# Minimal Model To Account for the Membrane Conductance Increase and Desensitization of γ-Aminobutyric Acid Receptors Synthesized in the *Xenopus* Oocytes Injected with Rat Brain mRNA†

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ABSTRACT: γ-Aminobutyric acid (GABA) receptors, which translocate chloride anion with binding GABA, were synthesized in *Xenopus* oocytes by injecting rat brain mRNA. GABA-elicited responses in the oocytes were measured electrophysiologically by the current-clamped method. Five different measurements were made to establish the relationship between GABA concentration and the electrical responses: (1) the GABA-elicited conductance increase before desensitization; (2) the rate of desensitization of GABA receptors; (3) the rate of recovery of desensitized receptors upon removal of GABA; (4) the GABA-elicited conductance increase after desensitization equilibrium; (5) the fraction of the active form of GABA receptors after desensitization equilibrium. These results were interpreted on the basis of the minimal model proposed for nicotinic acetylcholine receptor in *Electrophorus electricus* electroplax [Hess, G. P., Cash, D. J., & Aoshima, H. (1983) *Annu. Rev. Biophys. Bioeng. 12*, 443–473]. Estimated equilibrium and rate constants in the model for GABA receptors could successfully explain the results of the five above measurements.

γ-Aminobutyric acid (GABA) receptors and nicotinic acetylcholine receptors (nAChR) are known to induce ionotrophic transmission by binding an agonist, GABA or acetylcholine. GABA receptors translocate chloride anion selectively by binding GABA, while nAChRs translocate monovalent metal ions such as $Na^+$, $K^+$, $Rb^+$, or $Li^+$ by binding acetylcholine. The relationship between the physiological response and agonist binding to the receptor is complicated, since the agonist binding to the receptor causes not only ion translocation but also inactivation (desensitization) of the receptor.

nAChR has been studied extensively and is the best characterized channel protein gated by a ligand (Cold Spring Harbor Laboratory, 1983). The relationship between agonist concentration and the response was studied by using the membrane vesicles (Kasai & Changeux, 1971) prepared from *Electrophorus electricus* or *Torpedo* and a quenched-flow method (Cash & Hess, 1980; Hess et al., 1979; Neubig & Cohen, 1980). The minimal reaction model (Figure 1) to explain ion translocation and desensitization of nAChR in *E. electricus* was proposed from the measurements of following five different kinetics (Aoshima et al., 1981; Cash et al., 1981, 1985; Hess et al., 1983): (1) the rate of fast ion flux mediated by nAChR; (2) the rate of desensitization of nAChR; (3) the rate of recovery of the desensitized nAChR upon removal of an agonist; (4) the rate of slow ion flux mediated by equilibrium mixtures of active and desensitized receptor forms; (5) the fraction of active form of nAChR when the equilibrium of desensitization is reached.

In this paper, we examined whether the minimal model proposed for nAChR could also explain the respnse and desensitization of the GABA receptor in the presence of various concentrations of GABA. Since the measurement system of GABA receptor mediated ion translocation using membrane vesicles was not established, electrical responses induced by GABA were observed on *Xenopus* oocytes injected with rat brain mRNA (Houamed et al., 1984; Miledi et al., 1982; Parker et al., 1986). The *Xenopus* oocyte (a globular form with a diameter of more than 1 mm) is larger and more simple shaped than nerve cells in the nervous system, which makes the electrophysiological measurements easy and simple (Barnard et al., 1982; Dascal et al., 1986; Gundersen et al., 1984; Sakmann et al., 1985). Intracellular ion activities of the oocyte were estimated by Kusano et al. (1982). Five different measurements of electrical responses of the injected oocyte elicited by GABA receptors were carried out, which are similar to those carried out for nAChR by using the membrane vesicles of *E. electricus* electroplax. The results of these measurements were explained by the minimal reaction model proposed for nAChR (Hess et al., 1983). Equilibrium and rate constants in the model for GABA receptors were estimated from the results of five different measurements according to a procedure similar to that described in previous papers (Aoshima et al., 1981; Hess et al., 1983).

## Materials and Methods

*Preparation of Rat Brain mRNA and Xenopus Oocyte.* The whole brain was obtained from male adult Wistar rat (about 100 g) after anesthetizing it with ethyl ether. The brain was rinsed in distilled water and was frozen and smashed in liquid nitrogen immediately. Poly(A) mRNA was extracted from the rat brain by homogenization in guanidine thiocyanate solution, ultracentrifugation on 5.7 M CsCl solution, phenol–chloroform extraction, and oligo(dT)–cellulose (type 3; Collaboractive Research, Inc., Lexington, KY) chromatography (Maniatis et al., 1982). The concentration of mRNA was estimated from the absorption at 260 nm, assuming $A_{1\,cm}^{1\,mg/mL} = 25$.

