mouth are shown in Fig. 3. The responses were depolarizing or hyperpolarizing. The mean amplitudes of acid responses in taste cells, non-taste cells in the fungiform papillae and non-taste cells in the filiform papillae were 27.0, 15.9 and 20.3 mV, respectively, which did not show any statistical difference. These values were much larger than those in non-taste cells of the ventral surface of the tongue and the palate. The time to peak of a depolarization induced by 0.5 mM acetic acid was 1–5 sec in taste cells as well as non-taste cells. These values were the smallest compared with the peak times of depolarizations induced by the other basic taste and water stimuli. Some non-taste cells in the ventral surface of the tongue and the palate were hyperpolarized or did not respond to acid (Table 1).

Sucrose responses. Electrical responses to 1 M sucrose of taste cells and non-taste cells are shown in Fig. 4. The

<table>
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<th>10 mM Q-HCl</th>
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ventral surface of the tongue, and the palate, respectively.

Non-Taste Cell Responses in Frog

Fig. 4. Electrical responses of taste cells and non-taste cells to 1 M sucrose. (A) Taste cell response. (B)-(E) Non-taste epithelial cell responses at various portions described. The inset graph shows the mean amplitudes of the sucrose responses.

Fig. 5. Electrical responses of taste cells and non-taste cells to 0.5 M NaCl. (A) Taste cell response. (B)-(E) Non-taste epithelial cell responses at various portions described. The inset shows the mean amplitudes of the NaCl responses.
response magnitude in taste cells was 8.0 ± 2.0 mV, which was significantly larger than those in non-taste cells in four different areas. As shown in Table 1, the hyperpolarized responses appeared in non-taste cells in a high percentage. The time to peak of a depolarization in response to the sucrose was a range of 11-21 sec in both types of cells.

NaCl responses. Figure 5 shows an example of responses to 0.5 M NaCl in taste cells and non-taste cells. Both types of cells investigated were mostly depolarized by 0.5 M NaCl (Table 1). The mean amplitudes of the responses were a range of 10.2-14.6 mV, where no significant difference was found. The peak time of a depolarization in both cells was from 26 to 34 sec, which was the longest of all taste stimuli used.

Q-HCl response. Figure 6 shows an example of Q-HCl responses in taste cells and non-taste cells. The amplitude of the Q-HCl responses in 22 taste cells was 3.2 ± 0.5 mV, which was the smallest value of responses evoked by four basic taste stimuli. The Q-HCl responses in the other non-taste cells were almost the same values. Excepting non-taste cells in the filiform papillae, the hyperpolarized and no responses appeared in 20-30% of the non-taste cells in the other epithelia (Table 1). The peak time of a depolarization in both cells evoked by Q-HCl was a range of 7-21 sec.

Water responses. As shown in Table 1, many taste cells and non-taste cells responded to deionized water with hyperpolarizing responses. These responses are due to removal of the adapting Ringer solution covering each epithelium by deionized water. Some taste and non-taste cells were depolarized by water. As shown in the inset of Fig. 7, the mean magnitudes of responses in taste and non-taste cells for water were all negative (Fig. 7). A hyperpolarization range was from −2.7 to −14.7 mV. The hyperpolarizing response of the filiform papilla cells was generally larger than that of the other non-taste cells.

DISCUSSION

It has been reported that supporting cells in the taste bud and the taste disk are depolarized by various chemical stimuli [17, 18, 24]. Depolarizing responses in non-taste cells besides the taste organ by various chemical stimuli are reported in mudpuppy epithelial cells [24], neuroblastoma cells [6], Tetrahymena and Nittela cells [2].

In the time course of electrical responses in taste and non-taste cells, the peak time of depolarization evoked by acid stimulus (Fig. 3) was much shorter than that by the other taste stimuli (Fig. 4-7). This result is consistent with the
previous study with frog supporting cells in taste disk [17]. The amplitude of resting potentials of non-taste cells investigated in the present study was, on the average, 55% of that of the taste cells. The response amplitudes of taste cells and non-taste cells were almost the same when NaCl and Q-HCl were used. However, the response magnitude of taste cells for acetic acid was the same as those of non-taste cells in the dorsal surface of the tongue, but was much larger than that of non-taste cells in the ventral surface of the tongue and the palate. The sucrose responses in taste cells were much larger than those in non-taste cells at every region, suggesting that sweet-binding receptors are formed mostly in the taste cells, but hardly in non-taste cells (Fig. 4).

