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Agonist and Potentiation Actions of *n*-Octanol on γ-Aminobutyric Acid Type A Receptors

YASUTAKA KURATA,[1] WILLIAM MARSZALEC, JAY Z. YEH, and TOSHIO NARAHASHI

Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, Illinois

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ABSTRACT

The *n*-octanol effects on the γ-aminobutyric acid type A ($GABA_A$) receptor were studied in human embryonic kidney 293 cells transfected with α1, β2, and γ2S subunit cDNAs. GABA-evoked currents had an EC_{50} of 13.3 ± 1.7 μM and a Hill coefficient (n_H) of 1.4 ± 0.1. *n*-Octanol was also capable of evoking a small current with an EC_{50} of 1000 μM and an n_H of 2. In addition, *n*-octanol modulated GABA-induced currents in a concentration-dependent manner. Coapplications of *n*-octanol increased peak currents evoked by 3 μM GABA with an EC_{50} of 190 μM and an n_H of 1.8. The extent of potentiation decreased with increasing GABA concentrations and no potentiation was observed when *n*-octanol was coapplied with 1000 μM GABA. One-minute preapplication of 1000 μM *n*-octanol slightly potentiated 3 μM GABA-induced current, whereas it suppressed 300 μM GABA-induced current to 16% of the control, suggesting that 84% of the receptors underwent desensitization. Two models were used to explain *n*-octanol agonistic and potentiating actions on the α1β2γ2S $GABA_A$ receptor: *n*-octanol binds to multiple sites to exert multiple actions, or *n*-octanol acts as a partial agonist to manifest these actions. The partial agonist model is unique because it is a simpler model to explain *n*-octanol actions on the $GABA_A$ receptor.

The γ-aminobutyric acid type A ($GABA_A$) receptor mediates the majority of inhibitory synaptic responses in the mammalian brain (Sivilotti and Nistri, 1991) and is the site of action for many drugs (Burt and Kamatchi, 1991; Macdonald and Olsen, 1994). Ethanol has been reported to potentiate GABA-induced responses in several preparations (Aguayo 1990; Nishio and Narahashi, 1990; Nakahiro et al., 1991; Reynolds et al., 1992; Sigel et al., 1993; Marszalec et al., 1994; Weiner et al., 1994; Harris et al., 1995). In most of these studies, the focus was on the alcohol-induced potentiation of GABA-induced peak current. However, alcohol-mediated changes in other properties of GABA-induced current have not received much attention.

In rat dorsal root ganglion (DRG) neurons, alcohols have been shown to modify GABA currents in several ways (Nakahiro et al., 1991; Arakawa et al., 1992). The effects include: 1) potentiation of GABA-induced peak currents with a brief alcohol coapplication; 2) inhibition of peak currents with extended alcohol preperfusions; 3) apparent acceleration of desensitization of GABA-induced currents; 4) inhibition of the steady-state currents that follow desensitization; and 5) direct generation of current by alcohols. The interpretation of alcohol-GABA channel interactions is difficult because of a variety of $GABA_A$ receptor subtypes that exist in the native neurons, each composed of different combinations of receptor subtypes (Burt and Kamatchi, 1991; Macdonald and Olsen, 1994). Thus, it remains to be seen whether alcohols exert these multiple actions on a single type of $GABA_A$ receptor.

To explore whether alcohols exert multiple effects on a single type of $GABA_A$ receptor, we have initiated our study by using human embryonic kidney (HEK) cells transfected with cDNAs encoding for the α1, β2, and γ2S rat $GABA_A$ receptor subunits. The α1, β2, and γ2S subunit combination has been reported as the most prevalent form of the $GABA_A$ receptor in the mammalian brain (Burt and Kamatchi, 1991). In our previous studies with this combination of $GABA_A$ receptor subunits, we found that alcohols potentiate peak currents without changing the maximal GABA response. In other words, alcohols shift the GABA dose-response curve toward lower concentrations (Kurata et al., 1993; Marszalec et al., 1994).

All experiments reported here made use of the eight-carbon alcohol *n*-octanol, because ethanol has a weak effect on GABA-induced currents in α1β2γ2*S*-transfected HEK cells (Marszalec et al., 1994). However, the previous study with DRG neurons indicates that *n*-alcohols having less than 10 carbons (including ethanol) produce qualitatively similar effects on GABA-induced currents, but differ in their potency

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[1] Current affiliation: Department of Physiology, Kamazawa Medical University, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-02, Japan.

ABBREVIATIONS: HEK, human embryonic kidney; DRG, dorsal root ganglion; EC_{50}, concentration producing 50% maximal response; IC_{50}, concentration producing 50% inhibition.

(which correlates with lipid solubility) (Nakahiro et al., 1991). Furthermore, recent single-channel patch clamp experiments have clearly shown that ethanol and *n*-octanol have identical effects to modulate GABA receptor channels in DRG neurons (Tatebayashi et al., 1998).

In the present study, alcohol actions on the $\alpha1\beta2\gamma2S$ receptor were examined during a brief period of coapplication and after prolonged pretreatment with either GABA or *n*-octanol. *n*-Octanol exerted multiple actions on the receptor depending on the experimental condition. We have attempted to explain various effects of *n*-octanol on the $\alpha1\beta2\gamma2S$ receptor using two models. One is based on the classical allosteric model in which *n*-octanol binds to a site different from the GABA binding site to modulate the GABA-induced response. The other is based on a partial agonist model in which *n*-octanol acts as a weak partial agonist to bind to the same site as GABA does. The binding to the agonist site manifests multiple actions as has been demonstrated with nicotinic receptors (Cachelin and Rust, 1994; Steinbach and Chen, 1995; Fletcher and Steinbach, 1996). The latter model is unique in that many features of *n*-octanol action can be satisfactorily accounted for, whereas the allosteric model can only explain limited observations.

Materials and Methods

Preparation. HEK293 cells were transfected with $\alpha1$, $\beta2$, and $\gamma2S$ subunit cDNAs derived from rat brain $GABA_A$ receptors. The techniques for the stable expression of these $GABA_A$ receptors were detailed in a previous report (Hamilton et al., 1993). All cells were grown in modified Eagle's minimum essential medium supplemented with 10% fetal calf serum in the presence of humidified air containing 5% CO_2. These cells exhibited GABA dose-dependent responses similar to those reported by other groups for this subunit combination, and were sensitive to benzodiazepines requiring the presence of $\alpha1$, $\beta2$, and $\gamma2S$ subunits (Kurata et al., 1993).