Adult female frogs (*Xenopus laevis*) were purchased from Nihonseibutukyozai Co., Tokyo. *Xenopus* oocytes were dissected from the ovaries of female frogs anesthetized in ice water containing 0.2 mg/mL MS 222 (Sankyo, Tokyo). They

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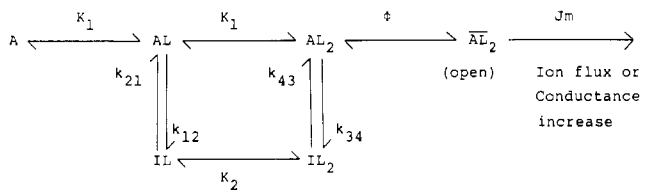


FIGURE 1: Minimum reaction model to account for the function and desensitization of nAChR and GABA receptor. The active form (A) and the desensitized form (I) of the receptor bind the ligand (L) more rapidly than the rate of desensitization of the receptor. The active receptor with two bound ligands ($AL_2$) converts rapidly to an open-channel form ($\overline{AL_2}$) with an equilibrium constant for channel opening ($1/\Phi$). $\overline{AL_2}$ permits ion flux or conductive increase through channel opening. The fraction of the receptor in the open channel form after time 0 ($[\overline{AL_2}]_0$) and that at the time when equilibrium is reached ($[\overline{AL_2}]_\infty$) are given in eq 1 and 2, where $L$ represents the concentration

$$[\overline{AL_2}]_0 = \frac{L_2}{L^2(1 + \Phi) + L(2K_1)\Phi + K_1^2\Phi} \quad (1)$$

$$[\overline{AL_2}]_\infty = \frac{K_{c2}L^2}{K_{c2}[L^2(1 + \Phi) + L(2K_1)\Phi + K_1^2\Phi] + \Phi[L^2 + L(2K_2)]} \quad (2)$$

of the ligand (agonist). The rate constant of ion flux or the conductance increase before desensitization, $J_A$, and that after equilibrium of desensitization has gone to completion, $J_I$, are expressed in eq 3 and 4, where Jm represents the maximum flux or the maximum

$$J_A = \mathrm{Jm}[\overline{AL_2}]_0 \quad (3)$$

$$J_I = \mathrm{Jm}[\overline{AL_2}]_\infty \quad (4)$$

conductance increase obtained if all the receptors are converted to the open-channel form (Hess et al., 1981). The fraction of the active form of the receptor is expressed in eq 5 when equilibrium of de-

$$[A] + [AL] + [AL_2] + [\overline{AL_2}] = [\overline{AL_2}]_\infty/[\overline{AL_2}]_0 \quad (5)$$

sensitization has gone to completion. The existence of two different desensitized forms of nAChR in *E. electricus* electroplax was reported (Aoshima, 1984), but this can be ignored unless the incubation time of the agonist with nAChR is very long. The first-order rate constant of desensitization, $\alpha$, is given in eq 6. The rate and equilibrium

$$\alpha = \frac{k_{43}L + k_{21}(2K_2)}{L + 2K_2} + \Phi\left[\frac{k_{34}L^2 + k_{12}L(2K_1)}{L^2(1 + \Phi) + L(2K_1)\Phi + K_1^2\Phi}\right] \quad (6)$$

constants pertaining to the model are defined as follows: $K_1 = 2[A][L]/[AL] = [AL][L]/2[AL_2]$; $K_2 = [IL][L]/2[IL_2]$; $K_{c1} = k_{21}/k_{12} = [AL]/[IL]$; $K_{c2} = k_{43}/k_{34} = [AL_2]/[IL_2]$; $\Phi = [AL_2]/[\overline{AL_2}]$.

were detached manually from the inner ovarian epithelium and follicular envelope after incubation in collagenase (Sigma type I, 1 mg/mL) solution for 1–2 h according to the procedure of Kusano et al. (1982). Oocytes at stages V and VI were microinjected with about 50 ng of the mRNA in water and incubated in modified Barth solution [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 7.5 mM Tris, pH 7.6] containing 25 mg/L penicillin G and 50 mg/L streptomycin, at 20 °C for 2–4 days before electrophysiological measurements.

