 2-choice preference tests (Supplementary Figure S2).

Figure 1  Behavioral screening tests with flies expressing RNAi against different G-protein mRNAs in Gr5a-positive cells. Flies had the choice between 2 and 5 mM sucrose. A PI of 1 indicates a total preference for the higher concentrated sucrose solution, whereas a PI of 0.5 indicates a lack of preference. Control strains were CantonS wild type (wt), homozygous Gr5a-GAL4, and homozygous UAS-Goα RNAi. Differences between the indicated data points were statistically checked by the unpaired Student’s t-test, *P ≤ 0.05. Error bars represent standard error of the mean.
Neural and behavioral responses to sucrose are affected in mutant flies expressing reduced amounts of Goα

Due to the involvement of Goα in the development of heart epithelium and axon growth, a null mutation in Goα is lethal (Katanaev et al. 2005). In order to find mutants for a functional characterization, we queried the database for P-element insertions in the Goα gene locus because these insertions often result in reduced amounts of gene transcription. Two different Goα proteins, DGo1 and DGo2, have been identified that are transcribed from this gene locus (de Sousa et al. 1989; Schmidt et al. 1989; Thambi et al. 1989; Yoon et al. 1989). Two P-element insertions in a genomic intron of dGo2 and a potential promoter region of dGo1, P{GawB}NP3200 (Brand and Perrimon 1993) and P{EP}2154 (Rorth 1996), were selected for further analysis (Figure 3A). The P-element integration in EP2154/EP2154 flies results in absence of detectable dGo2 transcription, whereas both transcripts could be detected in heterozygous NP3200/+ transgenic flies by RT-PCR (Figure 3B). Q-PCR analysis revealed that the relative amount of total Goα mRNA was reduced to 57% in heterozygous NP3200/+ flies and 47% in homozygous EP2154/EP2154 flies (Figure 3C). dGo1 transcription was reduced to 70% and dGo2 transcription to 53% in NP3200/+ flies. The expression of the dGo2 transcript was almost completely suppressed in EP2154/EP2154 flies (0.0004%), whereas the amount of dGo1 equates to the wild-type level (Figure 3C). In addition, we analyzed the relative expression rates of these transcripts between labella and heads of wild-type flies. Relating to the amount in the heads, we found 24% of dGo1 and 82% of dGo2 expression in the labellum of the fly (Figure 3D).

Next, we aimed to investigate the neuronal responses of the P-element lines upon sucrose stimulation using tip recordings. The electrophysiological responses of the GRNs of both NP3200/+ and EP2154/EP2154 flies were significantly decreased at each sucrose concentration tested in comparison to the control strain (Figure 3E,F). In a rescue experiment, we complemented the NP3200/+ mutant, which is a GAL4 enhancer trap strain, by driving the expression of wild-type dGo2 under control of the enhancer-specific GAL4 activation (Brand and Perrimon 1993). Q-PCR experiments indicate that the amount of dGo2 transcripts in the rescue line (NP3200/UAS-Go) attained the amount of dGo2 in wild-type flies (Figure 3C). The reduced neuronal activity in NP3200/+ flies was likely caused by the low Goα expression levels because the impaired phenotype of these flies was partially rescued by dGo2 expression (Figure 3E,G). At each sucrose concentration tested, the rescue line displayed a significantly increased spike rate in comparison to the control strain (Figure 3E, square). For each sucrose concentration, 9 ≤ n ≤ 12. Error bars represent SEM. The asterisks indicate statistically significant differences between the associated data points; *P ≤ 0.05.
to the deficient NP3200/+ flies. Notably, the neural responses of NP3200/\textit{UAS-Go} were highly similar to those of wild-type flies at higher sucrose concentrations.

Two-choice preference tests revealed that solutions with high sucrose concentrations can be restrictedly distinguished from solutions with low sucrose concentrations by NP3200/+ and EP2154/EP2154 flies (Figure 3H). The impaired behavioral response of NP3200/+ was rescued by transgenic expression of \textit{dGo2} in \textit{Goa}-positive cells (NP3200/\textit{UAS-Go}) (Figure 3H). We additionally performed an established PER assay (Dethier and Goldrich-Rachman 1976), in which we applied a 100 mM sucrose solution to the first legs of the fly and monitored the induced extensions of the proboscis. Twenty-four percent of the NP3200/+ flies displayed a PER to 100 mM sucrose, whereas 93% of the wild-type flies showed a PER in response to stimulation with sucrose (Supplementary Figure S3). Taken together, these data support the participation of \textit{Goa} in sucrose perception.

