

Destruction and Reorganization of the Receptor Membrane in Labellar Chemosensory Cells of the Blowfly

Recovery of Responses to Sugar After Destruction

MITSUYOSHI NINOMIYA, MAMIKO OZAKI,
YASUHIRO KASHIHARA, and HIROMICHI MORITA

From the Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan

ABSTRACT Recovery from destruction by sodium deoxycholate (DOC) was studied with the receptor membrane of the blowfly, *Phormia regina*. The recovery can be divided into two processes, colchicine dependent and colchicine independent. The colchicine-dependent process was completely depressed by pretreatment with colchicine at 5 mM for 2 min (partially at 0.1 mM for 10 min), but the colchicine-independent one persisted. Vinblastine also caused depression but lumicolchicine did not. Records of responses obtained from the DOC-treated sugar receptor showed long response latencies that gradually became indistinct with recovery. Colchicine also affected this change in response latency after the DOC treatment. These results suggest that the colchicine-dependent recovery process is related to microtubules in the distal process of the receptor cell. The recovery time course and the change in response latency could be quantitatively explained by the simple assumptions that DOC underwent desorption from the receptor membrane (colchicine-independent recovery process) and that regeneration of the disrupted distal process of the receptor cell accompanied recovery in the number of available receptor sites (colchicine-dependent recovery process).

Address reprint requests to Dr. Hiromichi Morita, Dept. of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812, Japan. Dr. Ninomiya's present address is Shionogi Research Laboratories, Shionogi & Co., Ltd., Sagisu, Fukushima-ku, Osaka 553, Japan. Dr. Ozaki's present address is Dept. of Biological Sciences, Lilly Hall of Life Science, Purdue University, West Lafayette, IN 47907. Dr. Kashiwara's present address is Laboratory of Learning and Memory Research, Dept. of Information Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki 444, Japan.

INTRODUCTION

The sugar receptor response in the fleshfly can be recovered after a complete loss of the response caused by treatment with detergents (Shimada, 1975). This recovery is important to the contact chemosensory cells, which can be exposed to many dangerous substances while the fly searches for food. The mechanism of this recovery process is interesting in itself and should be related to the maintenance of responsiveness.

In this work, we studied some properties of the recovery process in the blowfly after treatment with sodium deoxycholate (DOC) and obtained results suggesting the involvement of microtubules in the process. This is particularly interesting because microtubules have also been postulated to be important for the maintenance of membrane excitability in squid giant axons (Matsumoto and Sakai, 1979a, b). The present results on the recovery of the sucrose response and on the response latency were quantitatively examined with the equation proposed by Morita (1969) for the sucrose response. The excellent agreement led us to estimate some important constants. One of them was the dissociation constant between sucrose and the receptor molecule, which should be especially valuable for biochemical studies.

MATERIALS AND METHODS

The blowfly, *Phormia regina*, was used 2–7 d after emergence. The chemosensilla used were of the largest type located on the outer margin of the labellum. A glass capillary containing a stimulus substance (sucrose) dissolved in 10 mM NaCl was used for the stimulation and for the recording of impulses (Hodgson et al., 1955). An isolated head was mounted on a piece of platinum wire, which served as an indifferent electrode, and the glass capillary was made to cover the chemosensillum at the tip. The duration of the stimulus was ~1 s, and the intervals between stimuli were no shorter than 3 min. The response to sugar was defined as the number of impulses during a period from 0.15 to 0.35 s after the initiation of the impulse discharge, during which the response could be roughly regarded to be in a steady state. To record the receptor potential, two glass microelectrodes filled with Waterhouse's solution (Buck, 1953) were inserted into the outer lumen (the compartment containing no sensory cilia) of the labellar chemosensillum, with the proximal microelectrode being grounded (Morita, 1969). The potential difference between the two electrodes was recorded through DC amplification with appropriate positive feedback at ~1 kHz at the input stage. For all other records, electric signals were cut off below 100 Hz.

For the long-lasting experiments (Fig. 2), a live fly was mounted on the stage of a microscope with its wings pinched with a small clip. Its proboscis was fixed in the extended position by inserting the base of the haustellum into a gap between two pieces of steel wire, which also served as an indifferent electrode (Getting, 1971). No difference was seen in either the r_{∞} (the maximum response) or the K_b (the concentration at which the sugar evoked the half-maximal response, i.e., the half-maximal concentration) of the sensilla of flies mounted alive and those on isolated heads. All the electrophysiological experiments were done at 22–24°C and at a relative humidity of 70–90%.

The sugar receptor was treated with various reagents, as for the stimulation using a glass capillary filled with these solutions. Vinblastine (Sigma Chemical Co., St. Louis, MO) in glycerol solution was used after dilution with distilled water (final concentration, 10% glycerol and 1% vinblastine). All other reagents were dissolved in 67 mM Na phosphate

buffer at pH 7.6. The treatment with sodium deoxycholate (DOC) was for 2 min at 7.2 mM. Colchicine (Nakari Chemicals, Ltd., Osaka, Japan) solution was used within a day after dissolution. Lumicolchicine was prepared by irradiating a 1-mM solution of colchicine in ethanol for 15 h with an ultraviolet lamp (Wilson and Friedkin, 1966) and concentrating it by evaporation. The conversion of colchicine into lumicolchicine could be monitored spectrophotometrically.