Electrical Recording. GABA-induced currents were recorded with the whole-cell configuration of the patch clamp technique. Patch electrodes were made from 1.0-mm (o.d.) borosilicate glass capillary tubes using a vertical pipette puller (Narishige PP-83, Tokyo, Japan). Electrode resistance ranged from 2 to 5 MΩ when filled with internal solution. All currents were recorded with an Axopatch-1C amplifier (Axon Instrument Co., Foster City, CA) and were stored on an PDP 11/73 computer (Digital Equipment, Pittsburgh, PA).

A 5- to 10-min period following membrane rupture allowed the pipette solution to equilibrate with the cell interior before starting current recording. GABA-evoked inward currents were recorded at a membrane potential clamped to −60 mV, which was near the resting potential of most cells. The GABA reversal potential occurred between 0 and +10 mV, near the equilibrium potential for chloride ions in the internal and external media used. All experiments were performed at room temperature (22°C).

Solutions. Cells were dialyzed with an internal (electrode) solution of the following composition: 140 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 5 mM EGTA, and 5 mM Mg-ATP. The normal external solution contained: 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM HEPES, and 10 mM D-glucose. The pH of both solutions was adjusted to 7.3 with NaOH.

n-Octanol (Aldrich, Milwaukee, WI) was applied at concentrations of 10 to 1000 μM. It was first dissolved in dimethyl sulfoxide and then diluted with external solution. The dimethyl sulfoxide concentration was kept at 0.01 to 0.1% (v/v), which had no effect on GABA-induced currents. External solutions containing GABA (Sigma, St. Louis, MO) or *n*-octanol were prepared immediately before use.

Drug Application. External solutions were perfused into the recording chamber at a normal rate of 1 to 2 ml/min and were increased to 10 ml/min when solutions were changed. GABA-containing solutions with or without *n*-octanol were directly applied to the cell by a variant of the U-tube method (Marszalec et al., 1994). This allowed localized solution exchanges within a range of 20 to 30 ms. For prolonged application of GABA or *n*-octanol, bath application was used.

Data Analysis. The dose-response relationship for agonistic action was analyzed by the following logistic equation:

$$y \equiv \frac{100\% \cdot [C]^{n_H}}{[C]^{n_H} + [EC_{50}]^{n_H}} \tag{1}$$

where y represents the percentage of maximal response induced by GABA or *n*-octanol at a concentration of C, with 100% being the maximal obtainable response. EC_{50} and n_H represent the half-maximal effective concentration of GABA and the Hill coefficient, respectively.

The dose dependence of modulatory effects of *n*-octanol on GABA-induced current was analyzed by two equations, one relating to potentiation (eq. 2) and the other for suppression (eq. 3).

$$\frac{I_{oct} - I_{cont}}{I_{cont}} \equiv \frac{A \cdot [C]^{n_H}}{[C]^{n_H} + [EC_{50}]^{n_H}} \tag{2}$$

$$\frac{I_{oct}}{I_{cont}} \equiv \frac{[IC_{50}]^{n_H}}{[C]^{n_H} + [IC_{50}]^{n_H}} \tag{3}$$

Here, A is a scaling factor and I_{oct} is the amplitude of *n*-octanol modified GABA-induced currents. The amplitude of the paired control GABA current is denoted as I_{cont}. EC_{50} represents the half-maximal concentration for the potentiating action of *n*-octanol and IC_{50} represents its half-maximal inhibitory concentration.

The dose-response relationship for GABA to open the channel can also be visualized as follows:

$$\begin{array}{ccccc} R & \overset{K_G}{\longleftrightarrow} & GR & & \\ \updownarrow K_G & & \updownarrow aK_G & & \\ RG & \overset{aK_G}{\longleftrightarrow} & GRG & \overset{X_G}{\longrightarrow} & GRG^*\ (OPEN) \end{array}$$

Scheme 1. Dose-response relationship for GABA to open channel.

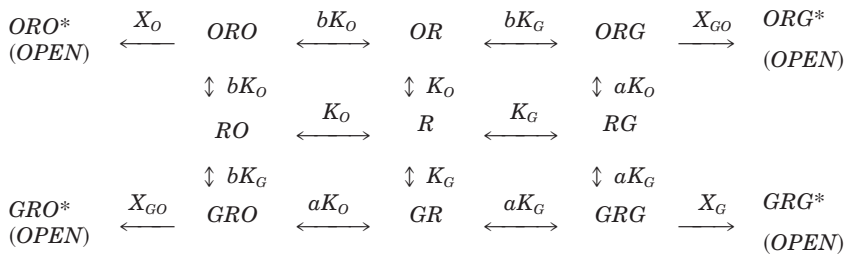
where K_G is a microscopic dissociation constant for GABA (G), to bind to the GABA binding site on the receptor, R; a is a cooperative factor for altering the second binding site; and X_G is an equilibrium channel opening ratio. GR and RG are the receptor, which is singly bound to GABA. The doubly bound receptors are referred to as GRG in the closed state and GRG^* in the open state. These parameters were estimated from the dose-response relationship (eq. 4), which is based on a velocity equation (Segel, 1975) with incorporation of the efficacy concept of channel opening (Colquhoun and Ogden, 1988).

$$y = \frac{\dfrac{X_G[G]^2}{aK_G^2}}{1 + 2\dfrac{[G]}{K_G} + \dfrac{[G]^2}{aK_G^2} + \dfrac{X_G[G]^2}{aK_G^2}} \tag{4}$$

where y is a normalized response in the presence of a given concentration of GABA, $[G]$.

Similarly, octanol acts as a partial agonist as defined in the following eq. 5:

$$y' = \frac{\dfrac{X_O[O]^2}{bK_O^2}}{1 + 2\dfrac{[O]}{K_O} + \dfrac{[O]^2}{bK_O^2} + \dfrac{X_O[O]^2}{bK_O^2}} \tag{5}$$



Scheme 2. An alternative explanation for direct, potentiating, and inhibitory actions of *n*-octanol on $\alpha1\beta2\gamma2s$ $GABA_A$ receptor.

where [*O*] represents the concentration of *n*-octanol, K_O is a microscopic dissociation constant for *n*-octanol to bind to the GABA binding site on the receptor, *b* is a cooperative factor, and X_O is an equilibrium channel open ratio as defined above for the agonist GABA.

