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

Recovery of Responses to Sugar After Destruction

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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).

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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 \( R_a \) (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.

![Graph](image1)

**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 (± 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 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 (± 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.
Fig. 3 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-
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, 1983). Therefore, we examined the effect 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
Here, the response is normalized so that the maximum response in the control is unity. The $K_b$ value increased and the $r_m$ value decreased after the treatment. The averaged $K_b$ values ($\pm$ SEM) in the control and the colchicine-DOC-treated sugar receptor were $14 \pm 4$ (n = 6) and $75 \pm 4$ 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 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
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 7](image)

**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.

**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 (± SEM) from 7–10 separate experiments. The continuous lines were calculated from Eq. 5 with the constants given below (see Discussion). Colchicine treatment: ○, 0 mM, 2 min, γ2 = 0.57, θ = 1.0; ●, 25 mM, 2 min, γ2 = 0, θ = 0. τ1 = 15 min, τ2 = 50 min, α = 4.0, γ1 = 0.25, δ = 0.1, x0 = 0.44 s.

![Figure 8](image)


**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 $r_m$ 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
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): \( r = r_m/(1 + K_b/a) \), where \( a \) is the sucrose concentration in the stimulus solution. According to Morita (1969), the sucrose response is expressed by the following equation:

\[
    r = kV = k\alpha V_m/(1 + \alpha + K/a),
\]

(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, \( r_m \), and the half-maximal concentration, \( K_b \), were expressed as follows (Morita, 1969): \( r_m = k\alpha V_m/(1 + 1/\alpha) \); \( K_b = K/(1 + \alpha) \). In these equations, the decrease in the total number of receptor sites, \( s \) to \( s' \) (\( s > s' \)), is expressed as \( \alpha \) to \( \alpha' \) (\( \alpha > \alpha' \)). The decrease in the total number of available receptor sites for pyranose on the receptor membrane, \( s \), decreases the \( r_m \) 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 \( r_m \) 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 \( \epsilon = a'/a = erfc(x/2\sqrt{Dt^*}) \), 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

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

(2)

where \( \gamma = a'/a \) 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
where the sets \((\gamma_1, \tau_1)\) and \((\gamma_2, \tau_2)\) correspond to the colchicine-independent and -dependent recovery processes, respectively, \(\epsilon_0\) is the initial value of \(\epsilon\), and \(\theta (0 \leq \theta \leq 1)\) 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 \(\theta\) and \(\gamma_2\). 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.

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 \(c_T\), as proposed by Getchell et al. (1980). Therefore, if the time course of \(x(t)\) is

\[
\gamma(t) = \gamma_1(1 - e^{-t/\tau_1}) + \gamma_2(1 - e^{-t/\tau_2}),
\]

\[
\epsilon(t) = \epsilon_0 + \theta(1 - \epsilon_0)(1 - e^{-t/\theta}),
\]

FIGURE 9. Theoretical curves calculated from Eq. 2 for the results of Figs. 1 and 3. \(\tau_1 = 15\) min; \(\tau_2 = 50\) min; \(\alpha = 4.0; \gamma_1 = 0.25; \epsilon_0 = 0.6. \bigcirc, 0.1\) 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), \(\gamma_2 = 0.32, \theta = 1.0. \bigtriangleup, 0.1\) M sucrose, 5 mM colchicine (2 min), \(\gamma_2 = 0, \theta = 0.\)
assumed to obey a single exponential characterized by the time constant of \( \tau_\varphi \), \( t_L^\varphi \) can be expressed by the following equation (see Appendix):

\[
t_L^\varphi = x_0^\varphi [1 - \theta(1 - e^{-\varphi(t)})^2]/[\text{erfc}^{-1}(a_1/a)]^2.
\]  

(5)

where \( x_0^\varphi = x_0^2/4D \), \( x_0^\varphi \) has the dimension of time, \( \text{erfc}^{-1} \) 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

\[
a_T = K_b(1 + a)/[\gamma(1/\delta - 1) - 1].
\]  

(6)

The smooth curves in Fig. 8 were drawn according to Eqs. 5 and 6. The values of the constants other than \( \delta \) and \( x_0^\varphi \) 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 \( \sim 30 \mu \text{m} \).

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 \( r_\infty \) 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:

\[
x(t) = x_0 - \theta x_0 (1 - e^{-\varphi(t)}),
\]  

(A1)

where \( \theta (0 \leq \theta \leq 1) \) 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 \( \epsilon \) 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

\[
(1 - \epsilon_0)/x_0 = (\epsilon - \epsilon_0)/(x_0 - x).
\]  

(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, $t^*$, can be expressed by the following equation, as proposed by Getchell et al. (1980):

$$t^* = \frac{x^2}{4D} \left[ \text{erfc}^{-1}\left(\frac{aT}{a}\right)\right]^2.$$  \hspace{1cm} (A3)

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

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

$$V_T = \frac{a'V_m}{1 + a' + K/a}. $$  \hspace{1cm} (A4)

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

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