Desensitization of Cl\textsuperscript{−}-dependent GABA Response
Observed in Ganglion Cells of \textit{Aplysia}

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Abstract There are many cells in the abdominal ganglion which show a
fast, Cl\textsuperscript{−}-dependent hyperpolarizing response to \textit{γ}-aminobutyric acid
(GABA). This response is characterized by an initial rapid increase in
membrane conductance followed by an exponential decay to the original
value despite a sustained application of GABA. The decay of the response
was found to be largely due to the desensitization of the GABA receptor
(binding site-ionophore complex) since the equilibrium potential for
chloride remained unchanged when the conductance response was de-
pressed. The apparent or measurable rate constant of desensitization (K\textsubscript{D})
increased when the concentration of GABA increased, showing a satu-
ration in K\textsubscript{D}-[GABA] relationship at higher concentration of GABA. The
rate of desensitization did not change significantly when the resting
membrane was hyperpolarized from −40 to −80 mV, though the con-
ductance response was markedly depressed due to a characteristic voltage-
dependence in the receptor activation (Matsumoto, 1982). These obser-
vations are discussed in terms of an hypothesis in which the desensitization
of GABA receptor is the result of an additional binding of a GABA
molecule to the activated receptor.

Key words: GABA receptor, Aplysia, desensitization, Cl\textsuperscript{−}-channel.

The gradual decay of the response to a sustained application of \textit{γ}-aminobutyric
acid (GABA) has been observed in the neuromuscular junction of crab (Epstein
and Grundfest, 1970; Hochner et al., 1976), in goldfish Mauthner neurons
(Diamond and Roper, 1973), in spinal cord neurons of the cat (Krnjević et al.,
1977), and in \textit{Aplysia} ganglion cells (Yarovsky and Carpenter, 1978). This
phenomenon might be due to a desensitization of GABA receptor (binding site-
onophore complex), the mechanism of which is comparable to that proposed by
Katz and Thesleff (1957) for the desensitization of acetylcholine (ACh) receptor at
the frog endplate. However, it should be noted that the desensitization-like
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phenomena were not confirmed with the GABA receptors in the neuromuscular junctions of the crayfish (TAKEUCHI and TAKEUCHI, 1965), the lobster (CONSTANTI, 1977a, b), the locust (BROOKES and WERMAN, 1973), and the Ascaris (MARTIN, 1980). In all preparations, GABA selectively increases the permeability of the postsynaptic membrane toward Cl⁻, regardless of whether the receptor exhibits the desensitization or not. The discrepancy among these results may indicate the structural difference of the receptors in preparations; however, it may also reflect the difference in methods of evaluating the effects. For example, some investigators used membrane potential change, whereas others used membrane conductance change as the criteria for GABA-induced responses. These criteria must be used carefully because the potential change is rather secondary to the conductance change, and even the conductance change produced by agonists is rather a complex function of not only the membrane permeability toward the specific ions involved but also the equilibrium potentials of the ions (MATSUMOTO et al., 1984). Sustained application of agonist may cause an increase in intracellular ionic concentration, which would decrease the driving force for the ions involved (KUBA and KOKETSU, 1976).

The aim of the present study was to examine a characteristic fading of the GABA-induced response in the Aplysia ganglion cells, and to analyze some possible causes for this decay. Preliminary reports of this work have been presented by MATSUMOTO et al. (1982a, b).

METHODS

Preparation and perfusing solution. The abdominal ganglion of Aplysia kurodai was dissected out and placed in a perfusion chamber. The cells were exposed to the perfusing solution after removing the connective tissue by means of binocular microscopic surgery. The cells were normally perfused with the artificial Aplysia blood (SATO et al., 1968); Na⁺ 587, K⁺ 12, Cl⁻ 671, Ca²⁺ 14, and Mg²⁺ 52 mM. The effective perfusing volume of the chamber was 0.2 ml, and the rate of perfusion was 8 ml per min. The pH of perfusing solution was adjusted to 7.4 with Tris and HCl. The experiments were carried out at temperatures between 15 and 18°C maintained by means of a water bath surrounding the chamber.

Measurement of the membrane conductance. Two glass microelectrodes filled with 1.8 M potassium citrate (5–15 MΩ) were inserted into an identified single cell within the cell clusters of R_B and R_C after FRAZIER et al. (1967). One of the electrodes was connected to a high impedance preamplifier (gain 1) for the purpose of recording the membrane potential. The other was connected to a current source in order to control the level of membrane potential before and during the GABA receptor activation. The GABA-induced responses were evaluated in two ways. One was a constant current method in which a constant inward current pulse of 500 ms was given intracellularly every 5 s; the voltage drop across the receptor membrane was compared before and during the response to GABA. The other was a constant
voltage method in which the resting potential was clamped at a given level during the response to GABA, and GABA-induced current was recorded. In addition to this conventional voltage clamp method, we employed a "modulated resting clamp" method (MATSUMOTO, 1982), in which the resting membrane was periodically hyperpolarized by a voltage pulse with a duration of 500 ms; the current required for this constant hyperpolarizing pulse was recorded continuously before and during the response to GABA in order to evaluate the change in slope conductance.

