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REVIEW ARTICLE

Application of the Co-Agonist Concerted Transition Model to Analysis of $GABA_A$ Receptor Properties

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Abstract: The co-agonist concerted transition model is a simple and practical solution to analyze various aspects of $GABA_A$ receptor function. Several model-based predictions have been verified experimentally in previous reports. We review here the practical implications of the model and demonstrate how it enables simplification of the experimental procedure and data analysis to characterize the effects of mutations or properties of novel ligands. Specifically, we show that the value of EC_{50} and the magnitude of current response are directly affected by basal activity, and that co-application of a background agonist acting at a distinct site or use of a gain-of-function mutation can be employed to enable studies of weak activators or mutated receptors with impaired gating. We also show that the ability of one GABAergic agent to potentiate the activity elicited by another is a computable value that depends on the level of constitutive activity of the ion channel and the ability of each agonist to directly activate the receptor. Significantly, the model accurately accounts for situations where the paired agonists interact with the same site compared to distinct sites on the receptor.

Keywords: $GABA_A$ receptor, ion channel, agonist, potentiator, activation, potentiation, modulation, model.

1. INTRODUCTION

The γ-aminobutyric acid type A ($GABA_A$) receptor is a member of the Cys-loop family of transmitter-gated ion channels and the major ionotropic inhibitory ion channel in the central nervous system. $GABA_A$ receptors are membrane proteins consisting of five highly homologous subunits arranged around the central pore [1]. Receptor activation leads to opening of the gate of an anion-selective pore resulting in increased conductance for chloride ions and leading, in mature neurons, to hyperpolarization or dampening of the effects of excitatory ion channels.

The $GABA_A$ receptor is a key target of anesthetic, sedative and anxiolytic drugs, such as benzodiazepines, propofol and etomidate [2]. Furthermore, many endogenous and synthetic steroids modulate the $GABA_A$ receptor [3]. These compounds interact with unique binding sites located in the extracellular (benzodiazepines) or transmembrane regions (intravenous anesthetics, neurosteroids) of the receptor (Fig. **1A-B**). The interplay between the transmitter, endogenous neuroactive steroids, and exogenous and clinical GABAergic agents determines the functional output of the $GABA_A$ receptor system and associated behavior.

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The co-agonist concerted transition model (the "co-agonist model") is based on the Monod-Wyman-Changeux model first introduced in the seminal paper by Monod, Wyman and Changeux [4] as a way to study the activity of multimeric regulatory proteins. A subsequent work introduced the model to analyze transmitter-gated ion channels [5, 6]. The co-agonist model has been used to analyze the behavior of mutant $GABA_A$ receptors [7-9] and the mechanisms of activation and potentiation of the $GABA_A$ receptor by several clinically relevant agents [10-13].

The co-agonist model (Fig. **1C**) is a cyclic model with N identical sites for a ligand. The sites have different affinities for the ligand when the receptor is in the closed (C) or open (O) states. When the receptor switches from one state to another, the properties of all sites change, *i.e.*, there cannot be a mixture of sites with differing affinities for the ligand on the same receptor. Receptor activation in the presence of agonist X is described by the state function:

$$P_{open} = \frac{1}{1 + L \times \left[\frac{1 + [X]/K_{C,X}}{1 + [X]/(K_{C,X} c_X)} \right]^{N_X}} \quad \text{Eq. 1}$$

where P_{open} is the open probability of the peak response, [X] is the concentration of agonist X, $K_{C,X}$ is the equilibrium dissociation constant for X in the closed receptor, c_X is the

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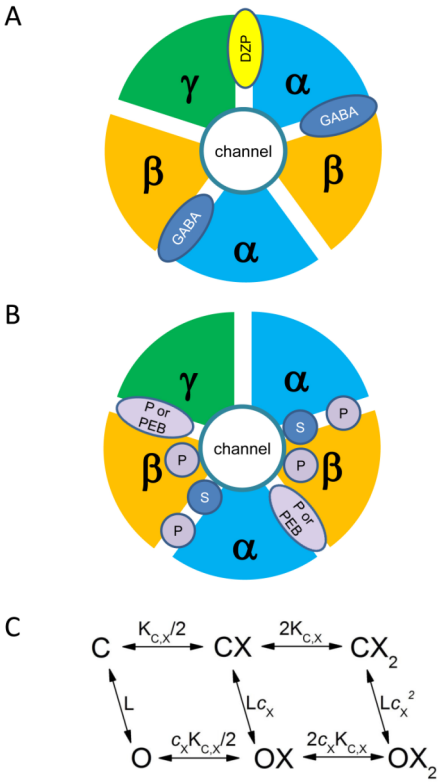