The response characteristics such as the amplitude of response and the peak time of response were, on the whole, very similar between taste cells and non-taste cells. We have been studying ionic mechanisms of receptor potentials in frog taste cells induced by four taste stimuli and deionized water. We have proposed the following mechanisms: (1) In case of NaCl stimulation, the receptor potentials are generated by functions of cationic and anionic channels at the receptive membrane and second messenger-dependent cation channels at the basolateral membrane of the taste cells [8, 9]. (2) In case of acid stimulation, Ca$$^{2+}$$ channels and H$$^+$$ transporters such as H$$^+$$ pump at the receptive membrane play an important role in generating acid-induced receptor potentials [7, 13]. (3) In case of bitter stimulation, the depolarization is produced by a secretion of intracellularly accumulated Cl$$^-$$ through the apical receptive membrane [10]. (4) In case of sugar stimulation, the receptor potential is generated by an entry of extracellular H$$^+$$ through the apical receptive membrane [11]. (5) In case of water stimulation, the receptor potential is generated by a secretion of Cl$$^-$$ through the apical membrane and by a blockage of K$$^+$$ outflow through the basolateral membrane [12].

It has been reported that a taste cell responds to odorants with a depolarization [4], while an olfactory cell responds to tasters with a depolarization [22]. However, the mechanisms underlying these responses have not yet been understood.

Although some non-taste cells in lingual epithelia and other tissues respond to chemical stimuli of very low concentrations [2, 17, 24], other non-taste cells slightly respond to chemical stimuli of very high concentrations alone (for example: frog striated muscle fibers, *Drosophila* salivary gland cells and frog stomach epithelial cells, unpublished data by Sato T).

Since the response characteristics of non-taste cells in the frog mouth examined in the present experiments are, on the whole, very similar to those of taste cells, it is probable that tastant-induced responses in both taste cells and non-taste cells are induced by some common chemo-electrical transduction mechanisms, which involve receptor sites and ionic channels of voltage-sensitive and ligand-sensitive types. Molecular transduction mechanisms in non-taste cells have to be clarified in the next step.

ACKNOWLEDGMENTS

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Immunogold Colocalization of Opsin and Actin in *Drosophila* Photoreceptors That Undergo Active Rhabdomere Morphogenesis

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ABSTRACT—This paper describes the localization of visual pigment opsin and its association with actin in the photoreceptors of newly emerged (within 12 hr after emergence) *Drosophila melanogaster*. The photoreceptor of newly emerged flies was characterized by the rich content of rough-surfaced endoplasmic reticulum (rER) and the small rhabdomere: the photoreceptor is actively constructing rhabdomere, and therefore suitable to study the mechanism of rhabdomere morphogenesis. The photoreceptor specifically contained opsin-bearing structures some of which were enclosed by several layers of membranes. The structure became sparse in 10 d old flies. Opsins in the structure may be incorporated into the new rhabdomere. The antiopsin also labeled the plasma membrane facing to the intramembratal space and the endomembranes in the cell body. Both regions were furnished by uniformly oriented actin filaments with the plus ends towards the rhabdomere. Such orientation makes the actin filaments possible to be involved in the vectorial transport of materials towards the rhabdomere by a presumptive interaction with the myosin-like *ninaC* proteins identified in *Drosophila* photoreceptors.

INTRODUCTION

Photoreceptor function is maintained throughout the life by continuous turnover of the photoreceptive membrane both in vertebrates [6, 9, 18] and invertebrates [7, 21]. In the arthropod compound eye, old membranes are removed from the phototransductive rhabdomere and digested by the lysosomal system in the photoreceptor itself [7, 25]. The removal of photoreceptive membranes is accompanied by the reciprocal addition of new membranes to the rhabdomeral microvilli. The new membranes should contain visual pigment opsin as an integral membrane protein, and should be transported from the cell body towards the base of the rhabdomeral microvilli where the membrane addition is taking place [8, 21–23].

The transport requires force. The force in this case must be able to transport materials towards the rhabdomere. A possible candidate of such a force-producing system is the actin-myosin interaction, because the photoreceptor cell body is furnished by actin filaments [2] and the myosin-like *ninaC* proteins (NINAC) [13, 17]. If the conventional actin-myosin interaction occurs between the actin and NINACs, the produced force could transport materials along the actin filaments towards their plus ends, which attach to the tip of the rhabdomeral microvilli in arthropod photoreceptors [2, 5, 12]. In fact, mutations in the myosin domain of a NINAC isoform disrupt the accumulation of calmodulin in the rhabdomere [19], suggesting that the actin-NINAC interaction is involved in the calmodulin transport into the rhabdomere. The interaction may also transport other rhabdomeral proteins such as opsin.