RESULTS

Recovery of Responses After DOC Treatment

Treatment of the sugar receptor with 7.2 mM DOC for 2 min led to the complete loss of its response to sucrose for 10–20 min. Injury discharge of impulses was observed during this treatment but it usually disappeared before the treatment ended.

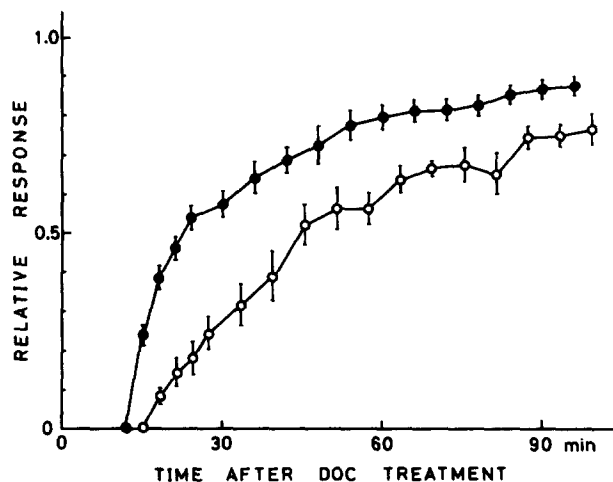


FIGURE 1. Recovery time course of the sucrose response after DOC treatment. The time course differs with different test stimulus concentrations. Each point is the mean value of seven (0.01 M sucrose, ○) or nine (0.1 M sucrose, ●) separate preparations and the bar indicates the SEM.

Fig. 1 shows the complete loss and subsequent recovery of the response to 0.1 or 0.01 M sucrose after the DOC treatment. Here, the relative response means the ratio of the response to that before the treatment. The response to 0.1 M sucrose was lost completely, then recovered gradually, and nearly attained a steady level in 90 min. The steady level lasted for several hours, with the recovered response being ~85% of the original level.

The same recovery process after DOC treatment seems to have been slower and to have reached a lower steady level when the response was tested with 0.01 M sucrose (Fig. 1). The DOC-treated sugar receptor was stimulated every 3 min after the treatment, and the period from the end of the DOC treatment to the last stimulation without a response was defined as the delay time of the recovery.

The averaged delay time (\pm SEM) for 0.1 or 0.01 M sucrose stimulation was 12.1 ± 1.6 ($n = 9$) or 15.4 ± 1.3 min ($n = 7$), respectively. Thus, the same recovery process displayed a different pattern when tested with a different stimulus concentration (see the Discussion for this difference).

Depression of the Recovery

Colchicine. The sugar receptor was pretreated with colchicine before the DOC treatment (colchicine-DOC treatment) in order to examine the effects of colchicine on the recovery process. The results of the long-lasting experiments are shown by the curve at the bottom in Fig. 2. The recovery response to 0.1 M sucrose was markedly depressed by 2 min pretreatment with 25 mM colchicine at pH 7.6. This depression continued for at least several hours, whereas the treatment with colchicine alone showed no effect on the response to 0.1 M

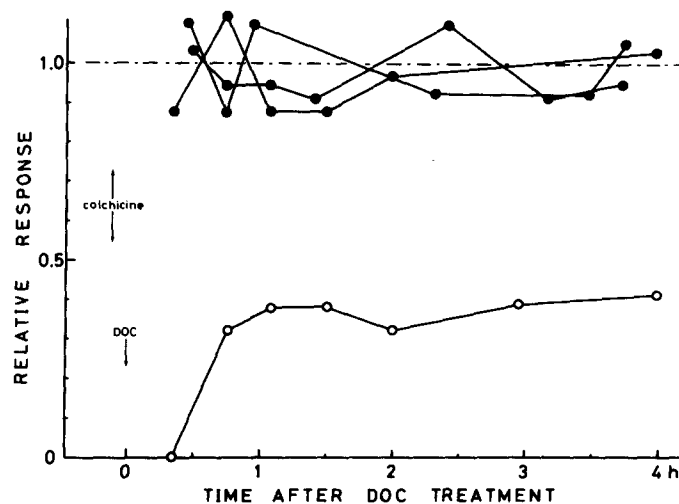


FIGURE 2. Effect of colchicine on the recovery of the DOC-treated sugar receptor or on the responses of DOC-untreated sugar receptors. The colchicine treatment was for 2 min at 25 mM. Live flies were used. Colchicine, ●; colchicine-DOC, ○.

sucrose during the same period (three curves at the top in Fig. 2). Thus, the colchicine treatment was effective only after the DOC treatment. Therefore, the depressive effect of colchicine on the sugar response during the recovery seems to result from its action on a certain recovery mechanism, but not on a sensory transduction mechanism.

Fig. 3 shows the recovery time course of the response to 0.1 M sucrose after DOC treatment, which had been preceded by pretreatment with 0 (the same as in Fig. 1), 0.5, 1.0, or 5.0 mM colchicine for 2 min. The averaged delay times (\pm SEM) for 0.5, 1.0, and 5.0 mM colchicine pretreatments were 10.2 ± 1.2 ($n = 9$), 16.1 ± 2.3 ($n = 8$), and 13.1 ± 2.2 min ($n = 7$), respectively (see above for 0 mM colchicine). There was no significant difference in the delay times for the various colchicine concentrations. This series of experiments shows that the main effect of colchicine on the recovery time course is to decrease the steady level it attains.