Fig. (1). Schematics of $GABA_A$ receptor structure and the state diagram of the concerted transition activation model. (**A**) Cross-section of the extracellular domain of the receptor showing interfacial locations of the binding sites for the transmitter GABA at the β-α interfaces and the binding site for the benzodiazepine diazepam (DZP) at the α-γ interface. (**B**) Cross-section of the transmembrane region of the receptor showing the binding sites for neurosteroids (S) at the β-α interfaces, six putative binding sites for propofol (P) at the β-α, γ-β and α-β interfaces and within the β subunits, and two sites for the barbiturate pentobarbital (PEB) at the γ-β and α-β interfaces. Photoaffinity labeling and functional data indicate that the sites for barbiturates overlap with the sites for propofol at the γ-β and α-β interfaces [29, 31, 34]. (**C**) Activation of the receptor by a single agonist, X, with two sites on the receptor. The agonist binds to a site on the inactive or closed receptor (C) with the dissociation constant of $K_{C,X}$, and on the active or open receptor (O) with the dissociation constant of $c_X K_{C,X}$. Analogous models for agonists with greater numbers of agonist binding sites would have N_X+1 horizontal layers. In the absence of X, the equilibrium between the C and O states is determined by L=C/O. Coapplication of a second agonist that interacts with distinct sites on the receptor shifts the equilibrium between the C and O states and so could be viewed as modifying the value of L.

ratio of the equilibrium dissociation constant for X in the open receptor to K_X, and N_X is the number of binding sites for X. The parameter L describes the extent of unliganded activity and equals to [C]/[O] in Fig. **1C**. It is to note that the use of the co-agonist model requires a conversion of the raw current amplitudes to units of open probability (P_{open}) [14, 15].

Recent efforts to analyze $GABA_A$ receptor properties employing the co-agonist model have focused on receptor activation by combinations of agonists. One goal of these studies is to enable analysis and prediction of native $GABA_A$ receptor function when the receptors are exposed to a mixture of various endogenous and exogenous active compounds. Despite its simplicity, the model capably accounts for many aspects of drug interactions and how they relate to inherent receptor properties. In this review, we concentrate on the practical implications of the two-state co-agonist concerted transition model. The theoretical underpinnings of the model, and extensions of the two-state model to include the pre-active high-affinity closed (*e.g.*, [16, 17]) or post-open desensitized states have been discussed in recent publications [18-20].

2. ACTIVATION OF THE RECEPTOR BY WEAK AGONISTS IN THE PRESENCE OF A BACKGROUND ACTIVATOR

Studies on direct activation of the synaptic-type α1β2γ2 $GABA_A$ receptor by weak agonists such as neuroactive steroids or benzodiazepines are technically difficult because of small current amplitudes. Even at micromolar concentrations, the steroid alfaxalone and benzodiazepine diazepam elicit responses with a channel open probability of <0.01 [13, 21]. Both drugs, however, potently and efficaciously potentiate responses to GABA. The maximal open probability, $P_{open,max}$, for a given agonist can be calculated as:

$$P_{open,max} = \frac{1}{1+L\left(\frac{K_{O,X}}{K_{C,X}}\right)^{N_X}} = \frac{1}{1+Lc_X{}^{N_X}} \qquad \text{Eq. 2}$$

where $K_{O,X}$ is the equilibrium dissociation constant for agonist X in the open receptor, and $K_{C,X}$, L and N are as described above. The lack of significant direct activation by steroids or benzodiazepines is explained, in the co-agonist model framework, by the small difference in the binding affinities of the compound for the open ($K_{O,X}$) and closed ($K_{C,X}$) receptors.

One potential approach to enable concentration-response studies of weak agonists, including steroids and benzodiazepines, is coapplication of the agonist of interest with a second, background activator that interacts with the receptor through different binding sites. The effect of the background activator is to reduce the value of L, that results in an increase in $P_{open,max}$ (Eq. 2). The modified value of L can be determined from the experimental data as:

$$L_{modified} = (1-P_{open,background})/P_{open,background} \qquad \text{Eq. 3}$$

where $P_{open,background}$ describes the level of activity due to the direct activating effect of the background activator.

In Fig. **2**, we show sample current traces from oocytes injected with cRNA for the wild-type concatemeric α1β2γ2L $GABA_A$ receptor, then exposed to the steroid allopregnanolone or the benzodiazepine diazepam, in the absence and presence of a low concentration of GABA. In the absence of GABA, neither allopregnanolone nor diazepam elicits a measurable response whereas both drugs robustly enhance responses to GABA.