As an alternative explanation for the direct, potentiating, and inhibitory actions of *n*-octanol on the $\alpha1\beta2\gamma2s$ $GABA_A$ receptor, a kinetic scheme based on the partial agonist model (Scheme 2), as previously used by other investigators (Cachelin and Rust, 1994; Steinbach and Chen, 1995; Fletcher and Steinbach, 1996), is proposed when GABA and *n*-octanol are applied together to the receptor. The following assumptions were made: 1) the binding of GABA to the first site changes the affinity of the second site by the same factor, *a*, regardless of whether GABA or *n*-octanol binds to the second site; 2) the binding of *n*-octanol to the first site changes the affinity of the second site by the same factor, *b*, regardless of whether GABA or *n*-octanol binds to the second site; and 3) the equilibrium open probability of heteroliganded receptor (*GRO* and *ORG*), X_{GO}, does not depend on the order of binding of GABA and *n*-octanol. At equilibrium, eq. 6 can be derived:

$$y = \frac{X_G\left(\frac{[G]^2}{aK_G^2}\right) + X_{GO}\left(\frac{[G][O]}{aK_GK_O}\right) + X_{GO}\left(\frac{[G][O]}{bK_GK_O}\right) + X_O\left(\frac{[O]^2}{bK_O^2}\right)}{\left[1 + 2\frac{[G]}{K_G} + 2\frac{[O]}{K_O} + \frac{[G][O]}{aK_GK_O} + \frac{[G][O]}{bK_GK_O} + \frac{[G]^2}{aK_G^2} + \frac{[O]^2}{bK_O^2} + \frac{X_{GO}[G][O]}{aK_GK_O} + \frac{X_{GO}[G][O]}{bK_GK_O} + \frac{X_G[G]^2}{aK_G^2} + \frac{X_O[O]^2}{bK_O^2}\right]} \quad (6)$$

eq. 6 is reduced to eq. 4 when GABA is present alone. Likewise, eq. 6 is reduced to eq. 5 when *n*-octanol is present alone.

All dose-response curves were fitted by a nonlinear least-squares method. Data are presented as mean ± S.E.M., unless otherwise stated.

Results

Characteristics of GABA Responses under Control Conditions. The dose-response relationship for GABA to induce inward currents was fitted by two methods as described under *Materials and Methods*. Figure 1A depicts the fit of data with a logistic eq. 1 yielding an EC_{50} of 13.3 μM and a Hill coefficient (n_H) of 1.40. Figure 1B illustrates the fit of data with eq. 4. The open probability was set to 80% as a scaling factor for the original dose-response data (Weiss and Magleby, 1989; Newland et al., 1991). A least-squares fit to the data by the velocity equation based on the partial agonist model yielded the following parameters: a cooperating factor, *a*, of 0.8, an intrinsic dissociation constant, K_g, of 22 μM and an equilibrium channel open ratio, X_G, was assumed to be 4 based on the assumed maximal open probability of doubly liganded $GABA_A$ receptor. These parameters will be used later for fitting the data obtained in the presence of GABA and *n*-octanol.

***n*-Octanol Generates Currents by Itself.** In the absence of GABA, *n*-octanol was capable of generating inward currents in a dose-dependent manner when the membrane potential was held at −60 mV (Fig. 2). The *n*-octanol-induced currents were small compared with GABA-induced currents. On the average, the current generated by 1000 μM *n*-octanol was about 35% of that generated by 3 μM GABA and 3.3 ± 0.7% ($n = 6$) of the maximal current generated by 300 μM GABA.

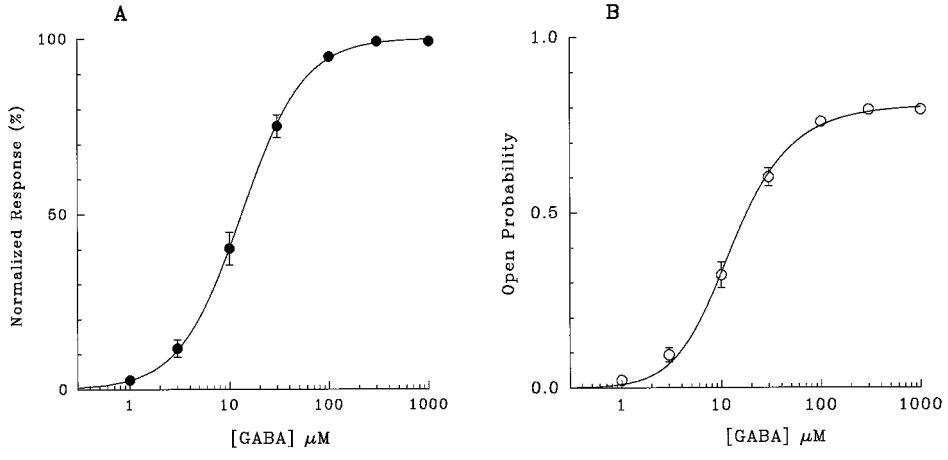


Fig. 1. GABA dose-response relationship observed in HEK293 cells expressing $\alpha1\beta2\gamma2S$ subunit combination. Two models were used to fit data: an allosteric model (A) and a partial agonist model (B). A, in the allosteric model, a logistic eq. 1 was used to fit data giving an EC_{50} of 13.3 μM and a Hill coefficient (n_H) of 1.40. Current amplitude was normalized to its maximum value. B, Percentage of response in A was divided by 125 to convert to open probability, assuming that maximal open probability was 0.8. Data was fitted by eq. 4 yielding the following parameters: cooperative factor, *a*, of 0.8 ± 0.4, intrinsic dissociation constant, K_G, of 22.2 ± 8.0 μM, and an equilibrium opening ratio, X_G, of 4.