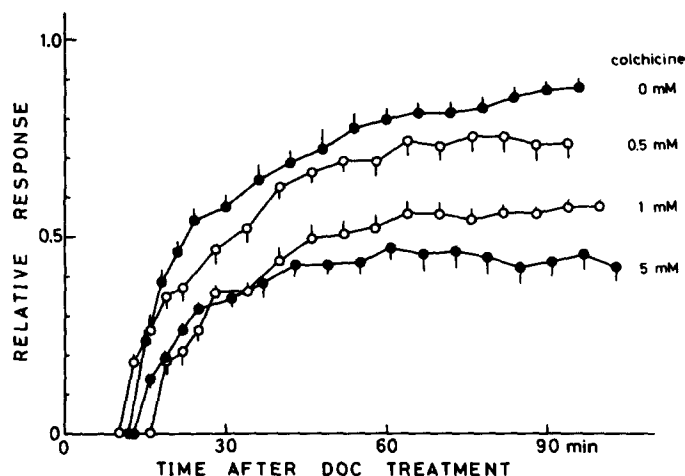


FIGURE 3. Effect of colchicine concentration on the recovery time course of the 0.1 M sucrose response after DOC treatment, which was preceded by pretreatment for 2 min with a given concentration of colchicine. Each point is the mean value (\pm SEM) of 7–10 separate preparations.

Fig. 4 shows the responses at 90 min after the DOC treatment, which were regarded as those at a steady level of recovery, as plotted against the colchicine concentration used for the pretreatment. Pretreatment for 10 min was more effective than that for 2 min. Even 0.1 mM colchicine was effective with 10 min pretreatment. Thus, the duration of colchicine treatment was critical to the exertion of its depressive effect.

Fig. 4 also shows that the recovery after the DOC treatment consisted of colchicine-dependent and colchicine-independent components. The colchicine-

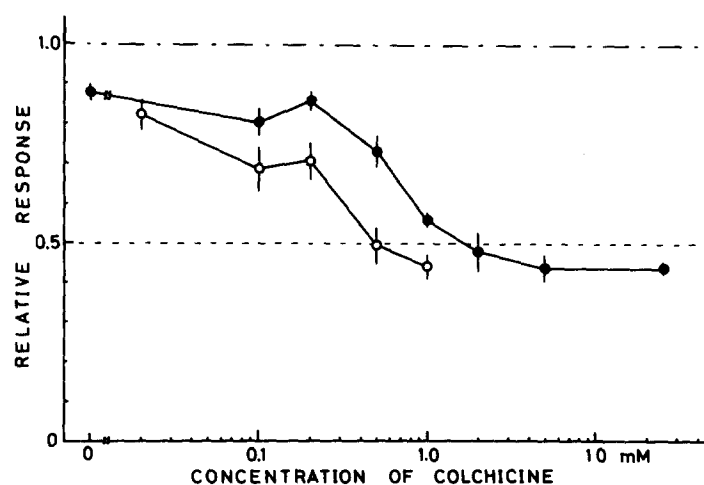


FIGURE 4. Effect of duration of pretreatment on the recovery responses to 0.1 M sucrose at 90 min after DOC treatment. Each point is the mean value (\pm SEM) of 7–10 separate experiments. 2 min treatment, \bullet ; 10 min treatment, \circ .

dependent component was completely depressed by colchicine pretreatment at 5 mM for 2 min or at 1 mM for 10 min. The colchicine-independent component persisted even after 25 mM colchicine pretreatment, and retained as much as 0.45 of the relative response. The recovery time course of the colchicine-independent component fits well a single exponential with a time constant of 15 min (Fig. 3; 5 mM colchicine). The recovery time course of the colchicine-dependent component was clearly slower, its time constant being 50 min if expressed by a single exponential (Fig. 3; 0, 0.5, 1 mM colchicine). Quantitative analyses are presented in Fig. 9 (Discussion).

Other related compounds. Colchicine affects the microtubule assembly-disassembly system and binds to tubulin, the protomer of microtubules (see, for reviews, Wilson et al., 1974; Snyder and McIntosh, 1976; Correia and Williams,

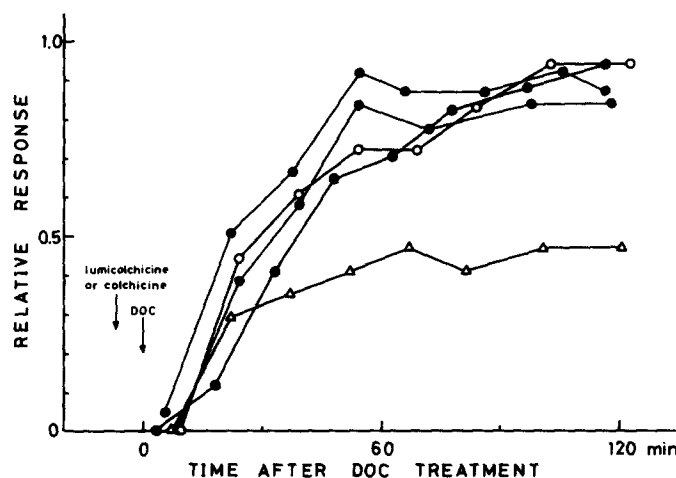


FIGURE 5. Effect of 1 mM lumicolchicine pretreatment for 10 min on recovery of the 0.1 M sucrose response after DOC treatment (●). For comparison, the recovery time courses are also shown for DOC treatment (O) and colchicine-DOC treatment (1 mM, 10 min) (Δ).