*Electrophysiology.* The membrane depolarization and conductance increase of the oocytes evoked by GABA were measured by the current-clamped method (Houamed et al., 1984). The *Xenopus* oocyte was continuously perfused with normal frog Ringer solution (115 mM NaCl, 1 mM KCl, 1.8 mM $CaCl_2$, 5 mM Tris, pH 7.2) at 20 °C via a gravity-feed system. The perfusing system was a slight modification of that used previously for brain slices (Kobayashi & Murakami, 1982). The oocyte was placed on the bottom of a small chamber and was impaled with two microelectrodes filled with 3 M KCl, one for monitoring membrane potential and another for injecting a hyperpolarizing current for current clamping the membrane. A rectangular pulse current ($I$) was injected every 6 s to create a hyperpolarizing membrane potential ($\Delta V$) from which the membrane conductance ($g$) was estimated by the equation $g = I/\Delta V$. GABA was dissolved in the perfusing solution and bath applied to the oocyte at a flow rate of 2 mL/min. The conductance increase ($\Delta g$) elicited by GABA was obtained by subtracting the conductance when perfused with frog Ringer solution from that when perfused with GABA solution.

*Estimation of GABA-Induced Conductance Increase and Desensitization.* The conductance of the oocyte injected with mRNA was measured electrophysiologically in the presence of various concentrations of GABA. GABA application was repeated after washing the oocyte with frog Ringer solution for more than 20 min to prevent the effect of desensitization. The maximum conductance near the peak of membrane depolarization was taken as a response elicited by GABA (Kobayashi & Aoshima, 1986). The responses were normalized to that elicited by 1 mM GABA (100%), since the injected oocytes showed some variation in the amount of synthesized GABA receptor. When it took several hours to measure the responses of the same oocyte, the control responses evoked by 1 mM GABA were obtained both at the beginning and at the end of the measurement to revise the time-dependent variation of the amount of synthesized GABA receptors in the oocyte.

The conductance decline evoked by continuous application of GABA was plotted in a semilogarithmic coordinate against perfusing time after the peak conductance. The rate constant of desensitization ($\alpha$) induced by GABA was estimated from the slope of this plot (Kobayashi & Aoshima, 1986).

*Reactivation of Desensitized GABA Receptor.* The electrical response of the injected oocyte was obtained as a control by application of 1 mM GABA. Then, the GABA receptor in the oocyte was desensitized completely by application of 1 mM GABA for about 4 min. After perfusion of frog Ringer solution for various time periods, 1 mM GABA was again applied to the oocyte and the conductance increase was obtained. The amount of reactivated GABA receptor was estimated from the ratio of this conductance increase to the control.

*Conductance Increase and the Fraction of Active Form of the Receptor When Equilibrium of Desensitization Is Reached.* After the response of the injected oocyte to 1 mM GABA as a control was obtained, the oocyte was continuously perfused with frog Ringer solution containing various concentrations of GABA for 20 min. Then the oocyte was perfused with 1 mM GABA solution, and the response was measured electrophysiologically. The membrane conductance increase after perfusion of GABA at various concentrations for 20 min was taken as the conductance increase when equilibrium between the active and desensitized forms of the receptor is reached. The fraction of the active form of the receptor, after the equilibrium of desensitization was reached, was estimated from the ratio between the preincubated and nonpreincubated conductance increase of the oocyte elicited by 1 mM GABA application.

## RESULTS

mRNA prepared from rat brain was injected into the *Xenopus* oocytes. The injected oocytes showed the dose-dependent responses to the application of GABA at various concentrations (Figure 2a) as reported in other papers (Houamed et al., 1984; Miledi et al., 1982), while noninjected oocytes showed no response to GABA application. The maximum conductance increase near the peak of membrane