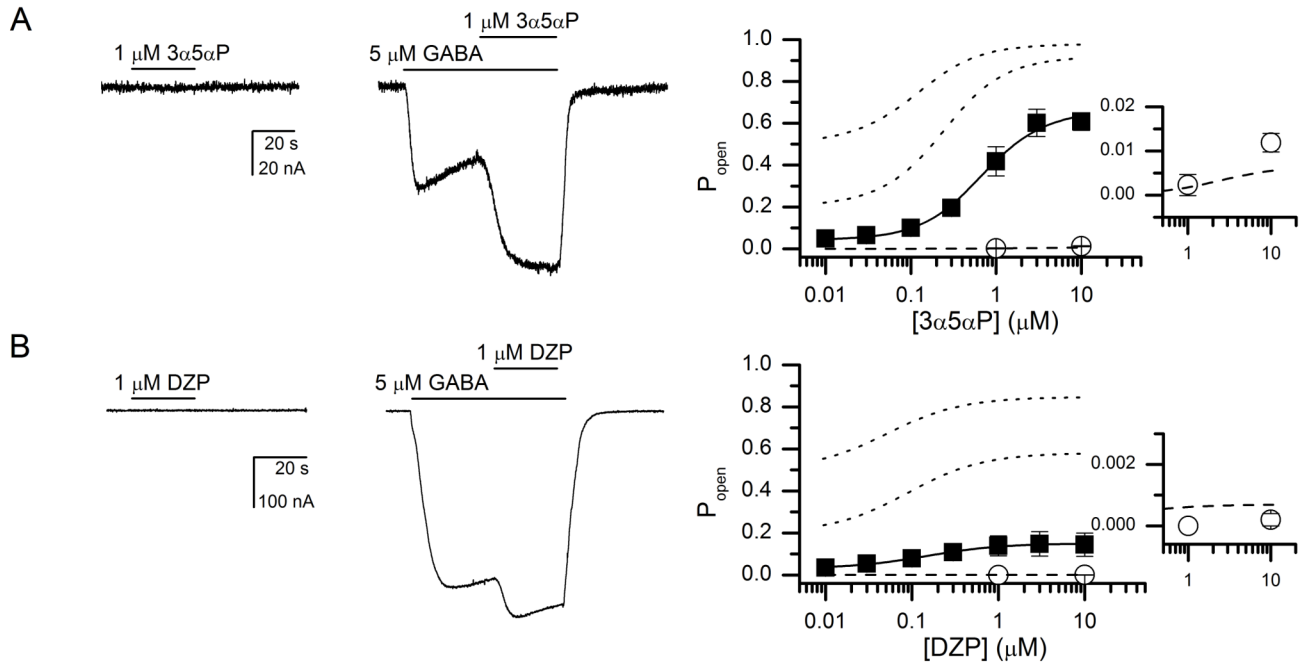

Fig. (2). Activation of the wild-type GABA$_A$ receptor by weak agonists in the presence of a background activator. (**A**) The steroid allopregnanolone (3α5αP) is minimally effective when applied alone (left trace) but potentiates the response to GABA (right trace). The graph shows concentration-response data for receptor activation by allopregnanolone in the absence (direct activation; circles) and presence of a low concentration of GABA (potentiation; squares). The data points show mean ± S.D. from 4-5 cells. The curve for potentiation (solid line) shows a fit to Eq. 1 with the best fit parameters of L = 23.6 ± 8.5, $K_{C,3\alpha5\alpha P}$ = 0.88 ± 0.19 μM and $c_{3\alpha5\alpha P}$ = 0.145 ± 0.025. The number of steroid binding sites was constrained to 2. The dotted lines show simulated concentration-response curves at background open probability of 0.2 or 0.5. The curve for direct activation (dashed line) shows the result of simulation using an L of 8000 [9], and $K_{C,3\alpha5\alpha P}$ of 0.88 μM and $c_{3\alpha5\alpha P}$ of 0.145. The inset shows the direct activation data at higher resolution. (**B**) The benzodiazepine diazepam (DZP) is minimally effective when applied alone (left trace) but potentiates the response to GABA (right trace). The graph shows concentration-response data for receptor activation by diazepam in the absence (circles) and presence of a low concentration of GABA (squares). The data points show mean ± S.D. from 4-5 cells. The potentiation data are from a previous study that yielded a $K_{C,DZP}$ of 0.17 μM and a c_{DZP} of 0.18 [21]. The dotted lines show simulated concentration-response curves at background open probability of 0.2 or 0.5. The curve for direct activation (dashed line) was simulated using an L of 8000, $K_{C,DZP}$ of 0.17 μM, c_{DZP} of 0.18, and N_{DZP} of 1. The inset shows the direct activation data at higher resolution. For both allopregnanolone and diazepam, there is good accordance between the simulated direct activation curves and the observed data at 1 and 10 μM of an agonist. The experiments were conducted on wild-type β2α1γ2L+β2α1 concatemeric GABA$_A$ receptors expressed in *Xenopus* oocytes. Generation and properties of the concatemeric receptor have been described previously [35, 36]. Currents were recorded using standard two-electrode voltage clamp. Data acquisition and analysis have been described in detail in previous reports [9, 14].

Analysis of the currents obtained in the presence of GABA yielded a K_C of 0.88 μM and a c (K_O/K_C) of 0.145 for allopregnanolone and a K_C of 0.17 μM and a c of 0.18 for diazepam (further details are provided in the figure legend). These values, combined with L estimated in the absence of the background activator (GABA), were then used to simulate the predicted concentration-response curves for direct activation by allopregnanolone and diazepam. The simulated curves are in good agreement with the observed data at 1 and 10 μM of an agonist (inset in Fig. **2**).

The open probability of the response to background activator + allopregnanolone (or diazepam) depends on the open probability of the response to the background activator applied alone. A larger response to the background activator, *i.e.*, lower L, results in a larger response to the combination of background activator and the primary agonist (dotted lines in Fig. **2**). The nature of the background activator is not critical, as long as it and the primary agonist bind to distinct sites, *i.e.*, do not compete for a shared site. This was indirectly demonstrated in a previous study [22] that found that diazepam similarly potentiates GABA$_A$ receptors activated by GABA, pentobarbital, etomidate, or alfaxalone.

The inherent assumption in the co-agonist model is that there is no interaction or direct effect of binding of one agonist on the binding of the other agonist. Specifically for the examples shown in Fig. **2**, the model assumes that the binding of GABA does not modify receptor interaction with allopregnanolone or diazepam other than indirectly through an effect on L.