The type of GABA receptor used in this experiment. As mentioned above, most of the GABA receptors identified previously in different animals are the type which when activated, produce a Cl- dependent response. According to YAROWSKY and CARPENTER (1978), there are five types of GABA receptors in the ganglion cells of Aplysia. Three are excitatory types, whereas the other two are inhibitory types. One of the inhibitory types is the receptor, activation of which produces a Cl- dependent response whereas the other is the receptor, activation of which produces a K+ dependent response. Among these GABA receptors in the ganglion cells of Aplysia, only the inhibitory, Cl- dependent type was selected for this experiment because this type of receptor has been studied most extensively in different animals as described above.

Identification of the cells which include Cl- dependent type of GABA receptors. Many cells in the abdominal ganglion show a biphasic hyperpolarizing response when GABA is applied by perfusion. The initial phase of hyperpolarization is known to be associated with a permeability increase toward Cl-, whereas the subsequent phase is due to a permeability increase toward K+ (YAROWSKY and CARPENTER, 1978). However, there are a certain number of cells in the group of Rβ and Rγ, which respond to GABA with a monophasic, rapid hyperpolarization. This hyperpolarization is exclusively due to an increase in permeability of the postsynaptic membrane toward Cl-. Only these cells were selected and used for this experiment. The selective increase in Cl- permeability was confirmed by measuring the expected change in reversal potential of the response after altering the Cl- concentration of the perfusing solution, and by observing a powerful blocking effect of bicuculline which is specific for the Cl- dependent response according to YAROWSKY and CARPENTER (1978).

RESULTS

Presence of desensitization

Generally, GABA induces a hyperpolarization associated with an increase in membrane conductance. However, this response gradually disappears when the application of GABA is prolonged, as shown in the upper trace of Fig. 1. This phenomenon first suggested the presence of desensitization in the GABA receptors of the ganglion cells. Another feature of the receptor desensitization may be confirmed by observing the depressing effect of a preceding GABA application on the response to the higher concentration of GABA. This is shown in the bottom
traces in Fig. 1. The response to a brief application of 0.1 mM GABA was markedly depressed following a sustained application of 0.01 mM GABA. Note that the response to 0.01 mM GABA demonstrated the desensitization, the response gradually disappearing during the continued application of GABA. The gradual decrease in GABA-induced hyperpolarization was always associated with a decrease in the conductance response. This fact suggested that the major cause of the decrease in GABA-induced hyperpolarization was the depression of the receptor activity. However, there remains a possibility that a prolonged application of GABA would induce intracellular accumulation of Cl⁻, which could partially contribute to the decrease in GABA-induced hyperpolarization. In order to examine this possibility, a voltage clamp experiment was performed on the neuron under study. In this experiment, the resting membrane was clamped stepwise at two voltage levels, every 5 s during a response to GABA, as shown in the bottom of Fig. 2; one level was the original resting potential and the other level was the reversal potential of the response, which was determined on the same cell in advance. This
method enabled us to detect any change in either the GABA-induced current response or the reversal potential during the response, if it occurred. The GABA-induced response recorded at the original resting potential was initially a distinct outward current but gradually decayed later during a prolonged application of GABA, as shown in middle of Fig. 2. The GABA-induced current response recorded at the equilibrium potential of Cl\(^-\) was a negligibly small outward current, which could be seen as an envelope interconnecting the tips of each downward
The rate of desensitization and GABA concentration

Figure 3 shows the current responses of the single cell to the sustained applications of GABA in different concentrations, obtained under the voltage clamp at the resting potential. When GABA concentration was increased from 0.1 to 10 mM, the GABA-induced responses tended to fade more rapidly. Since the time course of the decline was nearly exponential, we replotted the same data using a semi-log scale (not shown). For evaluating the rate of decline, the apparent or measurable rate constant \(K_D\) of desensitization was obtained from the slope of each line. The relation between \(K_D\) and GABA concentration was examined on five
different cells and shown in Fig. 4. The rate of desensitization increased with an increase in GABA concentration, and the curve showed a tendency to saturate at the higher concentration. Similar saturable nature of the rate of desensitization has been observed in the GABA receptor of the frog dorsal root ganglion cells (Akaike, 1985).

**Rate of desensitization and membrane potential**

The current responses to 0.3 mM GABA were measured from the same cell under the voltage clamp at -40, -50, -60, -70, and -80 mV, and illustrated as a, b, c, d, e in Fig. 5. The rate of decay calculated from the slope of each line showed only a minimal dependence on the membrane potential.