The dose-response relationship for *n*-octanol to generate inward currents is shown in Fig. 3. Again two models were used to fit the data. The classical logistic equation gave an EC_{50} of 1127 μM and a Hill coefficient of 2.0. The eq. 5 gave a cooperative factor, *b*, of 0.33, an intrinsic dissociation constant, K_o, of 2821 μM, and an equilibrium channel open ratio, X_O, of 0.15. This activation factor was adjusted to give an open probability of 0.13.

Several other observations indicated that *n*-octanol-induced currents were mediated by the $GABA_A$ receptor. First, no alcohol-induced current was observed in those cells that failed to respond to GABA. Second, the alcohol-induced currents decayed at higher concentration (Fig. 2D), and this tendency was especially pronounced with a prolonged application (see Fig. 10). Third, the reversal potential of the currents was between 0 and +10 mV, nearly identical with the equilibrium potential for chloride ions. Finally, the $GABA_A$ competitive antagonist bicuculline (see Fig. 4) and the noncompetitive antagonist picrotoxin (data not shown) reduced the current evoked by 1000 μM *n*-octanol.

Figure 4 illustrates competitive antagonism between bicuculline and GABA for GABA-induced currents. When coapplied with GABA, bicuculline at 10 μM almost completely eliminated the current induced by 3 μM GABA (Fig. 4B), reducing the GABA current to 1.4 $\pm$ 0.5% (n = 6) of the control, but reduced the 300 μM GABA-induced control current by 20.0 $\pm$ 5.0% of (Fig. 4E). Bath application, in addition to coapplication of bicuculline, enhanced antagonism at the beginning of current (Fig. 4F). These results are consistent with the competitive $GABA_A$ receptor antagonistic action of bicuculline.

Bicuculline at 10 μM was also very effective in eliminating *n*-octanol-induced current. The *n*-octanol-induced current was reduced to 3.44 $\pm$ 2.00% of the control (Fig. 4, G and H). As was seen with its action on GABA-induced response, the inhibitory action of bicuculline on *n*-octanol-induced current was reversible upon washing out bicuculline (Fig. 4, C and I). The fact that the degree of activation by 3 μM GABA was on the same of magnitude as that induced by 1000 μM *n*-octanol may explain the similar blocking action of bicuculline on their responses.

***n*-Octanol Potentiates GABA-Induced Peak Current.** When *n*-octanol was coapplied with GABA, the GABA-induced current was increased. The potentiating action of *n*-octanol on GABA-induced current was examined with respect to its dose dependence. As shown in Fig. 5, 30 to 1000 μM *n*-octanol increased the peak current evoked by 3 μM GABA in a dose-dependent manner. The potentiating action was reversible upon washing out *n*-octanol (Fig. 5G).

Figure 6 illustrates the dose-response relationship for *n*-octanol to exert its potentiating action on the currents induced by 3 μM and 10 μM GABA as fitted by two methods. By assuming *n*-octanol acting at an allosteric site to increase GABA-induced currents, the data were fitted with the logistic eq. 2 yielding an EC_{50} of 190 μM and a Hill coefficient of 1.49 for both 3 μM and 10 μM GABA but with different maximal responses (Fig. 6A).

A partial agonist is known to exert potentiating action on the response induced by a full agonist when the full agonist concentration is relatively low compared with its EC_{50} value (Cachelin and Rust, 1994; Steinbach and Chen, 1995; Fletcher and Steinbach, 1996). A kinetic scheme based on this concept is shown in Scheme 2 and a dose-response equation was derived with the incorporation of an assumption that the receptor bound by one GABA molecule and one *n*-octanol molecule is capable of opening the channel. Figure 6B depicts that such a partial agonist model (eq. 6) can fit the data as satisfactorily as the allosteric model. The parameters of GABA and *n*-octanol for binding to $GABA_A$ receptors were previously determined from Figs. 1 and 2 and the equilibrium channel opening ratio, X_{GO}, of the bi-ligand-bound receptor

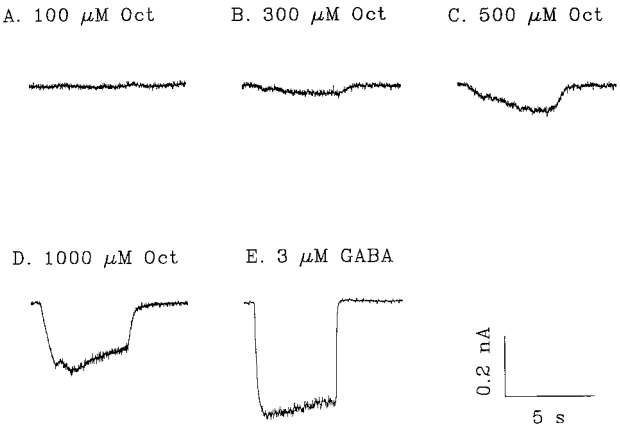


Fig. 2. *n*-Octanol generates currents in HEK293 cells expressing the $\alpha1\beta2\gamma2S$ $GABA_A$ receptors. A–D, Currents induced by 100 to 1000 μM *n*-octanol applied for 5 s. E, Current induced by 3 μM GABA. Current induced by 1000 μM *n*-octanol was about 50% of that produced by 3 μM GABA.

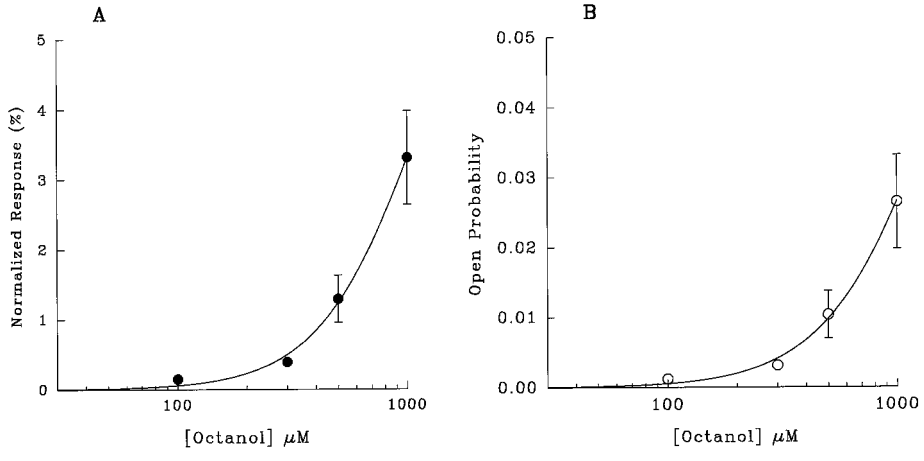


Fig. 3. Dose-response relationship for currents generated by *n*-octanol. A, amplitude of octanol-induced currents is given as percentage of control current evoked by 300 μM GABA. A, logistic eq. 1 was used to fit data yielding an EC_{50} of 1127 μM and a Hill coefficient (n_H) of 2.0. B, data were fitted with a partial agonist model (eq. 5) yielding the following parameters: cooperative factor, *b*, of 0.33, intrinsic dissociation constant, K_O, of 2821 μM, and an equilibrium opening ratio, X_O, of 0.15.