3. ACTIVATION OF A LOSS-OF-FUNCTION MUTANT RECEPTOR IN THE PRESENCE OF A BACKGROUND AGONIST

Studies on receptors containing loss-of-function mutations can be conducted analogously in the presence of a background agonist. The Y205S mutation in the β subunit

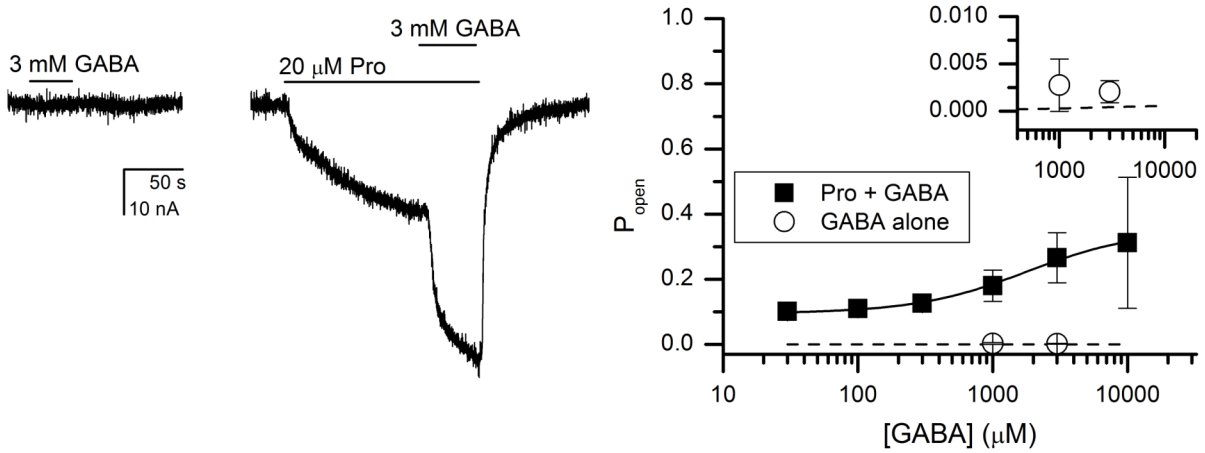

Fig. (3). Activation of the β(Y205S) mutant $GABA_A$ receptor by GABA in the presence of a background activator. The transmitter GABA is minimally effective on the β2(Y205S) mutant receptor when applied alone (left trace) but potentiates the response to propofol (Pro; right trace). The graph shows the concentration-response data for receptor activation by GABA in the absence (direct activation; circles) or presence of 20 μM propofol (potentiation; squares). The data points show mean ± S.D. from 4-6 cells. The curve for potentiation (solid line) shows a fit to Eq. 1 with the best fit parameters of L = 9.5 ± 0.6, and $K_{C,GABA}$ = 1.7 ± 0.3 mM and c_{GABA} = 0.44 ± 0.01 for GABA. The number of GABA binding sites was constrained to 2. The curve for direct activation (dashed line) shows the result of simulation using an L of 8000 [9], and $K_{C,GABA}$ of 1.7 mM and c_{GABA} of 0.44. The inset shows the direct activation data at higher resolution. The experiments were conducted on β2(Y205S)α1γ2+β2(Y205S)α1 receptors expressed in *Xenopus* oocytes. Recording of currents and data analysis were done as described in previous reports [9, 14].

renders the transmitter binding site ineffective and $GABA_A$ receptors containing the β2(Y205S) mutation do not respond to up to 20 mM GABA. The effect is specific to activation by orthosteric agonists as activation by the allosteric agonist pentobarbital is not affected [23].

To determine the mechanistic basis of how the β2(Y205S) mutation affects receptor activation by the transmitter, we analyzed GABA-elicited activity in the presence of a low concentration of an allosteric agonist propofol. The application of 20 μM propofol generated a response with a P_{open} of ~0.1. Co-application of GABA potentiated the response to propofol (Fig. **3**). Analysis of the concentration-response data yielded a K_C of 1.7 mM and a *c* of 0.44 for GABA in the mutant receptor. Compared to the wild-type concatemeric receptor [9], the mutant receptor exhibits a ~24-fold lower affinity to the transmitter in the closed configuration. The binding of two GABA molecules contributes 6.7 kcal/mol of free energy to stabilize the open state in the wild-type receptor but only 1.0 kcal/mol in the mutant. The $K_{C,GABA}$ and c_{GABA} values determined in the presence of background propofol could be used to simulate the direct activation curve (dashed line in Fig. **3**) and to calculate the $P_{open,max}$ for GABA ($6.5x10^{-4}$) in the β2(Y205S) receptor in the absence of a background activator using Eq. 2.

4. ACTIVATION OF GAIN-OF-FUNCTION MUTANT RECEPTORS

Introduction of a gain-of-function mutation whose sole effect is to modify the equilibrium between unliganded closed and open receptors, in principle, functionally mimics the presence of a background agonist. For example, substitution of the leucine residue at the 9' position in the second transmembrane domain (TM2) with a small hydrophilic residue, *e.g.*, serine or threonine, generates a receptor with elevated constitutive open probability [7] and increased sensitivity to GABA [24], propofol [25], and benzodiazepines [10]. As with exposure to a background agonist, introduction of a gain-of-function mutation can enable studies of weak agonists. The disadvantage of using a mutation for this purpose is that the P_{open} of background activity is limited to a single value imposed by the mutation. It also needs to be determined that the mutation employed is without a direct effect on activation by the agonist of interest.