**PTX impairs the behavioral and electrophysiological responses to sucrose**

PTX is well known to inhibit specifically \textit{Goa} and \textit{Gi} function, as it catalyzes ADP-ribosylation of the G-protein.
subunit (Moss and Vaughan 1988). In *Drosophila*, Goα is the only cellular target for PTX because it is the only G-protein subunit with a cysteine residue on the fourth position starting from the C-terminus, where ADP-ribosylation occurs (West et al. 1985). Using a combination of a transgenic and a pharmacological approach, we tested whether PTX impairs the electrophysiological and behavioral responses to sucrose. First, we created flies that expressed the transgene ptx delimited to sugar neurons (*UAS-pxtx/+; Gr5a-GAL4/*+) and investigated nerve responses of those flies employing tip recording. The responses to sucrose concentrations between 20 and 500 mM were significantly lower than those of the parental lines (Figure 4A). Remarkably, the strongest effect of PTX-induced inhibition of Goα was observed upon stimulations with 20 mM sucrose, with a spike frequency reduction to approximately 15% (Figure 4A). A 2-choice preference test revealed that *UAS-pxtx/+; Gr5a-GAL4/*+ flies completely lost the ability to discriminate between 2 and 5 mM sucrose (Figure 4B).

The responses to 20 mM sucrose were also monitored from *L*-type sensilla of wild-type flies that were preincubated with 10 μg/ml PTX for 20 min. Strikingly, neuronal responses were diminished to 5 spikes per second after the incubation with PTX (Figure 4C,D), whereas flies that were incubated with control solutions showed no effect on the firing rate elicited by 20 mM sucrose (data not shown). We also tested responses to 100 mM sucrose, a physiological concentration that is present in many fruits (U.S. Department of Agriculture, Agricultural Research Service 2006) and found these responses reduced to 73% after treatment with PTX (Figure 4C,D).

**Goα is localized in taste organs**

RT-PCR experiments revealed that Goα is expressed in the labellum of the fly (Supplementary Figure S4). We visualized Goα-positive neurons by expression of a membrane-located GFP reporter (*UAS-mCD8::GFP*) under control of the GAL4 enhancer trap strain NP3200. Goα expression was found in taste neurons of the labial palps and in the distal segments of the legs (Figure 5A,B). At least one neuron per sensillum was Goα positive, as each sensillum of the labellum exhibited GFP fluorescence. Using a higher magnification, we observed that *L*- and *S*-type sensilla were potentially innervated by 4 kinds of neurons because all 4 GFP-positive cells seem to extend their dendrite into the same sensillum. These are probably S1, L2, L1, and W cells, indicating that Goα promoter activity may be present in sugar-*, bitter-*, salt-*, and water-responding neurons (Figure 5C). Immunohistochemical staining of labella from *Gr5a-GAL4/UAS-mCD8::GFP* flies with Goα antibodies revealed that *Gr5a* and Goα were expressed in overlapping subsets of GRNs (Figure 5D–G). In whole-mount preparations, we observed fluorescence exclusively in the dendrites of *Gr5a*-positive neurons (Figure 5E,F).