1983). Therefore, we examined the effects of another antimicrotubular reagent, vinblastine, and a non-antimicrotubular colchicine isomer, lumicolchicine, on the recovery of the sucrose response after DOC treatment. Pretreatment with 1% vinblastine for 2 min remarkably depressed the recovery of the sucrose response as observed with 25 mM colchicine pretreatment for 2 min (not shown). However, no depression of the recovery was produced when the sugar receptors were pretreated with 1 mM lumicolchicine for 10 min (Fig. 5), although the same conditions of pretreatment with colchicine (1 mM for 10 min) were sufficient to cause the maximum depression.

These results with vinblastine and lumicolchicine as well as colchicine suggest that microtubules are involved in the mechanisms for the recovery.

Other Properties of the Recovery

Fig. 6 shows the sucrose concentration-response curves obtained before the colchicine-DOC treatment (control) and at the steady recovery phase after this

treatment. Here, the response is normalized so that the maximum response in the control is unity. The K_b value increased and the r_∞ value decreased after the treatment. The averaged K_b values (\pm SEM) in the control and the colchicine-DOC-treated sugar receptor were 14 ± 4 ($n = 6$) and 75 ± 4 mM ($n = 6$), respectively.

Neither a change in shape nor any grouping discharge of impulses was seen in the records of the sucrose responses obtained after the DOC treatment. However, there were two noticeable changes, i.e., a long latency and a lack of the phasic response in impulse discharge. In the DC records obtained from an untreated sugar receptor, the fast rise of receptor potential initiated the first impulse within

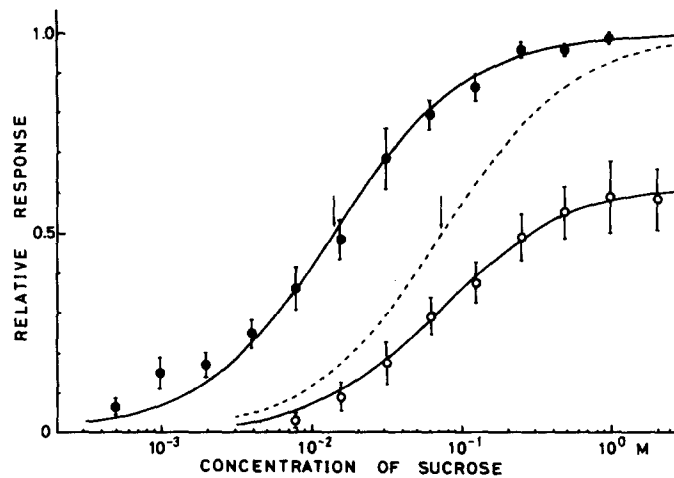


FIGURE 6. Concentration-response curves for sucrose stimulation obtained before (●) colchicine-DOC treatment (25 mM, 2 min) and during the steady recovery phase after (○) the treatment. The response is normalized so that the maximum response before the treatment is unity. Each point is the mean value (\pm SEM) of six separate preparations. The smooth curves represent the theoretical curves according to Eq. 1. The broken line represents the theoretical curve for recovered responses, which were normalized so that the maximum was unity. The values of K_b (shown by the arrow) in the curve before and after the treatment are 14 and 75 mM, respectively.

a few milliseconds after the onset of stimulation, and the initial high rate of discharge of impulses (phasic response) declined to a steady rate (tonic response) within 0.15 s (Fig. 7, top). In records obtained from the sugar receptor during the steady recovery phase after colchicine-DOC treatment, a long latency without the phasic response was associated with the slow rise of receptor potential, and the slow fall of receptor potential was accompanied by impulses over 1 s after the end of the stimulus (Fig. 7, bottom).

Systematic analyses of the changes in the response latency with recovery are shown in Fig. 8. The response latency was defined as the period from the onset of the stimulus to the discharge of the first impulse. Recovering from complete depression after the DOC treatment, the response latency decreased gradually

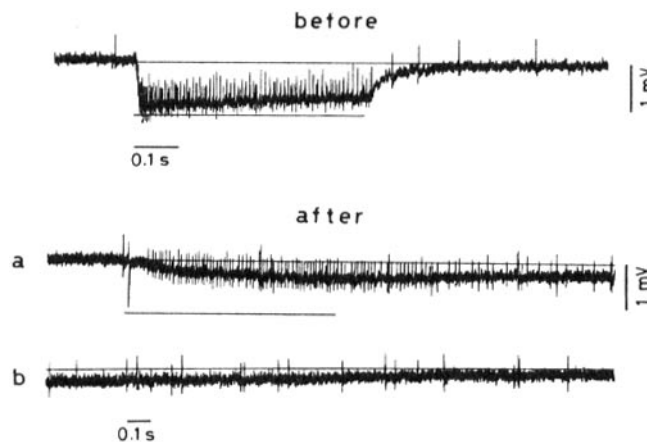


FIGURE 7. A typical DC record of the 0.1 M sucrose responses, obtained before and after colchicine-DOC treatment (25 mM, 2 min) (continuous *a* to *b*). The horizontal bar represents the period of sucrose stimulation. Note that the time scale differs in the two records.

and lasted a few milliseconds. However, a long latency was observed even at the steady recovery phase with colchicine pretreatment. Thus, the recovery in the response latency after DOC treatment was also depressed by colchicine pretreatment.