was assumed to be 12. The most intriguing result of this simulation is that the open probability of the receptor bound by one GABA and one *n*-octanol molecules is higher than that of the receptor doubly bound by two GABA molecules. Further tests for this notion await single-channel analysis.

Potentiating Action of *n*-Octanol Depends on GABA Concentrations. Figure 7 summarizes the augmentation of GABA-induced peak currents by 100 μM *n*-octanol as a function of GABA concentration. The *n*-octanol-induced potentiation of GABA currents decreased as the GABA concentration increased. Peak currents evoked by GABA at 1 and 3 μM were greatly increased but those at 100 to 300 μM GABA were not changed by 100 μM *n*-octanol. According to the allosteric model, this alcohol-induced peak current potentiation is related to a shift of the GABA dose-response relationship in the direction of lower agonist concentrations. That is, 100 μM *n*-octanol reduced EC_{50} from 13 μM to 5.5 μM for activating the $GABA_A$ receptor.

Figure 7B was fitted with the partial agonist model by using the same parameters used to fit the data in Fig. 6. Although both models are capable of fitting the potentiation data for GABA concentrations greater than 3 μM, the partial agonist model fits the data better at 1 μM GABA than the allosteric model does. Thus, the potentiation of the response induced by low GABA concentrations by octanol differentiates thes two models.

Another critical test of the partial agonist model is to plot the GABA dose-response relationship for GABA alone and in the presence of various concentrations of *n*-octanol. A prediction made from the partial agonist model is that a Hill coefficient of more than one is expected to be reduced to unity (Kopta and Steinbach, 1994). Figure 8 depicts such a plot in which the GABA dose-response curve in the absence of *n*-octanol was compared with that in the presence of 100 and 300 μM *n*-octanol. The Hill coefficient was reduced from 1.4 to 0.99 and 0.95 in the presence of 100 and 300 μM *n*-octanol, respectively.

Lack of Effects of *n*-Octanol on Maximal GABA Response. Both the allosteric model and the partial agonist model make a prediction that at high GABA concentrations *n*-octanol would lose its potentiating action. This prediction proved to be the case as illustrated in Fig. 9. When the receptor was activated by 1000 μM GABA, coapplication of 100 to 1000 μM *n*-octanol with GABA did not affect the maximal GABA-activated current. GABA-induced currents in the presence of *n*-octanol relative to the control GABA-

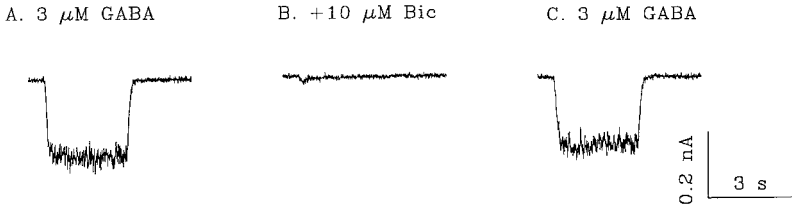


Fig. 4. Effects of bicuculline (Bic) on GABA- and *n*-octanol-induced currents. Bicuculline (10 μM) suppressed GABA-induced currents to 1.37 ± 0.45% (n = 6) of control when it was coapplied with 3 μM GABA (A and B) and inhibition was reversible after washing with bicuculline-free solution (C). Same concentration of bicuculline reduced current only to 80.0 ± 5.0% (n = 4) of control when coapplied with 300 μM GABA (D and E). Block was enhanced by bath application and coapplication of 10 μM bicuculline (F). Coapplication of 10 μM bicuculline with *n*-octanol reversibly suppressed *n*-octanol-induced current to 3.44 ± 2.0% (n = 5) of control (G, H, and I). Time interval between each application was 5 min.

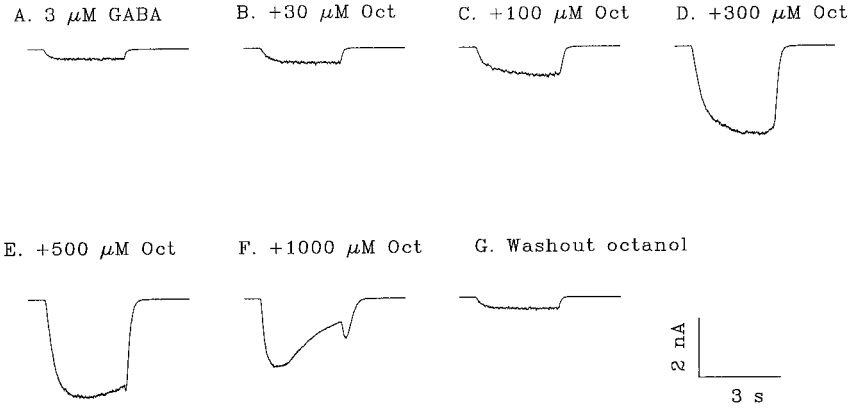


Fig. 5. *n*-Octanol reversibly potentiates GABA-induced currents in a dose-dependent manner. GABA was applied through the U-tube with or without *n*-octanol at 2-min intervals. B–F, *n*-octanol (30–1000 μM) was coapplied with 3 μM GABA for 3 s. Because there was pronounced decay in GABA-induced current in the presence of 1000 μM *n*-octanol, the maximum current was obtained by extrapolation to time zero (F). Potentiation and acceleration of decay phase were reversible upon washing out *n*-octanol (G).

current were 101 ± 4.0%, 105 ± 7.0%, 100 ± 4.0%, and 97 ± 4.0% in 100, 300, 500, and 1000 μM *n*-octanol, respectively.