**Responses to various sugars are dependent on different signaling pathways**

In addition to sucrose, we examined the neuronal activation in Goα mutant flies initiated by other sweet stimuli: maltose, trehalose, glucose, and fructose. Previous studies on wild-type flies have shown that the response spectra of *Gr5a* neurons to these sugars show wide variation in their stimulus-induced spike frequencies. Solutions of sucrose and maltose (100 mM) elicited more than 60 spikes per second, whereas solutions of glucose, trehalose, and fructose displayed rather moderate firing rates at the same concentration (Dahanukar et al. 2007). If the detection of all sugars depends on a Goα-related pathway, one would expect that the neural responses to all sugar stimuli were depressed in
EP2154/EP2154 and NP3200/+ flies. The electrical responses of sugar neurons from EP2154/EP2154 and NP3200/+ transgenic flies that were elicited by various concentrations of maltose and trehalose were identical to those of wild-type flies, whereas the spike rates elicited by different concentrations of glucose and fructose were significantly decreased (Figure 6A,B). The perception of maltose and trehalose does therefore seem not to be dependent on a Goα-related pathway. Similar observations were made with transgenic flies that expressed RNAi against Goα under control of the Gr5a promoter (Gr5a-GAL4; UAS-Goα RNAi) (Figure 6A,B). Remarkably, the extent of depression was greater at lower concentrations of glucose and fructose in EP2154/EP2154, NP3200/+ and Gr5a-GAL4; UAS-Goα RNAi, whereas the responses to 500 mM glucose and fructose were comparable with the control strains.

To confirm these data on a behavioral level, we performed 2-choice preference tests, in which flies had the choice between 5 mM sucrose and 13 mM maltose or alternatively between 5 mM sucrose and 75 mM trehalose. Wild-type flies did not prefer these maltose or trehalose solutions compared with the sucrose solution, which is in correspondence with the fact that the sugar concentrations elicited similar spike rates in these flies. NP3200/+ flies, which displayed the strongest phenotype in the electrophysiological responses to sucrose, preferred maltose and trehalose over sucrose in this test, indicating that the observed defective behavioral response is confined to an impaired sucrose perception in the Goα mutant flies (Figure 6C).

Caffeine and quinine perception is unaffected in Goα mutants

Bitter neurons express the receptor Gr66a (Thorne et al. 2004; Wang et al. 2004), which is required for the behavioral and physiological responses to caffeine (Moon et al. 2006). As Goα is also expressed in Gr66a-positive neurons, we investigated the behavioral responses to caffeine and quinine in flies defective in Goα signaling. In the performed avoidance tests, maltose was chosen as control solution because the electrophysiological response to this disaccharide was not affected in those flies. The behavioral tests revealed that flies expressing either RNAi against Goα (UAS-Goα RNAi) or ptx (UAS-ptx) in Gr66a-labeled cells (Gr66a-GAL4) show normal responses to caffeine and quinine (Figure 7A). The majority of flies avoided the solution with the bitter substance, indicating that the detection of this substance was not impaired. EP2154/EP2154 and NP3200/+ flies also did not display any anomaly in response to caffeine or quinine, because these flies had PIs comparable to wild-type flies (Figure 7A). Next, we investigated responses of bitter neurons from EP2154/EP2154 and NP3200/+ flies using tip recordings from S6 sensilla. Caffeine and quinine solutions evoked similar responses in the Goα mutant strains and the control strain (Figure 7B). It is therefore unlikely that Goα is required for the caffeine or quinine response, but we cannot exclude that the protein subunit is involved in the detection of other bitter compounds.

Discussion

Goα is involved in the reception of sucrose

The current analysis shows that the G-protein subunit Goα plays an important role in the reception of the carbohydrates sucrose, glucose, and fructose in Drosophila. We have systematically analyzed behavioral and electrophysiological responses to sugars in different transgenic flies and found these responses significantly altered in comparison to control strains. Notably, the strongest effect on the sucrose responses was observed at the lowest sucrose concentration tested (20 mM) in all transgenic or pharmacological approaches to affect Goα function. As the differences of neuronal activities in the mutant flies remained significant in
comparison to control strains even at the highest concentrations used (500 mM), we conclude that a Goα-related pathway is involved in the reception of low and high concentrations of sucrose.