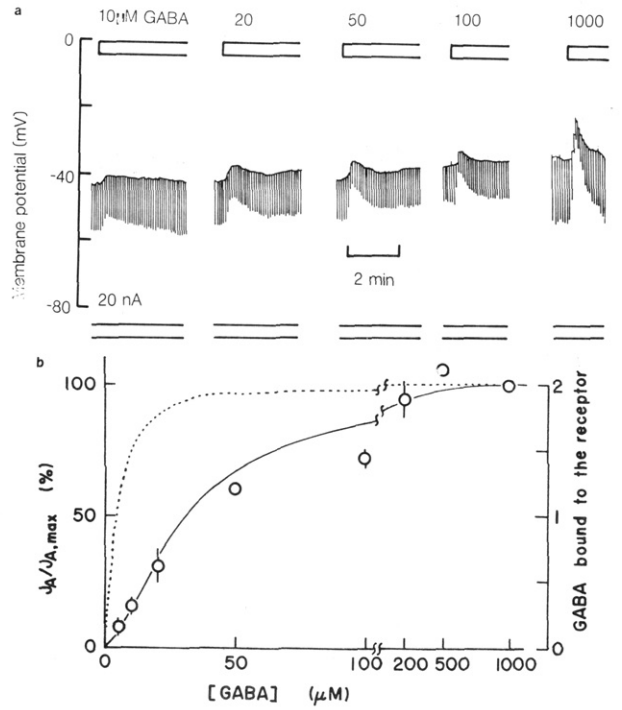


FIGURE 2: (a) Electrical responses elicited in a rat brain mRNA-injected oocyte by different concentrations of GABA. All traces were obtained under a two-electrode current clamp. Intermittent downward deflections are hyperpolarizations evoked by an intracellularly applied rectangular current (3 s, 0.1 Hz, 20 nA) for measuring input conductance. The lowest recording shows the duration of the intermittent current. GABA was applied at the concentrations indicated ($\mu$M) for the times shown by the upward bars, which indicated the times when a cock was changed. Slight time lag between the upward bar and the response was due to the volume between a change cock and a chamber. The responses to GABA were obtained from the same injected oocyte. (b) Dose dependence of the response for GABA and calculated number of GABA molecules bound to the receptor. Data were obtained from records similar to those in Figure 2a and were normalized as a fraction of the response in each oocyte to 1 mM GABA application. Each point is the mean of three experiments, and error bars represent the standard deviations. A solid curve indicating the dose–response relationship before desensitization equilibrium was drawn by eq 3 and the equilibrium constants in Table I. The dotted curve indicating the number of GABA bound to the receptor after desensitization equilibrium, which was obtained from a model-dependent calculation, was drawn by eq 7 and the equilibrium constants in Table I.

depolarization was plotted against bath-applied GABA concentration in Figure 2b. The membrane conductance increased with a sigmoidal curve at low concentrations of GABA and reached a plateau at the concentrations above about 200 $\mu$M.

As shown in Figure 2a, GABA receptors synthesized in *Xenopus* oocyte were inactivated dose dependently during the continuous application of GABA (Parker et al., 1986). In Figure 3a, the first-order rate constant of desensitization ($\alpha$) was estimated from the logarithmic plots of the conductance increase against bathing time of GABA solution. The concentration dependence of the rate of desensitization of GABA receptor was shown in Figure 3b.

After prolonged perfusion of the injected oocyte with 1 mM GABA, the desensitized GABA receptors were restored to the active form by washing with frog Ringer solution for various time periods. Then, the amount of the restored receptors was measured by application of 1 mM GABA as shown in Figure 4a. The reactivation process followed a single-exponential rate law, and its rate constant was estimated to be 0.0061 $s^{-1}$ from the slope of the line in Figure 4b.

After prolonged perfusion of the injected oocyte with various concentrations of GABA for 20 min, the conductance increase