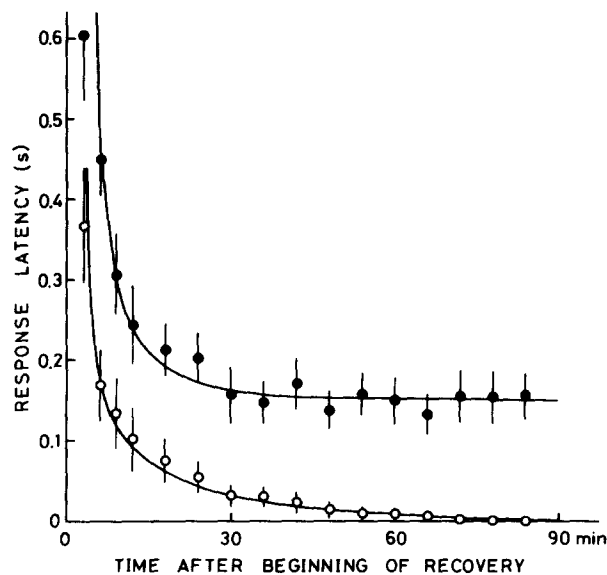


FIGURE 8. Change in response latency with recovery time after DOC treatment or colchicine-DOC treatment (25 mM, 2 min). Each point is the mean value (\pm SEM) from 7–10 separate experiments. The continuous lines were calculated from Eq. 5 with the constants given below (see Discussion). Colchicine treatment: O, 0 mM, 2 min, $\gamma_2 = 0.57$, $\theta = 1.0$; ●, 25 mM, 2 min, $\gamma_2 = 0$, $\theta = 0$. $\tau_1 = 15$ min, $\tau_2 = 50$ min, $\alpha = 4.0$, $\gamma_1 = 0.25$, $\delta = 0.1$, $x_0^* = 0.44$ s.

DISCUSSION

In the present study, we demonstrated that treatment of the sugar receptor with DOC reversibly suppressed its electrophysiological response (Fig. 1). We also characterized the recovery process after the DOC treatment by clarifying (a) different recovery time courses with different stimulus concentrations (Fig. 1), (b) the alteration of K_b and r_∞ values in the sucrose concentration-response curve obtained during recovery (Fig. 6), (c) the slow rise and fall of the receptor potential (Fig. 7), and (d) changes in the response latency with recovery (Fig. 8). Our results demonstrated that this recovery process was divided into two processes: a colchicine-dependent one and a colchicine-independent one. The former is completely depressed by 2 min pretreatment with colchicine above 5 mM, but the latter is not at all depressed (Figs. 2–4).

Depression of the Recovery

Both colchicine and vinblastine affect the microtubule assembly-disassembly system in vivo and in vitro, and have usually been used to study various cellular functions in which microtubules play a critical role, e.g., chromosomal movements, cell motility, intracellular movement of various materials, and maintenance of cell shape (see, for reviews, Olmsted and Borisy, 1973; Wilson et al., 1974; Snyder and McIntosh, 1976; Correia and Williams, 1983). However, these antimicrotubular reagents also affect cell functions not mediated by microtubules (Mizel and Wilson, 1972; Seeman et al., 1973). To exclude these cases, lumicolchicine has been used because it is an isomer of colchicine with no tubulin-binding activity (Price, 1974; Wilson et al., 1974; Yahara and Edelman, 1975). The effectiveness of colchicine and vinblastine and the ineffectiveness of lumicolchicine (Fig. 5) in the recovery of the sugar receptor after DOC treatment strongly suggest that microtubules are involved in the recovery mechanism. However, microtubules are not thought to be involved in the sensory transduction mechanism (cf. Matsumoto and Farley, 1978, 1980), because colchicine alone showed no effect on the response to sucrose (Fig. 2).

The colchicine concentration used in our recovery process is much higher than that (10^{-6} M) which is effective in microtubule assembly and disassembly in vitro (Wilson et al., 1974). However, we used a much shorter colchicine treatment, which may explain the high concentration requirement we found. In fact, in our recovery process (Fig. 4), as well as in the microtubule assembly-disassembly system (Wilson et al., 1974), the duration of colchicine treatment was critical to the exertion of its depressive effect. The results suggest that if pretreatment is prolonged, a lower colchicine concentration may be effective.

Other Properties of Recovery

Recovery time course. Morphological studies have established that there are five receptor cells in a single sensillum; one of them is a mechanoreceptor and four are chemoreceptor cells that send their distal processes into the inner lumen to reach the tip pore of the chemosensillum (see Zacharuk, 1980, for review). The receptor membrane that generates the receptor potential is located at the tip of the distal process (Tateda and Morita, 1959; Morita, 1959) and is exposed

to the external environment via the tip pore (Stürckow et al., 1973; Tominaga, 1975).

Based on our results and the findings mentioned in the previous paragraph, we made the following assumptions: (a) DOC treatment destroys the receptor membrane, which decreases the total number of available receptor sites, s , and also increases the distance, x , between the tip pore and the tip of receptor cell; (b) reorganization of the destroyed receptor membrane is accompanied by a recovery of the s and x values; (c) colchicine interferes with this reorganization of the receptor membrane, possibly by acting on the microtubules.