Effects of Prolonged Application of *n*-Octanol on GABA-Activated Currents. Figure 10A shows that when 1000 μM *n*-octanol was applied to the bath for 1 to 5 min before a coapplication of 3 μM GABA and 1000 μM *n*-octanol, *n*-octanol had little potentiating effect on GABA-induced peak currents. On the average, after a 1-min pretreatment of 1000 μM *n*-octanol, the current in the presence of both *n*-octanol and 3 μM GABA was 1.26 ± 0.37-fold (n = 5) the current in the presence of 3 μM GABA alone.

When 300 μM GABA was used to activate the maximal GABA current, a 1-min bath perfusion of 1000 μM *n*-octanol before a coapplication of 300 μM GABA and 1000 μM *n*-octanol reduced the GABA current to 16% of the control current induced by 300 μM GABA alone (Fig. 10B). This result suggests that 84% of the receptors have undergone desensitization following a 1-min pretreatment of 1000 μM *n*-octanol, which are presumably not available for activation by GABA. The remaining 16% would still respond to *n*-octanol with 7.4-fold potentiating action (Fig. 6). The overall response to coapplication of 1000 μM *n*-octanol and 3 μM GABA would become 1.18-fold the current induced by 3 μM GABA alone.

Discussion

The present study showed that *n*-octanol exerted multiple actions on the α1β2γ2S $GABA_A$ receptor expressed in HEK293 cells. In the absence of GABA, *n*-octanol was capable of inducing inward currents, and in the presence of GABA, it exerted a dual action on GABA-induced currents depending on the GABA concentration and on how long the receptor was exposed to GABA or *n*-octanol. For a brief exposure when GABA and *n*-octanol were coapplied to the cell and when GABA concentrations were lower than its EC_{50} value, *n*-octanol caused a great increase in GABA-induced currents. The potentiation diminished with increasing concentration of GABA and eventually disappeared as the GABA concentration was well above EC_{50} value. When the receptor was exposed to *n*-octanol for a prolonged period of time, a coapplication of GABA and *n*-octanol resulted in reduction of GABA-induced currents.

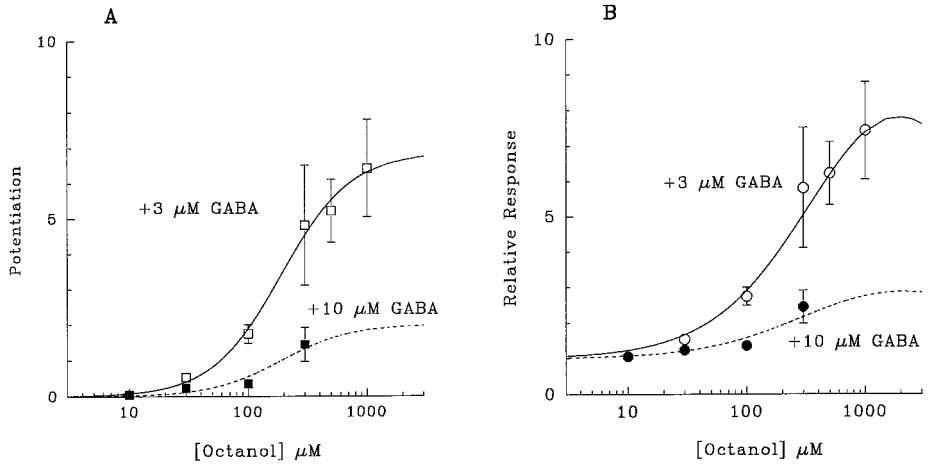


Fig. 6. Potentiation of GABA-induced currents by *n*-octanol and its simulation by two models. Potentiation by *n*-octanol was more pronounced in the presence of 3 μM GABA (open symbols) than in the presence of 10 μM GABA (filled symbols). Two models were used to fit data: an allosteric model (A) and a partial agonist model (B). In allosteric model, a logistic eq. 2 was used to fit data giving an EC_{50} of 191 ± 39 μM and an n_H of 1.49 ± 0.30 with A being 6.85 ± 0.06 and 2 ± 0.3 for 3 μM GABA and 10 μM GABA, respectively. Data for potentiation of currents induced by 3 μM and 10 μM GABA were also fitted by using partial agonist model (eq. 6): parameters used were determined from the dose-response relationship for GABA and *n*-octanol, i.e., a = 0.8, K_G = 22.2 μM, X_G = 4, b = 0.33, K_O = 2821 μM, and X_O = 0.15, and the parameter X_{GO} was assumed to be 12. Ordinate in B represents ratios of responses in the presence of GABA and n-octanol over control response, either in the presence of 3 μM GABA or 10 μM GABA alone. Ordinate in Fig. 6A is ratio determined in Fig. 6B − 1, representing degree of potentiation.

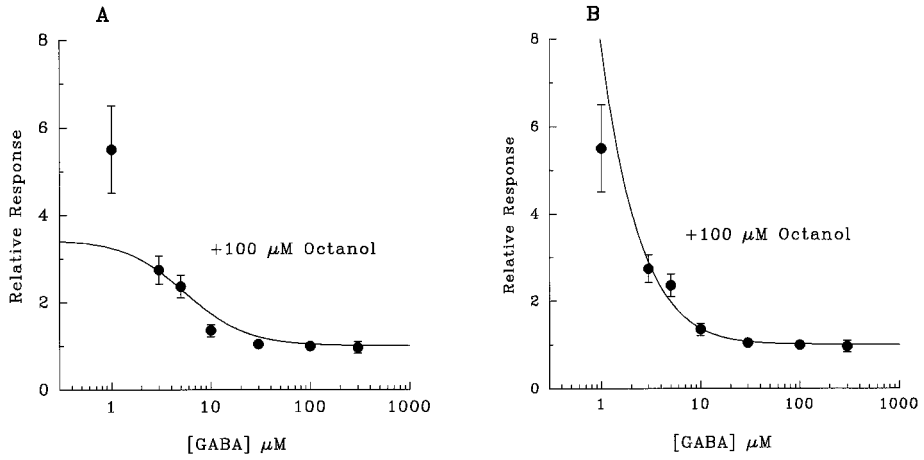


Fig. 7. Potentiation of GABA-induced currents by *n*-octanol depends on GABA concentration. Potentiation by 100 μM *n*-octanol was more pronounced at lower GABA concentrations than at higher concentrations. Two models were used to fit data: an allosteric model (A) and a partial agonist model (B). In allosteric model, 100 μM *n*-octanol reduced EC_{50} for GABA activation from 13 μM to 5.5 μM without altering n_H value and maximal response. Control parameters and those in the presence of 100 μM *n*-octanol were fitted into eq. 1. In partial agonist model, GABA-induced currents were fitted by eq. 6 with or without 100 μM *n*-octanol by using parameters used in Fig. 6. In both cases, solid line represents ratio of calculated responses in presence of *n*-octanol over control values without *n*-octanol.