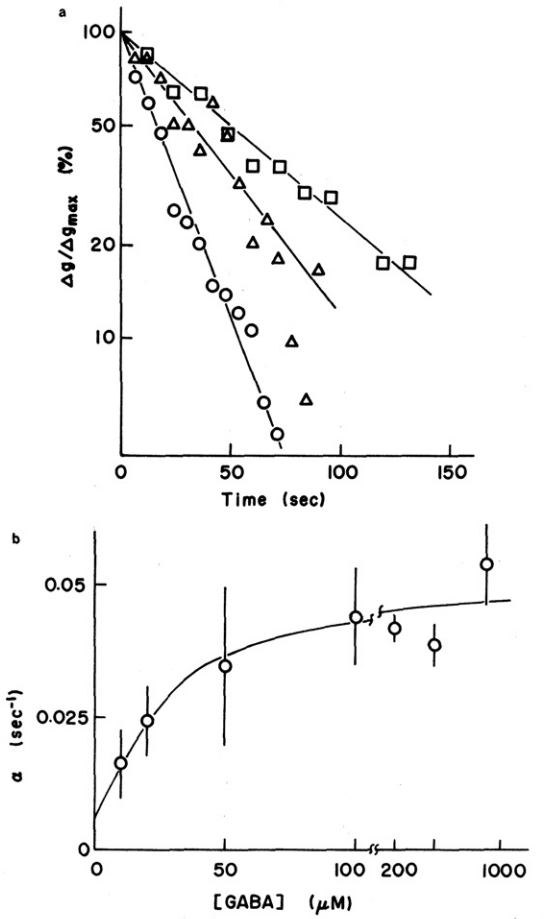


FIGURE 3: (a) Examples of semilogarithmic expression of desensitization of the receptor in the presence of GABA shown in Figure 2a. The rate constant of desensitization, $\alpha$, was estimated as follows from the slope of this straight line: ○, 1 mM GABA, $\alpha = 0.0498$ $s^{-1}$; △, 20 $\mu$M GABA, $\alpha = 0.0252$ $s^{-1}$; □, 10 $\mu$M GABA, $\alpha = 0.0166$ $s^{-1}$. (b) Dose dependence of the rate of desensitization of GABA receptor. Each point is the mean of three experiments, and error bars represent the standard deviations. The solid curve was drawn by using eq 6 and the rate and equilibrium constants in Table I.

elicited by 1 mM GABA was measured and was compared to the control, the response of nonpreincubated oocyte. One example perfused with 50 $\mu$M GABA is shown in Figure 5a. The conductance increase after equilibrium between the active and desensitized form is reached was estimated from the membrane conductance of the oocyte after 20-min incubation with various concentrations of GABA. The GABA-induced conductance increase of injected oocyte in the equilibrium was time independent after a few minutes of incubation with GABA until at least 30 min of examination. The dose dependence of the conductance increase after desensitization equilibrium was shown in Figure 5b. The fraction of the active form of the GABA receptor when equilibrium between the active and desensitized forms is reached was estimated from the ratio between the conductance increase of the preperfused oocyte and that of a nonpreperfused oocyte elicited by 1 mM GABA application. Figure 5c shows the dose dependence of the fraction of the active form of the receptor.

## Discussion

Electrophysiological measurements of *Xenopus* oocytes injected with rat whole brain mRNA gave the following information about GABA receptors: (1) the conductance increase induced by GABA (Figure 2b); (2) the rate of desensitization (Figure 3b); (3) the rate of reactivation (Figure 4b); (4) the conductance increase when equilibrium between the

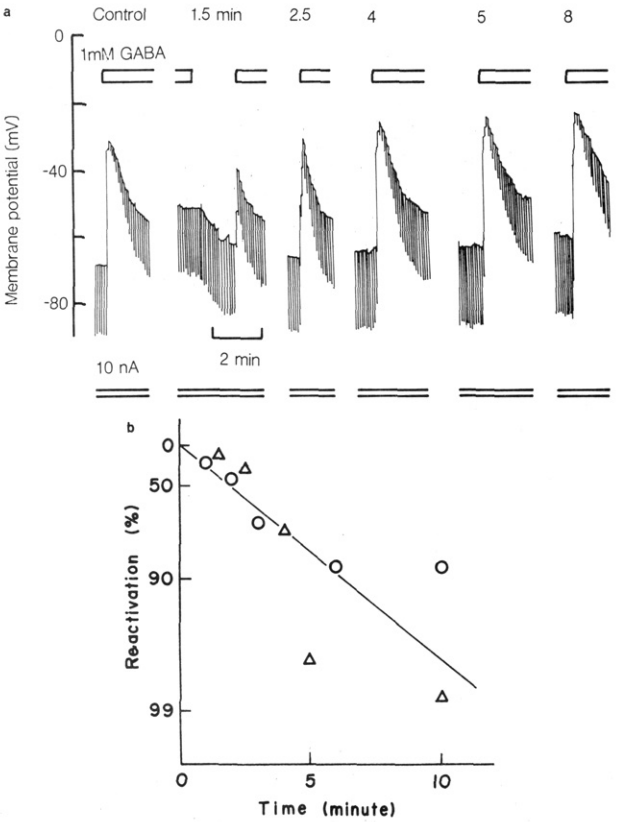


FIGURE 4: (a) Examples of recovery of desensitized GABA receptors upon removal of GABA. GABA receptors in the injected oocyte were desensitized by application of 1 mM GABA for about 4 min. After the oocyte was washed for the indicated times, the electrical responses elicited by 1 mM GABA were recorded with application of a hyperpolarizing rectangular current of 10 nA. (b) Evaluation of the rate constant of reactivation of the desensitized GABA receptor. From the slope of the straight line, the rate constant of reactivation of the GABA receptor was estimated to be 0.0061 $s^{-1}$ from two different injected oocytes (O and Δ).