If the transport of opsin is mediated by actin, the moving opsins are expected to be found close to the actin filaments. Detection of the situation must be easier in the photoreceptors that are actively constructing the rhabdomere than in the mature photoreceptors. The first aim of this paper is to demonstrate that the newly emerged flies undergo active rhabdomere morphogenesis. Furthermore we present the distribution pattern of opsin and its association with actin filaments in the photoreceptors of the newly emerged flies revealed by the electron microscopic histochemistry.

MATERIALS AND METHODS

Animals

Newly emerged (within 12 hr after adult eclosion) and 10 d old flies of wild-type *Drosophila melanogaster* (Canton S strain) were obtained from a laboratory stock culture kept under a 12 hr light/12 hr dark cycle at 25°C.

Conventional electron microscopy

Light-adapted compound eyes were fixed with 2% glutaraldehyde plus 2% paraformaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 (CB) overnight at 4°C. After a brief wash with CB, the tissues were post fixed with 2% OsO₄ in CB for 2 hr at room temperature. The tissues were then dehydrated through a graded series of ethanol and embedded in Epon. Ultrathin sections, cut at the level of photoreceptor nuclei, were double stained with 4% uranyl acetate in 50% ethanol and Reynolds' lead citrate solution. The electron micrographs were taken with a JEOL 1200EX electron microscope. We measured the size of the rhabdomeres and other structures on electron micrographs using a digitizer tablet connected to a computer.

Electron-microscopic immunogold labeling

Light-adapted compound eyes were fixed with 2% glutaraldehyde plus 2% paraformaldehyde in 0.1 M sodium phosphate buffer...
at pH 7.4 (PB) for 1 hr at room temperature. The tissues were then dehydrated through a graded series of methanol and embedded in L. R. White resin. Ultrathin sections were cut with a diamond knife and collected on nickel grids.

We used a monoclonal mouse IgG against Drosophila Rh1 (anti R1-6 opsins, provided by Dr. T. Tanimura) [10] and a monoclonal mouse IgM against chicken-gizzard actin (Amersham, code N.350). The antiactin detects a single band with apparent molecular weight of 42 kDa on a Western blot of Drosophila head homogenate; the antibody labels both G- and F-actins [2]. The labeling was done by the following two methods.

**Mixed-antibody method**: Each step of the labeling was done by floating the grid on 5–50μl drop of solution. The sections were first etched with saturated sodium metaperiodate in distilled water for 1 hr, and then blocked with 4% bovine serum albumin (BSA) in PBSG (0.1 M sodium phosphate buffer at pH 7.4 plus 0.5 M NaCl, 0.25% gelatin) for 30 min. The blocking was followed by incubation with the mixture of antiopsin (final conc. 1:200–1:400 of the original) and antiactin (final conc. 10–25μg/ml) in 1% BSA in PBSG overnight at 4°C. After washing with PBSG the primary antibodies were detected by the mixture of goat-anti-mouse (GAM) IgG-conjugated 15nm-gold (Janssen, final conc. 1:50) and GAM IgM-conjugated 5 nm-gold (Janssen, final conc. 1:50) in PBSG. Thus the GAM IgG-15 nm gold detects antiopsin (mouse IgG) whereas the GAM IgM-5 nm gold detects antiactin (mouse IgM). Control labeling was done by removing either the antiactin or antiopsin from the primary antibody-mixture.

**Two-surface method**: Each step of the labeling was done by floating the grid with the appropriate side down on 10–50μl drop of solution. Etched and blocked surface was incubated with antiactin in PBSG plus 1% BSA (10–25μg/ml) overnight at 4°C. The antiactin was detected by GAM IgM-5 nm gold in PBSG (1:50). After washing with distilled water and air-drying, the other surface was etched, blocked, and incubated with antiopsin in PBSG plus 1% BSA (1:200–1:400 of the original) overnight at 4°C. The antiopsin was detected by GAM IgG-conjugated 15 nm-gold in PBSG (1:50). Control labeling was done by replacing either antiopsin or antiactin with 1% BSA in PBSG. The sections were then stained and observed as described above.

**Decoration of actin filaments with myosin subfragment-1**

This procedure follows that of Arikawa and Williams [4]. Briefly, isolated light-adapted compound eyes were first incubated with 1.0% Triton X-100 in a buffer solution (150 mM KCl, 2 mM DTT, 20 mM Tris-HCl, pH 7.4) at room temperature for 60 min with gentle agitation. After a wash with the buffer for 30–40 min, the eyes were incubated with myosin subfragment-1 (S1, 10–15 mg/ml in the buffer) for 2 hr at room temperature. The tissues were then similarly processed as for the conventional electron microscopy.