At least three different sites exist in the sugar receptor membrane of the fly, i.e., pyranose, furanose, and a third site (Shimada et al., 1974; Shimada and Isono, 1978; Shimada and Tanimura, 1981). Sucrose reacts with the pyranose site, and the sucrose response, r , apparently obeys the Beidler theory (Beidler, 1954; Morita and Shiraishi, 1968): $r = r_{\infty}/(1 + K_b/a)$, where a is the sucrose concentration in the stimulus solution. According to Morita (1969), the sucrose response is expressed by the following equation:

$$r = kV = k\alpha V_m/(1 + \alpha + K/a), \quad (1)$$

where $\alpha = sg/G$, s being the total number of receptor sites, and g and G being the conductance per activated receptor site and that measured across the receptor membrane in the resting state, respectively. V is the membrane receptor potential, V_m is the theoretical maximum of V when the number of the activated sites is infinitely large, K is the dissociation constant between sucrose and the receptor molecules, and k is a proportionality constant. Comparing Eq. 1 and the Beidler theory, the maximum response, r_{∞} , and the half-maximal concentration, K_b , were expressed as follows (Morita, 1969): $r_{\infty} = kV_m/(1 + 1/\alpha)$; $K_b = K/(1 + \alpha)$. In these equations, the decrease in the total number of receptor sites, s to s' ($s > s'$), is expressed as α to α' ($\alpha > \alpha'$). The decrease in the total number of available receptor sites for pyranose on the receptor membrane, s , decreases the r_{∞} value and increases the K_b value in the sucrose concentration-response curve. Therefore, by assuming only a decrease in the s value after DOC or colchicine-DOC treatment, we can explain results such as the increase in the K_b value and the decrease in the r_{∞} value in the sucrose concentration-response curve obtained after colchicine-DOC treatment (Fig. 6).

However, the results of Figs. 7 and 8 suggest that the distance x from the tip pore to the receptor sites was increased by colchicine-DOC treatment. Accordingly, the actual stimulant concentration, a' , at the receptor sites should be given by the diffusion equation $\epsilon \equiv a'/a = \text{erfc}(x/2\sqrt{Dt^*})$, where t^* is the diffusion time, D is the diffusion coefficient, and erfc is the error function complement. The relative response (R), i.e., the ratio of the response (r') after the treatment to that (r) before the treatment, is obtained by considering Eq. 1 as

$$R \equiv r'/r = \gamma(1 + \alpha)(1 + 1/a^*)/[1 + \alpha\gamma + (1 + \alpha)/\epsilon a^*], \quad (2)$$

where $\gamma = \alpha'/\alpha$ and $a^* = a/K_b$. Thus, we can calculate the relative response as the function of time, t , after the beginning of recovery from destruction by DOC or colchicine-DOC treatment, if we know the time courses of $\gamma(t)$ and $\epsilon(t)$. Let us assume that

$$\gamma(t) = \gamma_1(1 - e^{-t/\tau_1}) + \gamma_2(1 - e^{-t/\tau_2}), \quad (3)$$

$$\epsilon(t) = \epsilon_0 + \theta(1 - \epsilon_0)(1 - e^{-t/\tau_2}), \quad (4)$$

where the sets (γ_1, τ_1) and (γ_2, τ_2) correspond to the colchicine-independent and -dependent recovery processes, respectively, ϵ_0 is the initial value of ϵ , and θ ($0 \leq \theta \leq 1$) is a constant that characterizes the final value of distance x , depending on the condition of colchicine pretreatment (see Appendix). Each curve in Fig. 9 calculated from Eqs. 2–4 fits the experimental data well (the same ones used in Figs. 1 and 3). The values of the constants used for the calculation are given below the figure. Thus, the recovery time courses, which differ with different treatments, can be described by changing only θ and γ_2 . Needless to say, the different time courses (displayed by the same recovery process) with different test stimulus concentrations (Fig. 1) can be described without any changes, i.e., by using the same set of constants.

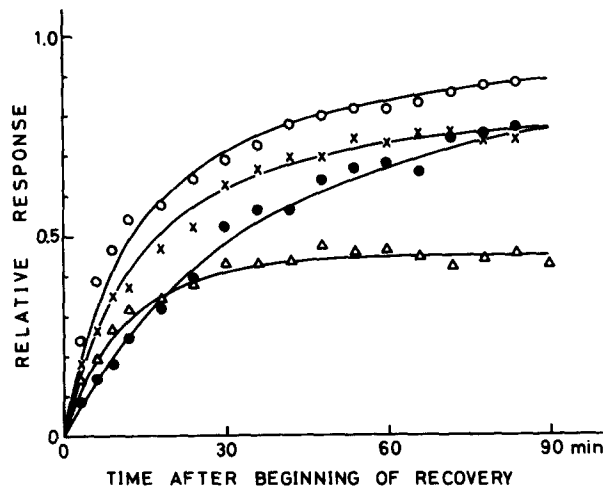


FIGURE 9. Theoretical curves calculated from Eq. 2 for the results of Figs. 1 and 3. $\tau_1 = 15$ min; $\tau_2 = 50$ min; $\alpha = 4.0$; $\gamma_1 = 0.25$; $\epsilon_0 = 0.6$. \circ , 0.1 M sucrose, 0 mM colchicine (2 min), $\gamma_2 = 0.57$, $\theta = 1.0$. \bullet , 0.01 M sucrose, 0 mM colchicine (2 min), $\gamma_2 = 0.57$, $\theta = 1.0$. \times , 0.1 M sucrose, 0.5 mM colchicine (2 min), $\gamma_2 = 0.32$, $\theta = 1.0$. Δ , 0.1 M sucrose, 5 mM colchicine (2 min), $\gamma_2 = 0$, $\theta = 0$.