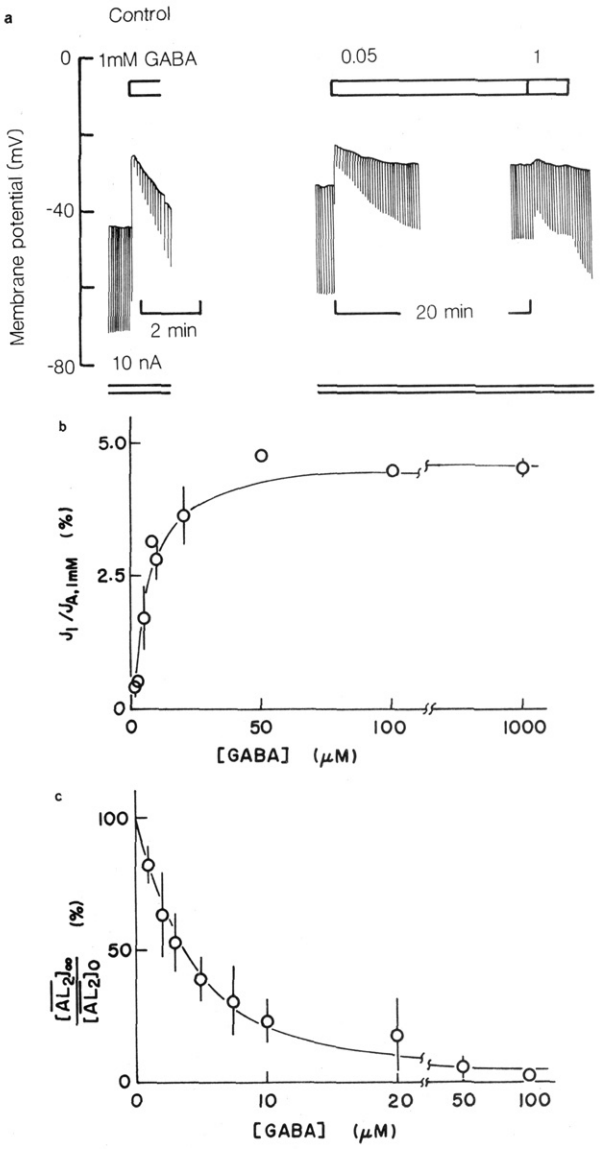


FIGURE 5: (a) Conductance increase and the fraction of active form of the receptor after desensitization equilibrium in the presence of 50 μM GABA. Conductance increase after desensitization equilibrium was estimated from the membrane conductance after perfusion of 50 μM GABA for 20 min. The fraction of active form of the receptor after desensitization equilibrium was estimated from comparison of the conductance increase of preincubated oocyte elicited by 1 mM GABA with that of the control, a nonpreincubated one. (b) Dose dependence of the conductance increase when equilibrium between active and desensitized forms of the receptor is reached. Each point is the mean of four experiments, and error bars represent the standard deviations. The solid curve was drawn by using eq 3 and 4 and the equilibrium constants in Table I. $J_{A,1mM}$ represents $J_A$ when 1 mM GABA was applied. (c) Dose dependence of the fraction of active form after desensitization equilibrium. Each point is the mean of four experiments, and error bars represent the standard deviations. The solid curve was drawn by using eq 5 and the equilibrium constants in Table I.

active and desensitized forms is reached (Figure 5b); (5) the fraction of the active form of the receptor after desensitization equilibrium (Figure 5c). These results of the GABA receptor had the following similarities to the results of nAChR-mediated ion flux measurements using membrane vesicles of *E. electricus* electroplax: (1) The dose–response relationship suggested that two or more agonist molecules were required to cause the opening of the channel (Akaike et al., 1985), so the simple model of Katz and Thesleff (1957) cannot explain the result. (2) Continuous application of agonist to the receptor caused the inactivation of the receptor, and the inactivated receptor was reactivated upon removal of an agonist. (3) Slight activity, conductance increase or ion flux, was observed even after desensitization equilibrium. (4) The activity before desensitization ($J_A$), after desensitization equilibrium ($J_I$), and the rate of desensitization ($\alpha$) showed different dose dependences, respectively. Since the measurements of nAChR in *E. electricus* were clearly explained on the basis of the minimal reaction model shown in Figure 1 (Aoshima et al., 1981), this model was applied for the interpretation of measurements of the GABA receptors.