Both Goα proteins play a role in the reception of sucrose

*Drosophila* has 2 Goα proteins, DGo1 and DGo2, that are generated from the same gene by alternative splicing of transcripts (de Sousa et al. 1989; Schmidt et al. 1989; Thambi et al. 1989; Yoon et al. 1989). These transcripts share the last 6 of overall 7 exons, whereas the first exon is found at different genomic regions in both variants; relative expression rates in the proboscis are 24% for dGo1 and 82% for dGo2. Because expression of dGo1 is unaffected in EP2154/EP2154 flies, loss of DGo2 must be responsible for the observed phenotypes in this strain. On the other hand, dGo2 transcription was affected to a smaller extent in NP3200/+ flies although the observed phenotypes are slightly stronger, indicating that DGo1 also plays a role in the signaling cascade. The simplest interpretation of our results is that both isoforms can complement each other, which is also in correspondence with the rescue experiments using UAS-Goα strains that contain dGo2 cDNA, which lead to a partial, but not complete rescue of the sugar responses.

Both Goα proteins differ in only 7 amino acids in the N-terminal region; the GTPase domains should therefore not show any difference in their function. Because the amino acid sequence of the N-terminal region is crucial for post-translational modifications that result in an anchoring of the α subunit to the plasma membrane (Jiang and Bajpayee 2009), we analyzed the sequence of both Goα proteins with regard to the consensus sequences for myristoylation and palmitoylation. Both proteins share the required sequences for these lipid modifications (data not shown); further studies are therefore required to explain the differences in function of the isoforms.
Goα signaling cascades

The Drosophila Goα also appears to be important for various neuronal functions. Recent studies indicate that Goα contributes to olfactory reception in the fruit fly (Chatterjee et al. 2009). It is moreover important in the development of the organism because Wnt and PCP Fz pathways can be transduced by trimeric G-protein complexes that contain this subunit (Katanaev et al. 2005). Goα is also required for the formation of heart epithelium in Drosophila (Fremion et al. 1999). Heterotrimeric Go is in general the most abundant G-protein in the brain and has been implicated in various functions such as embryogenesis, learning and memory, sensory organ development, and neurodegenerative diseases such as Parkinson and Alzheimer’s disease (reviewed in Jiang and Bajpayee 2009). In line with the ubiquitous expression in the brain, Goα protein subunit seems to be expressed in all taste neurons of the gustatory system, although only the sugar responses were affected in the mutants. Even though Goα seems to not to interact with all taste receptors, the protein might fulfill other functions in the neurons, besides the transduction of GR activation. Because the current understanding of its signaling mechanisms and its effectors is still either incomplete or unknown, these functions are at the current state of knowledge difficult to predict on a molecular level.

In Caenorhabditis elegans, Goα seems to act upstream of a DAG kinase in a serotonergic signaling pathway, where it antagonizes the Gq-phospholipase C beta DAG signaling cascade (Miller et al. 1999). Caenorhabditis elegans Goα was shown to be involved in sensory signal transduction because it is important for olfactory adaptation (Matsuki et al. 2006). Whether this PLC antagonizing effect may also exist in other species such as Drosophila is to date unknown. Goα could be involved in an IP3-activated signaling cascade because the application of inhibitors of the PLC-mediated IP3 transduction cascade yielded reduced responses to various sugars of GRNs in the flesh fly, Boettcherisca peregrina (Koganezawa and Shimada 2002). Moreover, 2 independent transgenic approaches revealed that IP3 signaling is indispensable for the reception of sucrose and trehalose in Drosophila (Usui-Aoki et al. 2005).

A second putative signaling mechanism may involve direct ion channel modulation by Goα proteins. PTX-sensitive Goα proteins can also modulate Ca2+ channels in different mammalian neurons (Hille 1994) and L-type Ca2+ channels via muscarinic receptors in myocytes (Valenzuela et al. 1997). Goα can also activate K+ channels in several signaling systems and in different organisms (VanDongen et al. 1988; Peleg et al. 2002).

Multiple gustatory signaling mechanisms in Drosophila

Despite their 7 transmembrane structure, Drosophila GRs lack sequence homology with mammalian taste receptors or other G-protein coupled receptors but are distantly related to the large family of Drosophila olfactory receptors (ORs) (Dunipace et al. 2001). It has been shown for the olfactory system of Drosophila that ORs form functional heterodimers, consisting of 1 of the 61 ligand-specific ORs and the coreceptor OR83b (Larsson et al. 2004; Neuhaus et al. 2005). Drosophila ORs are 7 transmembrane proteins, which appear to have an extracellular C-terminus and an intracellular N-terminus (Benton et al. 2006; Lundin et al. 2007) and...
function as ligand-gated ion channels (Sato et al. 2008; Wicher et al. 2008). Even so, Drosophila olfactory signal transduction involves the heterotrimeric G-proteins Gqα and Goα (Kain et al. 2008; Chatterjee et al. 2009).