The colchicine-independent recovery developed exponentially with a time constant of 15 min (Fig. 3). This property resembled that of the recovery of the response from the depression (without injury discharge) after treatment with a low concentration (0.5 mM) of DOC (not shown). Thus, it is very likely that such a depression is caused by the DOC monomers' adsorption to or penetration into the receptor membrane, and that the recovery proceeds with desorption or elimination of the monomers from the membrane.

Change in latency. If diffusion of the stimulant is rate-limiting, the response latency (t_l^*) should be dependent on the distance x from the tip pore to the receptor sites and on the threshold concentration of the stimulant (a_T), as proposed by Getchell et al. (1980). Therefore, if the time course of $x(t)$ is

assumed to obey a single exponential characterized by the time constant of τ_2 , t_L^* can be expressed by the following equation (see Appendix):

$$t_L^* = x_0^* [1 - \theta(1 - e^{-t/\tau_2})]^2 / [\operatorname{erfc}^{-1}(a_T/a)]^2 \quad (5)$$

where $x_0^* = x_0^2/4D$, x_0^* has the dimension of time, erfc^{-1} is the inverse of the error function complement, and x_0 is the initial value of x when $t = 0$. When V_T denotes the threshold membrane receptor potential for the generation of impulses as $V_T = \delta V_m$ ($0 < \delta < 1$), we can obtain a_T for the DOC-treated sugar receptor (see Appendix), considering Eq. 1 as

$$a_T = K_b(1 + \alpha)/[\gamma\alpha(1/\delta - 1) - 1]. \quad (6)$$

The smooth curves in Fig. 8 were drawn according to Eqs. 5 and 6. The values of the constants other than δ and x_0^* are the same as those used for the recovery time course. Thus, we could describe the change in latency as well as the recovery time course. If we assume $D = 5.0 \times 10^{-6} \text{ cm}^2/\text{s}$ to be the value for sucrose in a dilute aqueous solution (3% wt/vol) at 25°C (Gosting and Morris, 1949), the value of x_0 is $\sim 30 \text{ }\mu\text{m}$.

As discussed above, with the assumptions of the destruction and subsequent reorganization of the receptor membrane, we could consistently account for our findings of an alteration of the K_b and r_∞ values in the concentration-response curve, the different recovery time courses with different stimulus concentrations, the slowly rising receptor potential, and the long latency. These assumptions also allowed us to describe quantitatively the recovery time course and the change in the response latency. Furthermore, we can estimate the dissociation constant between sucrose and the receptor molecules, K . The theoretical curves agree best with the experimental data when $\alpha = 4.0$. Accordingly, the dissociation constant, K , for sucrose was estimated to be 70 mM from the relationship $K = K_b(1 + \alpha)$, where $K_b = 14 \text{ mM}$. Such an estimation should be useful to biochemical studies on the receptor molecule.

APPENDIX

Recovery Time Course

The change in distance, x , was also assumed to result from a reorganization of the receptor membrane (colchicine-dependent recovery process). Therefore, we adopted the following equation:

$$x(t) = x_0 - \theta x_0(1 - e^{-t/\tau_2}), \quad (\text{A1})$$

where θ ($0 \leq \theta \leq 1$) is a constant that characterizes the final value of distance, x , depending on the condition of colchicine pretreatment.

The actual sucrose concentration, a' , on the receptor sites is given by the diffusion equation. If $\epsilon (= a'/a)$ is sufficiently large ($\epsilon > 0.5$) at a given diffusion time, t^* , as is the case in the present work, the relation between ϵ and x can be regarded as linear at the given time t^* when calculated with the diffusion equation. Noticing that $\epsilon = 1.0$ when $x = 0$, and that $\epsilon = \epsilon_0$ when $x = x_0$, we obtain

$$(1 - \epsilon_0)/x_0 = (\epsilon - \epsilon_0)/(x_0 - x). \quad (\text{A2})$$

The introduction of Eq. A1 into Eq. A2 leads to Eq. 4.

Change in Latency

If the diffusion of sucrose is rate-limiting, the response latency, t_L^* , can be expressed by the following equation, as proposed by Getchell et al. (1980):

$$t_L^* = x^2/4D[\operatorname{erfc}^{-1}(a_T/a)]^2. \quad (\text{A3})$$

Introducing Eq. A1 into Eq. A3, we obtain Eq. 5.

According to Eq. 1, the relation between V_T and a_T in the DOC-treated sugar receptor is given as

$$V_T = \alpha' V_m / (1 + \alpha' + K/a_T). \quad (\text{A4})$$

With Eq. A4, and introducing the relations $V_T = \delta V_m$, $\alpha' = \gamma\alpha$, and $K = K_b(1 + \alpha)$, the a_T of the DOC-treated sugar receptor is given by Eq. 6.

Original version received 13 October 1982 and accepted version received 22 October 1985.