In estimation of Jm, Φ, and $K_1$ for nAChR, a least-squares computer program was used to obtain a Jm value that gives the best fit of data to the equation (Hess et al., 1983):

$$(\mathrm{Jm}/J_A - 1)^{1/2} = \Phi^{1/2} + \Phi^{1/2}K_1/L$$

However, since above equation includes three variables, this estimation is very susceptible to the error of $J_A$ and demands the exact measurements of $J_A$ values. So we used the result of different work, single-channel current measurements of GABA-gated channels, to estimate the value of Φ. From the single-channel current measurements of GABA-gated channels in the membrane of a rat hippocampal neuron, the equilibrium constant between the closed- and the open-channel states ($1/\Phi$) was estimated to be 0.15 (Sakmann et al., 1983). The ratio between the conductance increase after desensitization equilibrium and that before desensitization, $J_I/J_A$, is expressed as

$$J_{I,max}/J_{A,max} = K_{c2}(1 + \Phi)/[K_{c2}(1 + \Phi) + \Phi]$$

when the concentration of GABA, $L$, is large enough. The

Table I: Values of Constants for the GABA Receptor in Figure 1 (Experiments at 20 °C in Frog Ringer Solution)

| | |
|---|---|
| intrinsic ligand dissociation constant pertaining to active (A) state | $K_1 = 2[A][L]/[AL] = [AL][L]/2[AL_2] = 0.060$ mM |
| conformational equilibrium constant pertaining to equilibrium between closed, active (A) state and open-channel ($\bar{A}$) state | $\Phi = [AL_2]/[\overline{AL_2}] = 0.15$[a] |
| intrinsic ligand dissociation constant pertaining to desensitized (I) state | $K_2 = [IL][L]/2[IL_2] = 0.0018$ mM |
| conformational equilibrium constant pertaining to equilibrium between active (A) and desensitized (I) states, both with one ligand molecule bound | $K_{c1} = k_{21}/k_{12} = [AL]/[IL] = 0.21$; $k_{12} = 0.029$ s$^{-1}$; $k_{21} = 0.0061$ s$^{-1}$ |
| conformational equilibrium constant pertaining to equilibrium between active (A) and desensitized (I) states, both with two ligand molecules bound | $K_{c2} = k_{43}/k_{34} = [AL_2]/[IL_2] = 0.0063$; $k_{34} = 0.36$ s$^{-1}$; $k_{43} = 0.0023$ s$^{-1}$ |

[a] Estimated from the report of Sakmann et al. (1983).

equilibrium constant $K_{c2}$ was estimated to be 0.0063, using $\Phi$ of 0.15 and $J_I/J_A$ of 0.046 when $L = 1$ mM from this equation.

The equilibrium constant $K_1$ was estimated to be 0.060 mM, from the above values of $\Phi$ and $K_{c2}$ and the result in Figure 2b, where $J_A/J_{A,max}$ was plotted against the GABA concentration.

The equilibrium constant $K_2$ was estimated to be 0.0018 mM from the results in Figure 5, parts b and c, from the above estimated values of $\Phi$, $K_{c2}$, and $K_1$.

Since the equilibrium constant $K_{c1}$ is expressed as $K_{c1} = K_1K_{c2}/K_2$, $K_{c1}$ was estimated to be 0.21, from the estimated values of $K_1$, $K_2$, and $K_{c2}$.

The rate of desensitization at saturating concentrations of GABA, $\alpha_{max}$, is given by $\alpha_{max} = k_{34}\Phi/(1 + \Phi)$. So the rate constant of $k_{34}$ was estimated to be 0.36 s$^{-1}$, from the value of 0.047 s$^{-1}$ for $\alpha_{max}$. The rate constant $k_{43}$ was given by $k_{43} = K_{c2}/k_{34} = 0.0023$ s$^{-1}$.

The rate of reactivation of the receptor at low ligand concentration, $\alpha_{min}$, is given by $\alpha_{min} = k_{21}$ (Hess et al., 1983). So the rate constants $k_{21}$ and $k_{12}$ were estimated to be 0.0061 and 0.029 s$^{-1}$, respectively.

The estimated equilibrium and rate constants are summarized in Table I. The solid curves in Figures 2b, 3b, and 5 parts b and c, were drawn, from the equations derived from the minimal model in Figure 1 and the values in Table I. These curves fitted the experimental results very well. Unfortunately, our measurements of the conductance increase caused by GABA application included not only GABA-activated chloride channel activity but also the voltage-dependent channel activities, since we used the current-clamped recording (Houamed et al., 1984). However, the attribution of voltage-dependent channels to the conductance increase was relatively small, about 6% of the total increase at the maximum depolarization (Houamed et al., 1984) and that to the values of the ratio in Figures 2b, 3b, 4b, and 5 parts b and c, was less than the standard deviations. Actually, the dose-response curve for GABA in Figure 2b obtained by the current-clamped method was similar to that obtained by voltage-clamped method (Parker et al., 1986). The error of the equilibrium and rate constants in Table I due to the voltage-dependent channels was estimated to be at most 20%, though the responses and desensitization of GABA receptor evoked by various concentrations of the agonist were essentially explained by the minimal model. To remove the attribution of voltage-dependent channels, the voltage-clamped method is superior to the current-clamped method in a quantitative analyses of a ligand-gated channel. Thus, both nAChR and GABA receptors were interpreted by the same minimal model. It is interesting to investigate the similarities of molecular structures between nAChR and GABA receptors or to examine whether this minimal model can describe the reactions of other neurotransmitter receptors such as glycine or glutamate receptors in the future.