Mechanisms of Action of *n*-Octanol in Modulation of GABA-Induced Currents

The discussion in the following two sections will focus on the question of whether the multiple actions of *n*-octanol on the $GABA_A$ receptors involve multiple sites of action, or whether the direct and potentiation actions can be explained by the partial agonistic action of *n*-octanol as has been previously shown for other receptors (Steinbach and Chen, 1995; Fletcher and Steinbach, 1996). The multiple sites for multiple actions assume that the binding of *n*-octanol to each site is responsible for each type of *n*-octanol action, whereas, in the partial agonist model, *n*-octanol binds to the site identical with the GABA site manifesting its multiple actions.

Multiple Sites of *n*-Octanol Action. According to the multiple-site model for *n*-octanol action, the affinities of *n*-octanol for its binding site on the $GABA_A$ receptor were estimated by fitting the logistic Hill equation to the dose-response relationship for each type of *n*-octanol action. The EC_{50} values were 1127 μM (Fig. 3A) and 191 μM (Fig. 6A), respectively, for its direct action and potentiating action.

The GABA concentration-dependent potentiation of GABA-induced peak currents by a fixed concentration of *n*-octanol (Fig. 7A) was not well simulated by reducing EC_{50} for GABA activation. The extent of potentiation at 1 μM GABA is much greater than that predicated by the allosteric model, but can be approximated by the partial agonist model (Fig. 7B).

Partial Agonist Model: One Binding Site Manifesting Multiple Actions. Several observations suggest that *n*-octanol acts at the GABA site to induce inward currents. *n*-Octanol-induced currents have a reversal potential similar to that of GABA-induced currents. Both currents are inhibited by bicuculline and picrotoxin. The degree of bicuculline inhibition of the current induced by 3 μM GABA was similar to that induced by 1000 μM *n*-octanol, suggesting that the $GABA_A$ receptors are bound by GABA and *n*-octanol to the similar extent at their respective concentrations. Taking these results together, it is concluded that *n*-octanol acts on the GABA binding site as a weak partial agonist. The kinetic scheme for GABA to activate the receptor depicted in Scheme 1 is applicable to *n*-octanol.

***n*-Octanol Potentiation of GABA-Induced Currents.** One might wonder how a weak partial agonist is able to exert the potentiating action on the receptor activated by a full agonist as seen in Fig. 6. The interpretation is rather straightforward according to the partial agonist model out-

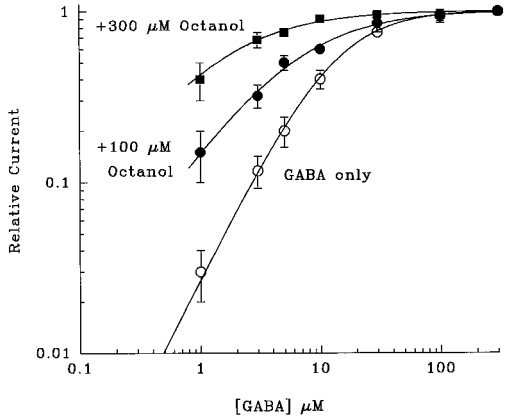


Fig. 8. *n*-Octanol reduces slope of GABA dose-response relationship. Dose-response relationship for GABA (○) was fitted to eq. 1 to give an EC_{50} of 13 μM and an n_H of 1.4. *n*-Octanol reduced EC_{50} to 5.9 μM and n_H to 0.99 when it was coapplied with GABA at 100 μM and reduced the EC_{50} to 1.4 μM and the n_H to 0.95 when coapplied with GABA at 300 μM.

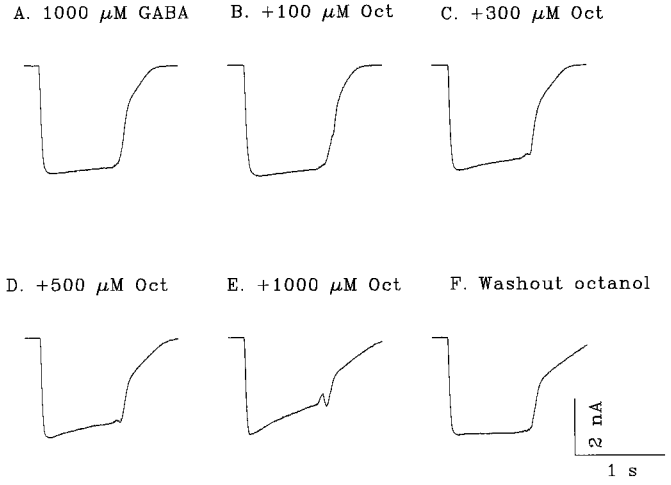


Fig. 9. Lack of effect of *n*-octanol on 1000 μM GABA-induced currents. GABA was applied through a U-tube with or without *n*-octanol at 5-min intervals. *n*-Octanol was coapplied with 1000 μM GABA for 1 s at concentrations of 100 to 1000 μM (B–E). There was pronounced decay in GABA-induced currents in the presence of 500 and 1000 μM *n*-octanol, and maximum current was obtained by extrapolation to time zero. Acceleration of decay phase was reversible upon washing out *n*-octanol (F).