**DISCUSSION**

A fading of the postsynaptic response to a sustained application of the transmitter has been considered as due to the reuptake of the transmitter (Kuffler and Edwards, 1958; Horwitz and Orkand, 1980), or the decrease in driving force for the ion involved in generation of the response (Kuba and Koketsu, 1976), and/or the desensitization of the receptor (binding site-ionophore complex) (Katz and Thesleff, 1957). It is obvious that the gradual fading of the GABA-induced response observed in the present study is not due to the decrease in GABA concentration by reuptake because the cells were perfused continuously with a solution containing a given concentration of GABA. A possibility of decrease in the driving force for Cl\(^{-}\) due to a gradual accumulation of intracellular Cl\(^{-}\) was also excluded by showing the equilibrium potential of Cl\(^{-}\) to be unaltered during the
depressed response. Consequently, we considered that the receptor desensitization is the major cause for fading of GABA-induced responses observed in the present study.

There are a few models describing kinetics of receptor desensitization, although most of them were proposed for the nicotinic acetylcholine (ACh) receptors. One model proposed by Adams (1975) is that the fading of the ACh-induced response is primarily due to plugging of the open Na⁺ channels by ACh molecules. According to his model, the apparent or measurable rate constant of desensitization (K₅₀) should increase linearly with the increase in agonist concentration [A] (K₅₀ = kₐ [A], where kₐ is a real or theoretical rate constant of desensitization). The present study demonstrated that K₅₀ of GABA receptor did increase with GABA concentration but not in a linear fashion. Instead, K₅₀ increased steeply at lower concentration of GABA, but tended to saturate at higher concentration (see Fig. 4). The saturable nature of K₅₀ may well be explained by a cyclic model originally proposed by Katz and Thesleff (1957), and later confirmed by Rang and Ritter (1970a, b). According to this model, K₅₀ is a complex function of not only kₐ but also kₐ, the affinity constant of receptor activation. Their theory predicts that any drugs or conditions which decrease kₐ would also decrease K₅₀. However, Magazanik and Vyskočil (1973) observed no effect of d-tubocurarine on the K₅₀ value of ACh receptors. Furthermore, they observed an increase in K₅₀ of ACh-receptor with α-bungarotoxin which is known to depress kₐ.

Recently the activation process of GABA receptor in ganglion cells of Aplysia was found to be voltage-dependent; nearly 3-fold increase in affinity with 10 mV depolarization (Matsumoto, 1982). A marked increase in K₅₀ is expected from the cyclic model proposed by Katz and Thesleff (1957), if K₅₀ is evaluated under the membrane depolarization. The present result, however, showed that K₅₀ remained unchanged when the membrane potential was depolarized from −80 to −40 mV. This result indicated that Katz and Thesleff's model is not directly applicable to the desensitization of GABA receptors in our preparation.

We modified a multi-site model originally proposed by Nastuk and Gissen (1966), postulating three binding sites on the GABA receptor. Two binding sites may be required for receptor activation since Hill coefficient is 1.8 (unpublished data).

\[
k_{a} \quad k_{a}'
\]

2A + R → A₂R ↔ A₂R* (1)

It was assumed that an additional agonist molecule (A) binds to the activated receptor (A₂R*), and that this complex changes to a transitional, but still active receptor (A₂R*A) before changing gradually to the desensitized state (A₂DA). Reaction kinetics are shown below:

\[
A + A₂R* \xrightarrow{K} A₂R*A \xrightarrow{kₐ} A₂DA . \quad (2)
\]
If the receptor activation and additional binding to $A_2R^*$ are assumed to be much faster processes than the time course of desensitization, the value of $K$ at equilibrium may be approximated as $K = [A][A_2R^*]/[A_2R^*A]$. The fading rate of agonist-induced response may be expressed as follows:

$$\frac{d([A_2R^*]+[A_2R^*A])}{dt} = k_d[A_2R^*A]$$

$$= k_d\frac{[A]}{K+[A]}([A_2R^*]+[A_2R^*A]).$$

Accordingly,

$$K_p = k_d\frac{[A]}{K+[A]}$$

(3)

Finally, the measurable rate constant ($K_p$) is expressed as a product of the theoretical rate constant ($k_d$) and an additional term, a function of GABA concentration. Our result shown in Fig. 4, namely the relationship between $K_p$ and GABA concentration, can be interpreted from the curve expressed by Eq. (3), including the saturable nature of the concentration dependence. In addition, the membrane depolarization, which has been proved to alter $k_a$ or $k_a'$ in the activation process of this receptor, did not alter the value of $K_p$, as shown in Fig. 5. This fact is also explained well in terms of Eq. (3), since neither $k_a$ nor $k_a'$ is included in the function of $K_p$. This model seems to explain some of our present results, particularly the kinetic aspect of the GABA receptor desensitization, though more supporting evidence is needed to confirm the validity of this model.

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REFERENCES