To illustrate this, we compared the effects of the α1(L263S) (L9'S in TM2 of the α1 subunit) and β2(Y97C) (Loop A at the transmitter binding site) mutations. Both mutations enhance constitutive open probability and could therefore conceivably be used in studies of weak agonists. However, the agreement between the concentration-response data in the presence of GABA and the concentration-response curves simulated assuming that the mutations solely affect L is quite different for the two mutants. For the receptor containing the α1(L263S) mutation, there is good agreement between the experimental data points and the curve simulated using the value of L estimated for the mutant receptor (7.3; [25]) and the $K_{C,GABA}$ (72 μM) and c_{GABA} (0.0033) estimated for the wild-type receptor [9] (Fig. **4**). This finding supports the notion that the α1(L263S) mutation modifies the equilibrium between the unliganded closed and open states without affecting receptor affinity to GABA [7, 9].

In contrast, when the GABA concentration-response curve is simulated in the same fashion for the receptor containing the β2(Y97C) mutation, the predicted curve is shifted by several orders of magnitude to lower concentrations of the transmitter (Fig. **4**). Analysis of the activation data from the mutant receptor using Eq. 1 indicate a 10-fold higher $K_{C,GABA}$ (353 μM vs. 35 μM in the wild-type α1β2γ2L recep-

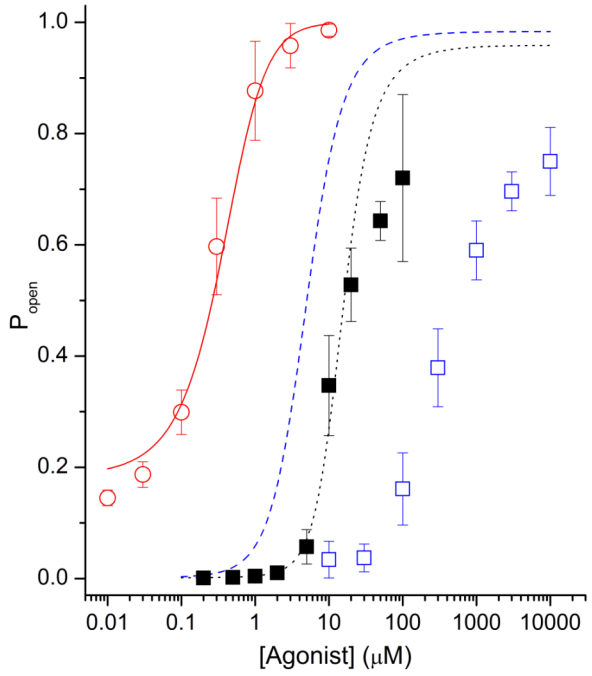

Fig. (4). Effects of mutations that modify unliganded gating on receptor activation by GABA or propofol. The plot shows the concentration-response relationships for β2α1(L263S)γ2+β2α1(L263S) receptors activated by GABA (circles), and α1β2(Y97C)γ2 receptors activated by GABA (open squares) or propofol (filled squares). The symbols show mean ± S.D. from 4-6 cells. The lines show the results of simulations using Eq. 1 and assuming that the mutations modify only constitutive activity with no effect on affinity (K_C) or efficacy (c) for the respective agonist: solid line, gating by GABA of β2α1(L263S)γ2+β2α1(L263S); dashed line, gating by GABA of α1β2(Y97C)γ2 receptors; and dotted line, gating by propofol of α1β2(Y97C)γ2 receptors. The $K_{C,GABA}$ (72 μM) and c_{GABA} values (0.0033) for the wild-type concatemeric receptor are from [9]. For the α1β2γ2 receptor, the $K_{C,GABA}$ (35 μM) and c_{GABA} (0.0045) values, and $K_{C,Propofol}$ (19 μM) and $c_{Propofol}$ (0.139) values are from [26]. The agreement between the circles and solid line indicates that the α(L263S) mutation modifies receptor activation by GABA predominantly through an effect on the unliganded closed-open equilibrium. The β2(Y97C) mutation increases constitutive activity and decreases affinity and efficacy for GABA but has a relatively small effect on affinity and efficacy for propofol. Accordingly, the imposed change in L without changes in K_C or c reasonably accounts for the mutant receptor activation for propofol (filled squares and dotted line) but not by GABA (open squares and dashed line). The receptors were expressed in *Xenopus* oocytes. Recording of currents and data analysis were done as described in previous reports [9, 14].

tor; [26]) and moderately reduced gating energy provided by the binding of two GABA molecules (4.6 kcal/mol vs. 6.4 kcal/mol in the wild-type; [26]). We infer that the mutation, besides modifying the unliganded closed-open equilibrium (L), additionally modifies the activation properties by GABA. This is in agreement with prior structural and functional reports for this receptor [27, 28].