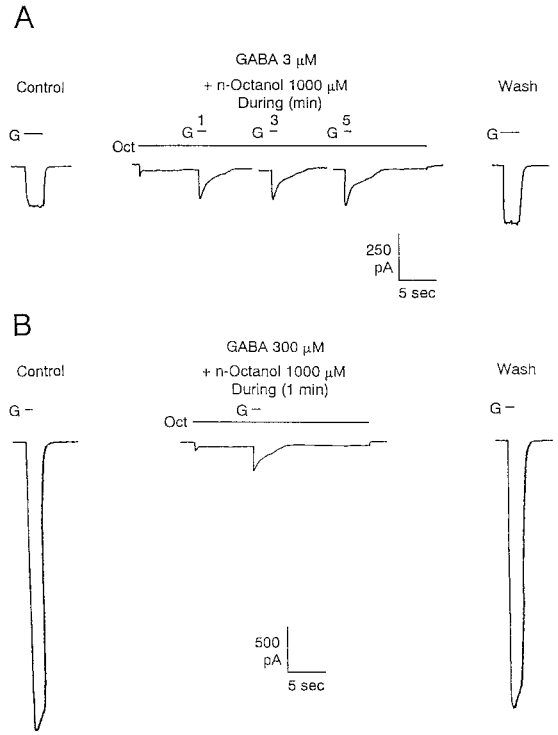


Fig. 10. Effects of prolonged application of *n*-octanol on GABA-induced currents. A, coapplication of 1000 μM *n*-octanol and 3 μM GABA to a cell has little or no potentiating effect on GABA-induced currents. GABA was coapplied with *n*-octanol 1, 3, and 5 min after beginning of bath application of 1000 μM *n*-octanol (horizontal line). B, coapplication of 1000 μM *n*-octanol and 300 μM GABA reduces GABA-induced current to 20% of control. Protocol was same as A, but with a higher GABA concentration (300 μM) and coapplication after 1 min of exposure to *n*-octanol. Time scale calibrations refer to periods of coapplication.

lined in Scheme 2. At low GABA concentrations, not many $GABA_A$ receptors are doubly bound by GABA, and with an increasing concentration of *n*-octanol, more receptors will be bound by one GABA and one *n*-octanol molecule. Because the mixed ligand-bound receptor has a high probability of opening, the whole-cell current becomes larger as the concentration of *n*-octanol is increased (Figs. 6B and 8). This may account for the observation that the partial agonist model is better than the allosteric model to account for the potentiating action of *n*-octanol on the response induced by 1 μM GABA as well (Fig. 7).

The potentiation disappeared at GABA concentrations greater than 100 μM. Because *n*-octanol has a weak affinity for GABA-binding site, the partial agonist model does not expect that the alcohol molecule can effectively displace high concentrations of GABA from the GABA binding sites (Fig. 7). When most of the receptors are bound by *n*-octanol, one would expect that the current starts to decline, because the receptor doubly bound by *n*-octanol is less likely to open, as simulated by the concentrations higher than 2000 μM. Unfortunately, we could not test this prediction, because this concentration is higher than its maximal solubility in aqueous solution. However, other partial agonists on the nicotinic receptors have been shown to exhibit such biphasic potentiation (Cachelin and Rust, 1994; Steinbach and Chen, 1995).

Thus, the response in the presence of low GABA concentrations is enhanced and the response in the presence of high GABA concentrations is not altered. The slope of dose-response relationship is reduced reflecting a decrease in Hill coefficient in the presence of *n*-octanol as seen in Fig. 8.

The Hill coefficient for potentiation higher than 1 suggests that there is positive cooperativity in the octanol binding, which could occur in the receptor bound by one GABA and one octanol, because the potentiation experiment was carried out at low GABA concentrations.

Inhibition of GABA-Induced Currents by Alcohol Pretreatment. Consistent with the previous report by Arakawa et al. (1992), high concentrations of *n*-octanol (300–1000 μM) preperfused before GABA application inhibited peak currents induced by high concentrations of GABA. This implies that the alcohol-induced current can undergo desensitization, which is consistent with the notion that alcohol acts as a partial agonist. After 1 min of application of 1000 μM *n*-octanol, about 84% of the $GABA_A$ receptors have undergone desensitization (Fig. 10B). The remaining 16% of receptors can still respond to the potentiating action of *n*-octanol, with a 7-fold increase in GABA-induced currents when GABA was applied at 3 μM. The overall amplitude of GABA-induced current under this condition would be only slightly greater than the current induced by 3 μM GABA alone when all receptors are available.

Comparison with Previous Studies. Many agents are known to exert multiple actions on transmitter-gated receptors. For examples, barbiturates (Schulz and Macdonald, 1981; Parker et al., 1986; Akaike et al., 1987, 1990; Amin and Weiss, 1993), propofol (Hales and Lambert, 1991; Hara et al., 1993, 1994; Orser et al., 1994; Jones et al., 1995; Sanna et al., 1995), and halothane (Yang et al., 1992; Sincoff et al., 1996) have been found to have the direct action on the $GABA_A$ receptor in the absence of GABA. Some of them exert a biphasic potentiating action in the presence of GABA at low concentrations relative to its EC_{50} value (Parker et al., 1986; Akaike et al., 1990) and inhibit the sustained residual current following exposure to a high desensitizing concentration of GABA (Nakahiro et al., 1989; Hall et al., 1994).

Recent studies have suggested that GABA and pentobarbital act at nearby but nonidentical sites (Uchida et al., 1996) or at different sites (Amin and Weiss, 1993; Ueno et al., 1997). A model has yet to be developed to account for the direct action, potentiating action, and inhibitory action of general anesthetics. Because the three actions cannot easily be separated from each other, the EC_{50} value of a drug estimated from the dose-response relationship for its apparent action would not reflect the affinity of the drug for each site of action.

This study represents the first model that provides a plausible illustrative explanation of the multiple actions of anesthetics on the $GABA_A$ receptors. *n*-Octanol acts as a partial agonist manifesting multiple actions on the $GABA_A$ receptor just like *d*-tubocurarine does on the fetal nicotinic acetylcholine receptors (Cachelin and Rust, 1994; Steinbach and Chen, 1995; Fletcher and Steinbach, 1996).

Acknowledgments

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Send reprint requests to: Dr. Toshio Narahashi Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, 303 East Chicago Ave., Chicago, IL. E-mail: tna597@anima.nums.nwu.edu