Although not unambiguously shown until now, Drosophila GRs may also exhibit ligand-gated channel properties besides activation of G-proteins. The existence of a G-protein–independent signaling pathway in insect sugar neurons was already suggested by in situ patch clamp recordings showing that an ion channel is directly gated by sucrose in the flesh fly (Murakami and Kijima 2000). Such ionotropic properties of GRs would provide a reasonable explanation why we did not observe an elimination of sucrose-induced responses in our electrophysiological experiments with Goα mutant flies. Similar results were obtained in analogous experiments with G2:1 null mutants that showed a significantly decreased response to sucrose in comparison to control flies, but not a complete suppression of the sugar-evoked response (Ishimoto et al. 2005). According to a recently proposed dual-activation model (Nakagawa and Vosshall 2009), the primary response to sucrose in Drosophila could be generated by activation of sucrose-gated ion channels composed of different GRs, followed by a G-protein–mediated potentiation of the ionotropic response. The fact that CO2 detection, mediated by a heterodimer consisting of GR21a and GR63a expressed in the fly antenna, requires Gqα and Gγ30a in vivo provides additional evidence for GR-mediated signal transduction via a metabotropic mechanism (Yao and Carlson 2010).

According to this model, Goα would play a modulatory part in the physiological detection of sugars. As demonstrated before, the severest defects of Goα mutants in tip recordings were observed at low sucrose concentrations, supporting a role of Goα in potentiating receptor function.

Role of other G-proteins in the perception of sugars

Goα was found to be involved in the perception of sucrose, glucose, and fructose, whereas trehalose and maltose seem to be independent from a Goα-related pathway, suggesting that there are heterogeneous pathways for the detection of sugars in Drosophila. These findings complement previous studies, in which a Gsα- and a Gqα-related pathways were suggested for trehalose perception (Ueno et al. 2006; Kain et al. 2010). Ueno et al. (2006) performed an electrophysiological approach and found significantly reduced spike rates in Gr5a-GAL4/UAS-Gsα RNAi and heterozygous Gsα null mutants upon stimulation of L-type sensilla with low trehalose concentrations. In a behavioral assay, the authors accessibly found a depressed intake of sucrose, fructose, and glucose in these flies. Using a different approach to study gustatory behavior, we found that Gr5a-GAL4;UAS-Gsα RNAi flies performed like wild-type flies in 2-choice preference tests. Despite the existence of minor differences that might be explained by the different experimental paradigms, one can conclude that Gsα-, Gqα-, and Goα-related pathways are involved in sugar perception of the fly.

The different G-protein pathways could be activated via different types of sugar receptors, although until now no type of G-protein is assigned to a certain GR in Drosophila. Whereas a Gr5a-initiated pathway in response to trehalose may involve Gsα and/or Gqα coupling in vivo, sucrose could activate a Goα-related pathway via other GRs, most likely involving a receptors of the Gr64 cluster. Because GRs were shown to interact (Jiao et al. 2008), different types of receptor heteromultimers may couple preferentially to different types of heterotrimeric G-proteins, depending on their subunit composition.

Activation of different, stimulus- and concentration-dependent signal pathways in sugar-sensitive cells was also observed in mammals (reviewed in McCaughey 2008). Whereas sucrose elicits, for example, an intracellular increase of the Cyclic adenosine monophosphate and subsequent Ca2+ influx, saccharin stimulation induces an intracellular increase in the secondary messenger IP₃ and results in Ca2+ release from internal stores (Bernhardt et al. 1996). However, there is evidence that the second pathway can also be activated by sucrose in mice (Zhang et al. 2003).

This report may help to understand the role of GR signaling in the insect gustatory system. Insight into the molecular determinants underlying receptor/G-protein interactions may help to fully understand the mechanisms and consequences of processing taste stimuli in future studies.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/.

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