As control, we tested activation of the α1β2(Y97C)γ2 receptor by propofol. We reasoned that the mutation in the transmitter binding site is likely to have minimal effect on propofol actions and any modification of the propofol activation curve by the mutation reflects its effect on the unliganded closed-open equilibrium. Indeed, the curve predicted using L of the mutant and the $K_{C,Propofol}$ and $c_{Propofol}$ estimated for the wild-type receptor [26] reasonably agrees with the observed data (Fig. **4**). We infer that receptors containing the β2(Y97C) mutation may be used to study agonists acting at the propofol binding site but not those acting at the transmitter site.

5. RECEPTOR ACTIVATION BY AGONIST COMBINATIONS

The combination of two agonist species results in an augmented response. When the paired agonists interact with distinct sites the combined effect is described with Eq. 1 taking into consideration the modified L (Eq. 3) that describes direct activation by the potentiator. The designation of which agonist is primary and which is secondary or the potentiator, is arbitrary and has no effect on the results of analysis.

One implication of potentiation in the co-agonist model is that different potentiators applied at concentrations which produce the same direct-activating response also potentiate the response to the primary agonist by the same degree. This can be easily understood when one considers that the effect of a potentiator is to reduce L. Agents that reduce the value of L by the same degree enhance the response to the primary agonist by the same degree. The reverse is also true. Different potentiators which enhance the response to the primary agonist by the same degree also elicit a direct-activating response of the same amplitude.

This is illustrated in Fig. **5A-B**, where the potentiating effects of propofol and pentobarbital on GABA-activated receptors are compared. The concentrations of propofol and pentobarbital were selected to generate responses with approximately equal amplitudes. As expected, the potentiated responses also have similar amplitudes.

The extent of potentiation upon receptor exposure to an agonist combination depends on whether the paired agonists interact with the same or distinct sites. As pointed out above, when the agonists interact with distinct sites, the response amplitude is described by Eqs. 1 and 3. On the other hand, when the agonists interact with the same sites, for example when two orthosteric agonists are combined, the state function is described as follows:

$$P_{open} = \frac{1}{1+L\times\left[\frac{1+[X]/K_{C,X}+[Y]/K_{C,Y}}{1+[X]/(K_{C,X}c_X)+[Y]/(K_{C,Y}c_Y)}\right]^N} \quad \text{Eq. 4}$$

where X and Y are the two agonists, N is the number of shared binding sites, $K_{C,X}$ and $K_{C,Y}$ are the equilibrium dissociation constants for X and Y in the closed receptor, and c_X and c_Y are the ratios of the equilibrium dissociation constants for X and Y in the open receptor to $K_{C,X}$ and $K_{C,Y}$, respectively. L is as described above.

When the agonists compete for the same sites, the effect of coapplication is predominantly dependent on the relative

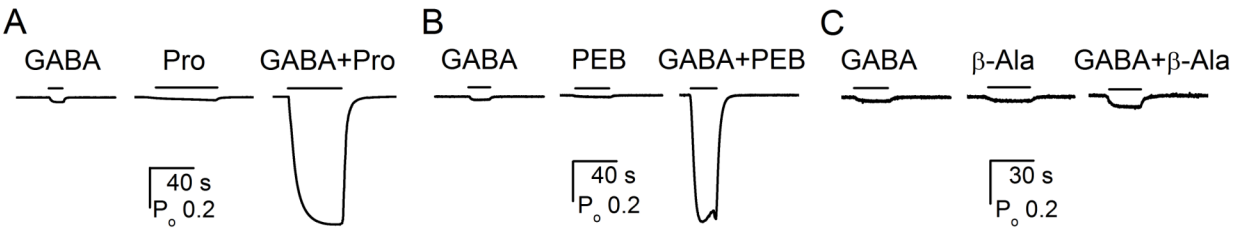

Fig. (5). Comparison of potentiation of GABA-activated receptors by combinations of allosteric and orthosteric agonists. The receptors were activated by GABA, propofol (Pro), or GABA + propofol (**A**), GABA, pentobarbital (PEB), or GABA + pentobarbital (**B**), and GABA, β-alanine (β-Ala), or GABA + β-alanine (**C**). The concentrations of the agonists were: 1.5 μM GABA, 15 μM propofol in A, 5 μM GABA, 400 μM pentobarbital in B, and 1 μM GABA, 200 μM β-alanine in C. The amplitudes of the current traces are given in units of open probability to demonstrate lack of saturation in potentiated responses. The data indicate that a potentiating effect resulting from coapplication of two agonists is greater when the paired agonists interact with distinct sites (as in A and B) than when the agonists interact with the same sites (C). The data also show that the amplitudes of responses to GABA + propofol and GABA + pentobarbital are similar when the response amplitudes to each agonist applied separately are similar. The experiments were conducted on wild-type β2α1γ2L+β2α1 concatemeric $GABA_A$ receptors expressed in *Xenopus* oocytes. The currents were recorded using standard two-electrode voltage clamp as described previously [9, 25].

efficacies and concentrations of the paired compounds. Coapplication of high concentration of a low-efficacy agonist is expected to reduce the response to the primary agonist because the low-efficacy agonist replaces the primary agonist at the binding site. In contrast, co-application of a high-efficacy agonist can result in potentiation due to concentration additivity.