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**Fig. 1** Transverse section of Drosophila photoreceptor. (a) Newly emerged fly (within 12 h after emergence). Note the rich content of rER (arrows) in the cell body. (b) 10 d old fly. I; intraommatidial space, LB; lysosomal body, LCB; large complex body, M; multivesicular body, m; mitochondria, R; rhabdomere. Scale bar = 1 μm.
RESULTS

Anatomy of the photoreceptors of the newly emerged flies

A Drosophila ommatidium contains eight photoreceptor cells (R1–8) each bearing a rhabdomere. R1–6 provide the six peripheral rhabdomeres, and R7 and R8 form a tiered rhabdomere in the center of the ommatidium. Thus, only seven rhabdomeres are observed in any given transverse section of an ommatidium.

The photoreceptor of the newly emerged flies (within 12 hr after emergence) has a well-organized rhabdomere (Fig. 1). The rhabdomere is, however, significantly smaller than the fully developed rhabdomeres of 10 d old flies (Fig. 2): the rhabdomeres are still developing in this stage. The periphery of the photoreceptor cell body is characterized by the rich content of rER (Fig. 1). The amount of rER was quantified as the length appeared in transverse sections. The newly emerged flies contained significantly more rER compared to 10 d old flies (Fig. 2, P<0.01, Student’s t-test).

Multivesicular bodies (MVBs, Figs. 1 and 3a) and lysosomal bodies (LBs, Figs. 1 and 3c) were commonly found. Also common was the large structure of irregular shape containing vesicles, ribosomes, and/or rER (Fig. 3e, g). The structures themselves were embedded in the rER mass. Several layers of membranes enclosed the structure in some cases (Fig. 3e). We hereafter refer the structure as the large complex body (LCB). We measured the areas occupied by the MVBs, LBs, and LCBs in R1–6 in transverse sections at the level of nuclei of the photoreceptors (Fig. 4). The LCB occupied about 1.6% (4.77±1.6μm²) of the total area of R1–6 in newly emerged flies. The area significantly decreased in 10 d old flies to about 0.1% (0.23±0.08μm², P<0.05, Student’s t-test), in which the LB reciprocally increased. The area occupied by the MVB remained constant (Fig. 4).

Distribution of opsins and actin

Figure 5 shows the results of control labeling for the mixed antibody method. Each section was first incubated with either antiactin (Fig. 5a) or antiopsin (Fig. 5b), and both were then reacted with the mixture of GAM IgG-15 nm gold and GAM IgM-5 nm gold. Since one of the primary antibodies was removed from the initial incubation, gold particles of only one size bound on each section, indicating that the detection system functioned properly. However, the density of antiactin labeling on the rhabdomeres was not consistent. Both in control and experimental double labeling, the density varied between rhabdomeres even in a single section (data not shown). The antiactin labeling in the cell body region and the antiopsin labeling are rather constant. Two surface method gave virtually the same result.

The antiactin recognizes actin in all photoreceptors [2], whereas the antiopsin specifically binds to Rh1, the opsin of R1–6 photoreceptors [10]. The following observations were therefore made on R1–6 photoreceptors.

Apparent colocalization of antiopsin and antiactin labeling was observed on the rhabdomere (e.g., Fig. 6). The antiopsin labeled the MVBs (Fig. 3b), LBs (Fig. 3d), and LCBs (Fig. 3f, h) in the cell body. The vesicles and the lamellated membranes contained in the LCBs were densely labeled with antiopsin, while the labeling was hardly detected on the associated ribosomes and rER. Other regions labeled with antiopsin were the plasma membrane facing to the intraommatidial space (Fig. 6a, b) and the endomembranes in the cell body (Fig. 6g, h). Opsin-bearing vesicles were found close to the plasma membrane (Fig. 6e). Although rarely, patchy labeling was detected on the rER (Fig. 6b, f). Table 1 summarizes the distribution of antiopsin labeling in a single photoreceptor. Nearly 45% of labeling was found outside the rhabdomere. Note the significant decrease in the labeling on the plasma membrane facing to the intraommatidial space in 10 d old flies (Table 1, P<0.05, Student’s t-test). The difference in the particle numbers is directly attributed to the difference in the labeling density on the membrane, because unlike the LBs and LCBs, the length of the plasma membranes in the sections does not change between newly emerged and 10 d old flies.