The number of GABA molecules bound to one GABA receptor is expressed as

$$[AL] + [DL] + 2([AL_2] + [\overline{AL_2}] + [DL_2]) = \frac{}{2(K_1\Phi/L + K_2\Phi/K_{c2}L + \Phi + 1 + \Phi/K_{c2})[AL_2]_\infty} \quad (7)$$

when equilibrium between active and desensitized forms of the receptor is reached (Aoshima et al., 1987). This number of bound GABA molecules was calculated from the above equation and the constants in Table I and was drawn by a dotted curve against GABA concentration in Figure 2b. As expected from the presence of desensitized forms in the model, the dose dependence of electrophysiological responses before desensitization equilibrium was different from that of GABA binding as calculated above from the minimal model. In fact, a large discrepancy was reported between the dose dependence of electrophysiological response and that of radioligand binding measurements (Olsen, 1982; Parker et al., 1986).

The presence of a second, slower desensitization process was observed for nAChR of *Torpedo* (Neubig & Cohen, 1980) or *E. electricus* (Aoshima, 1984) electroplax. Since a high-affinity binding site was reported for GABA receptors (Olsen, 1982), GABA receptors might also have another much slower desensitization process, i.e., the second higher affinity GABA binding site. However, the effects of the preincubation of the oocytes with GABA on electrophysiological response was not examined in the hour time region, as the amount of GABA receptors in the injected oocytes might vary. In some cases, it may also require an elaboration of the minimal model, for example the addition of the desensitized form without a ligand or the open-channel form with only one ligand, though their fractions must be small if they exist.

The rate of desensitization of nAChR in the injected oocyte measured by the electrophysiological method was slower than that measured by using membrane vesicles of *E. electricus* and a filtration assay, though both rates of desensitization showed similar dose dependence (Kobayashi & Aoshima, 1986). So, the possibility that the real rate of desensitization is larger than that obtained in this paper is not excluded. To clarify this problem, further improvement of measurements is necessary to obtain the responses of GABA receptors in the millisecond time region, since time resolution of our experiments was several seconds.

The electrical responses of GABA receptors synthesized in *Xenopus* oocytes under various conditions were clearly interpreted by the minimal model in Figure 1. This model will be useful in characterizing GABA receptors or in clarifying the effects of many drugs acting on GABA receptors, as reported for nAChR (Aoshima, 1983; Karpen & Hess, 1986).

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# Size and Shape of the *Escherichia coli* Lactose Permease Measured in Filamentous Arrays

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ABSTRACT: The *Escherichia coli* lactose permease has been purified on cation exchanger to contain a minimal amount of phospholipids, i.e., 4–5 mol/mol of permease, in the presence of the detergent dodecyl β-maltoside at its critical micelle concentration. This preparation is active in galactoside binding. When the detergent level is further reduced by dialysis, the lactose permease forms filaments one molecule wide and up to several micrometers long. The filaments tend to associate laterally to form sheets. Analysis of electron micrographs of negatively stained filamentous arrays indicates an average filament spacing of 51 Å and a subunit period of 26–30 Å along individual filaments. These values most probably correspond to the dimensions of the lactose permease molecule measured parallel to the membrane plane. In many filaments, the subunits show a stain-penetrated cleft. It suggests that the lactose permease molecule comprises two domains, which may be correlated with internal repeats between the N- and C-terminal halves of the polypeptide sequence.

The lactose permease is an integral protein of the *Escherichia coli* cytoplasmic membrane and has a molecular weight of 46 500. It catalyzes the cotransport of 1 mol of proton with 1 mol of galactoside across the membrane (Kaback, 1983; Overath & Wright, 1983). Therefore, at the expense of the electrochemical gradient of protons generated by the cell's metabolic activities, the cell can accumulate the sugar against a concentration gradient (Mitchell, 1968).

The *lac y* gene coding for the permease has been cloned into a multicopy plasmid (Teather et al., 1978), allowing the gene

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