Equations 1 and 4 predict qualitatively different response amplitudes when an agonist is combined with an activator binding to a distinct site vs. the same site. This can be seen when the response to the combination of two orthosteric agonists, GABA and β-alanine (Fig. **5C**), is compared to responses to GABA + propofol (Fig. **5A**) or GABA + pentobarbital (Fig. **5B**). Even though GABA and β-alanine both are high-efficacy agonists [29], the combination of the two results in relatively little potentiation. One practical implication of this finding is that the degree of potentiation resulting from combining agonists can be used to determine whether the paired agonists interact with the same or distinct sites. Using this approach, we have recently shown that 5α- and 5β-reduced steroids, and a natural steroid and its enantiomer interact with the same sites [29].

A case where one agonist interacts with some but not all of the sites available to the other can be considered an extension of these models. This mechanism predicts a mix of true potentiation and competition resulting in a potentiating effect that is intermediate between the cases described above. The exact contribution of the competitive component depends on the concentrations and efficacies of the paired agonists. The state function for this scenario is:

$$P_{open} = \frac{1}{1+L\times\left[\frac{1+[X]/K_{C,X,I}+[Y]/K_{C,Y,I}}{1+[X]/(K_{C,X,I}c_{X,I})+[Y]/(K_{C,Y,I}c_{Y,I})}\right]^{N_I}\left[\frac{1+[X]/K_{C,X,II}}{1+[X]/(K_{C,X,II}c_{X,II})}\right]^{N_{II}}} \quad \text{Eq. 5}$$

where agonist X can bind to both class I and class II sites but agonist Y binds only to class I sites. N_I describes the number of shared binding sites and N_{II} describes the number of sites to which only agonist X binds. Other terms are as described earlier. The case of partially shared binding sites may describe the actions of the combination of propofol and pentobarbital [29]. Biochemical studies have indicated that propofol and barbiturates bind with high affinity to overlapping sites at the α-β and γ-β interfaces but only propofol binds at the β-α interface with high affinity [30, 31].

6. MODIFICATION OF THE EC_{50} BY A BACKGROUND AGONIST

The effect of the background agonist on the concentration-response relationship of the primary agonist depends on whether the two compounds interact with the same or distinct binding sites. As shown in Fig. **6A**, coapplication of a background agonist (Agonist B) that binds to a distinct site shifts the concentration-response relationship for the primary agonist (Agonist A in Fig. **6**) to lower concentrations, and, depending on the efficacy of the primary agonist, can increase the maximal open probability of the receptor in the presence of A. The relationship between the level of background activity and the EC_{50} depends on multiple factors, including the number of binding sites for the primary agonist and the extent of background activity [9].

When agonists compete for a shared site, the effect of coapplication depends on the concentrations and efficacies of the paired agonists. Fig. **6B** compares the effects of coapplication of a high-efficacy or a low-efficacy agonist with the primary activator. The addition of the high-efficacy agonist (Agonist C) increases the level of background activity but does not modify the EC_{50} or the maximal response. Coapplication of the low-efficacy agonist (Agonist D) shifts