REFERENCES

- Beidler, L. M. 1954. A theory of taste stimulation. *Journal of General Physiology*. 38:133–139.
- Buck, J. B. 1953. Physical properties and chemical composition of insect blood. In *Insect Physiology*. K. D. Roeder, editor. John Wiley & Sons, New York. 147.
- Correia, J. J., and R. C. Williams, Jr. 1983. Mechanisms of assembly and disassembly of microtubules. *Annual Review of Biophysics and Bioengineering*. 12:211–235.
- Getchell, T. V., G. L. Heck, J. A. Desimone, and S. Price. 1980. The location of olfactory receptor sites. Inferences from latency measurements. *Biophysical Journal*. 29:397–411.
- Getting, P. A. 1971. The sensory control of motor output in fly proboscis extension. *Zeitschrift für Vergleichende Physiologie*. 74:103–120.
- Gosting, L. J., and M. S. Morris. 1949. Diffusion studies on dilute aqueous sucrose solutions at 1 and 25° with the Gouy interference method. *Journal of the American Chemical Society*. 71:1998–2006.
- Hodgson, E. S., J. Y. Lettvin, and K. D. Roeder. 1955. Physiology of a primary chemoreceptor unit. *Science*. 122:417–418.
- Matsumoto, D. E., and R. D. Farley. 1978. Alterations of ultrastructure and physiology of chemoreceptor dendrites in blowfly taste hairs treated with vinblastine and colchicine. *Journal of Insect Physiology*. 24:765–776.
- Matsumoto, D. E., and R. D. Farley. 1980. Decreased behavioral responsiveness to sucrose after treatment of blowfly taste receptors with vinblastine and colchicine. *Comparative Biochemistry and Physiology*. 66C:93–97.
- Matsumoto, G., and H. Sakai. 1979a. Microtubules inside the plasma membrane of squid giant axons and their possible physiological function. *Journal of Membrane Biology*. 50:1–14.
- Matsumoto, G., and H. Sakai. 1979b. Restoration of membrane excitability of squid giant axons by reagents activating tyrosine-tubulin ligase. *Journal of Membrane Biology*. 50:15–22.
- Mizel, S. B., and L. Wilson. 1972. Nucleoside transport in mammalian cells. Inhibition by colchicine. *Biochemistry*. 11:2573–2578.
- Morita, H. 1959. Initiation of spike potentials in contact chemosensory hairs of insects. III. D.C. stimulation and generator potential of labellar chemoreceptor of *Calliphora*. *Journal of Cellular and Comparative Physiology*. 54:189–204.
- Morita, H. 1969. Electrical signs of taste receptor activity. In *Olfaction and Taste*. C. Pfaffmann, editor. The Rockefeller University Press, New York. 3:370–381.
- Morita, H., and A. Shiraishi. 1968. Stimulation of the labellar sugar receptor of the fleshfly by mono- and disaccharides. *Journal of General Physiology*. 52:559–583.

- Olmsted, J. B., and G. G. Borisy. 1973. Microtubules. *Annual Review of Biochemistry*. 42:507–540.
- Price, M. T. 1974. The effects of colchicine and lumicolchicine on the rapid phase of axonal transport in the rabbit visual system. *Brain Research*. 77:497–501.
- Seeman, P., M. Chau-Wong, and S. Moyyen. 1973. Membrane expansion by vinblastine and strychnine. *Nature New Biology*. 241–22.
- Shimada, I. 1975. Chemical treatments of the labellar sugar receptor of the fleshfly. *Journal of Insect Physiology*. 21:1565–1574.
- Shimada, I., and K. Isono. 1978. The specific receptor site for aliphatic carboxylate anion in the labellar sugar receptor of the fleshfly. *Journal of Insect Physiology*. 24:807–811.
- Shimada, I., A. Shiraishi, H. Kijima, and H. Morita. 1974. Separation of two receptor sites in a single labellar sugar receptor of the flesh-fly by treatment with *p*-chloromercuribenzoate. *Journal of Insect Physiology*. 20:605–621.
- Shimada, I., and T. Tanimura. 1981. Stereospecificity of multiple receptor sites in a labellar sugar receptor of the fleshfly for amino acids and small peptides. *Journal of General Physiology*. 77:23–29.
- Snyder, J. A., and J. R. McIntosh. 1976. Biochemistry and physiology of microtubules. *Annual Review of Biochemistry*. 45:699–720.
- Stürckow, B., P. E. Holbert, J. R. Adams, and R. J. Anstead. 1973. Fine structure of the tip of the labellar taste hair of the blow flies, *Phormia regina* (Mg.) and *Calliphora vicina* R.-D. (Diptera, Calliphoridae). *Zeitschrift für Morphologie der Tiere*. 75:87–109.
- Tateda, H., and H. Morita. 1959. Initiation of spike potentials in contact chemosensory hairs of insects. I. The generation site of the recorded spike potentials. *Journal of Cellular and Comparative Physiology*. 54:171–176.
- Tominaga, Y. 1975. The taste pore of the fleshfly labellar hair. *Journal of Electron Microscopy*. 24:171–174.
- Wilson, L., J. R. Bamberg, S. B. Mizel, L. M. Grisham, and K. M. Creswell. 1974. Interaction of drugs with microtubule proteins. *Federation Proceedings*. 33:158–166.
- Wilson, L., and M. Friedkin. 1966. The biochemical events of mitosis. I. Synthesis and properties of colchicine labelled with tritium in its acetyl moiety. *Biochemistry*. 5:2463–2468.
- Yahara, I., and G. M. Edelman. 1975. Modulation of lymphocyte receptor mobility by concanavalin A and colchicine. *Annals of the New York Academy of Sciences*. 253:455–469.
- Zacharuk, R. Y. 1980. Ultrastructure and function of insect chemosensilla. *Annual Review of Entomology*. 25:27–47.