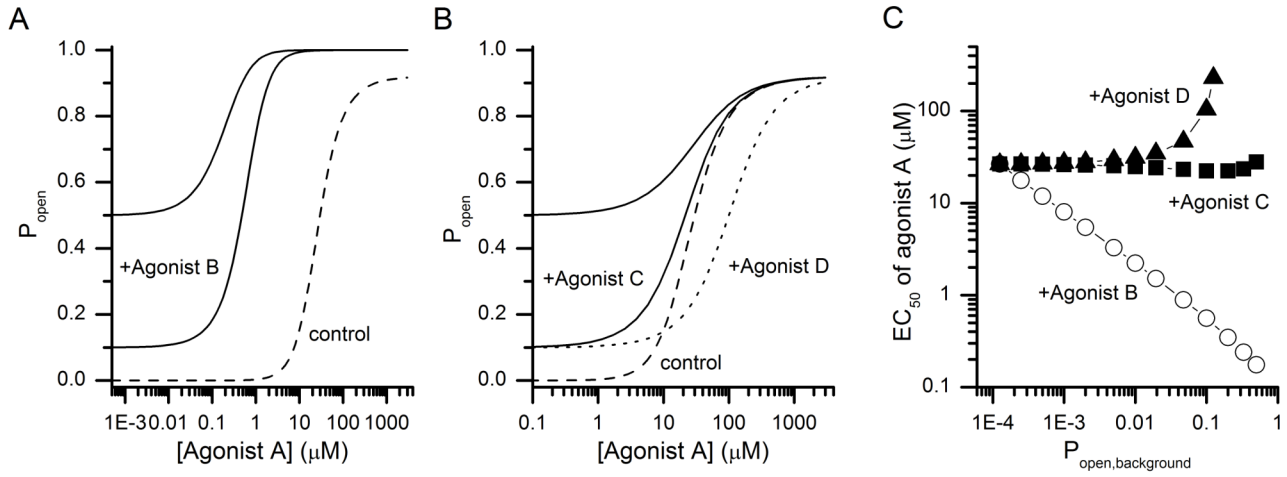

Fig. (6). Modification of concentration-response relationship in the presence of a secondary allosteric or orthosteric agonist. (**A**) The graph shows the effects of a secondary agonist that binds to sites distinct from the binding sites for Agonist A on the concentration-response relationship for Agonist A. Coapplication of the secondary Agonist B shifts the concentration-response curve for Agonist A to lower concentrations, and increases the maximal open probability. The dashed line, simulated using Eq. 1 at L = 8000, $K_{C,A}$ = 72 μM, c_A = 0.0033 and N_A = 2, indicates receptor activation by Agonist A in the absence of other activators (control). The solid lines show the results of simulations of activation by Agonist A in the presence of Agonist B at concentrations that elicit background activity with P_{open} of 0.1 or 0.5. In these simulations, the nature of the secondary agonist is not critical as long as it interacts with sites distinct from the binding sites for Agonist A. (**B**) The graph shows the effects of secondary agonists that bind to the same sites as Agonist A on the concentration-response relationship for Agonist A. The solid lines show the results of simulations of activation by Agonist A in the presence of 474 μM (P_{open}=0.1) or 1.7 mM (P_{open}=0.5) of the high-efficacy Agonist C with $K_{C,C}$ = 6.7 mM and c_C = 0.0023. The dotted line shows the results of simulations of activation by Agonist A in the presence of 151 μM of the low-efficacy Agonist D with $K_{C,D}$ = 38 μM and c_D = 0.027. The dashed line is reproduced from panel A and illustrates receptor activation by Agonist A in the absence of other activators (control). (**C**) The plot shows simulated EC_{50}s for Agonist A in the presence of Agonists B, C, or D. The concentrations of the secondary agonists were selected to generate background responses ranging from 0.000125 to 0.5 (0.000125 to 0.125 for the low-efficacy Agonist D). The K_C and *c* values for Agonists A, C, and D in panels A-C are given above and correspond to those determined for GABA [9], β-alanine [29], and piperidine-4-sulfonic acid [29], respectively.

the concentration-response curve for the primary agonist to higher concentrations but does not modify the maximal response. The summary of the effects of the three background agonists on the EC_{50} is given in Fig. **6C**. Exposure to an agonist with distinct binding sites (Agonist B) leads to a reduction in the EC_{50} for the primary agonist. Coapplication of a high-efficacy agonist (Agonist C) that shares the binding sites with the primary agonist has little effect on the EC_{50}. Finally, exposure to a low-efficacy agonist (Agonist D) that competes with the primary agonist increases the EC_{50}.

7. THE EFFECT OF BACKGROUND ACTIVITY ON POTENTIATION

The degree of potentiation upon coapplying two agonists, A and B, also depends on the level of background activity in the absence of either A or B. The background activity may be due to constitutive activity of the receptor, or originate from activation of the receptor by a background activator. At higher levels of background activity, the degree of potentiation resulting from combining A and B is reduced. On the other hand, as shown above [7, 9, 12], greater background activity is associated with more potent and efficacious direct activation by either A or B.

Mutant receptors with high constitutive activity show reduced potentiation by agents that efficaciously directly activate the receptor [32]. For example, Eq. 1 predicts that coapplication of 3.4 μM GABA and 11.6 μM propofol, that individually elicit a response with a P_{open} of 0.05 in the wild-type α1β2γ2L $GABA_A$ receptor (L=8000; [26]), generates a response with a P_{open} of 0.96 (~9-fold greater than the arithmetic sum of the individual responses). If the receptor contained a mutation (or a post-translational modification or a subunit switch) whose sole effect was to increase the constitutive open probability to 0.01 (L=99), only 0.2 μM GABA or 1.3 μM propofol would be needed to generate a response with a P_{open} of 0.05. However, the combination of the two agonists at these concentrations is predicted to generate a response with a P_{open} of 0.22 (a ~2-fold greater response than the sum of individual responses). The result is identical if a third activator, interacting with a unique site, is used to generate background activity with P_{open} of 0.01.

This finding may have physiological implications. Ambient (~0.5 μM) GABA elicits a response with P_{open} ~0.01 in synaptic-type α1β2γ2L receptors (*e.g.*, [33]). Accordingly, under physiological conditions, combinations of effective doses of allosteric agonists are expected to generate a relatively modest further increase in current amplitude even though the receptors are highly responsive to the individual agents in the presence of ambient GABA. An increase in the concentration of ambient GABA, for example by GABA uptake blockers, is predicted to enhance receptor sensitivity to a single allosteric agent but have a smaller effect on the ability of a combination of allosteric agents to potentiate the receptor.

CONCLUSION

The co-agonist concerted transition model has been instrumental in studies of activation and modulation of the $GABA_A$ receptor. Several model-based predictions have been individually verified in previous reports. We have reviewed here the practical implications of such analysis and how it enables simplification of the experimental procedure. Specifically, we have demonstrated that i) coapplication of a background agonist can be used to enable studies of weak agonists and loss-of-function mutants whereas introduction of a gain-of-function mutation functionally mimics the presence of a background agonist; ii) the degree of potentiation upon coapplication of two agonists depends on whether the paired agonists interact with the same binding sites; and iii) increased background activity is associated with increased direct activation but reduced potentiation. While the focus of this review was the synaptic-type α1β2γ2L $GABA_A$ receptor, the procedures are applicable to other subtypes of the $GABA_A$ receptor as well as other transmitter-gated ion channels.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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