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PERSPECTIVE

Perspective on the Relationship between $GABA_A$ Receptor Activity and the Apparent Potency of an Inhibitor

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ARTICLE HISTORY

Received: September 23, 2021
Revised: October 13, 2021
Accepted: October 13, 2021

DOI:
10.2174/1570159X19666211104142433

Abstract: ***Background*:** In electrophysiological experiments, inhibition of a receptor-channel, such as the $GABA_A$ receptor, is measured by co-applying an agonist producing a predefined control response with an inhibitor to calculate the fraction of the control response remaining in the presence of the inhibitor. The properties of the inhibitor are determined by fitting the inhibition concentration-response relationship to the Hill equation to estimate the midpoint (IC_{50}) of the inhibition curve.

***Objective*:** We sought to estimate sensitivity of the fitted IC_{50} to the level of activity of the control response.

***Methods*:** The inhibition concentration-response relationships were calculated for models with distinct mechanisms of inhibition. In Model I, the inhibitor acts allosterically to stabilize the resting state of the receptor. In Model II, the inhibitor competes with the agonist for a shared binding site. In Model III, the inhibitor stabilizes the desensitized state.

***Results*:** The simulations indicate that the fitted IC_{50} of the inhibition curve is sensitive to the degree of activity of the control response. In Models I and II, the IC_{50} of inhibition was increased as the probability of being in the active state (P_A) of the control response increased. In Model III, the IC_{50} of inhibition was reduced at higher P_A.

***Conclusion*:** We infer that the apparent potency of an inhibitor depends on the P_A of the control response. While the calculations were carried out using the activation and inhibition properties that are representative of the $GABA_A$ receptor, the principles and conclusions apply to a wide variety of receptor-channels.

Keywords: $GABA_A$ receptor, activation, inhibition, modeling, IC_{50}.

1. INTRODUCTION

Binding of the transmitter γ-aminobutyric acid (GABA) to the γ-aminobutyric acid type A receptor ($GABA_AR$) enhances activation of the receptor. As the concentration of GABA in the surrounding environment is increased, the probability of being in the active state (P_A) rises. In electrophysiological recordings, the increase in P_A manifests as higher whole-cell peak current. At saturating GABA concentrations, the peak P_A of the $GABA_A$ receptor varies between ~0.4 (α4β2δ; [1]) and ~0.9 (α1β2γ2; [2, 3]). The maximal P_A can vary considerably when the receptor is activated by other agonists. For example, the peak P_A of the α1β2γ2 $GABA_A$ receptor in the presence of a saturating concentration of piperidine-4-sulfonic acid is <0.2 [4]. The agonist concentration-response relationships are typically fitted to the Hill equation and characterized by estimating the midpoint (EC_{50}) and slope (n_{Hill}) of the curve.

In electrophysiological experiments, inhibition is described as fraction of the control response to agonist in the absence of an inhibitor. The concentration of agonist producing the control response is usually defined in terms of an "effective concentration" (EC) as the fraction of the maximal response elicited by a saturating concentration of the agonist. The properties of the inhibitor are presented in terms of a fitted Hill equation, described by IC_{50} (midpoint of the inhibition curve) and n_{Hill} of the inhibition curve. Comparison of the effects of different inhibitors, or the effects of mutations to the receptor on inhibition are then expressed through changes in the IC_{50} value [5-9]. Statistical approaches can be employed to determine if a change is statistically significant.

Here, we show that the fitted IC_{50} of an inhibitor is sensitive to the level of the control response. As a result, IC_{50}s measured at different activity levels cannot be meaningfully compared and statistical analysis is not appropriate. While EC and P_A values can be easily interconverted, we emphasize that any comparison of inhibition among subtypes of a receptor, including receptors with introduced mutations, needs to be conducted at a constant P_A rather than a constant EC value, because the latter may not equivalently correlate with P_A in different receptors.

We simulated the effects of an inhibitor employing three models with distinct mechanisms of inhibition. The models

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1570-159X/22 $65.00+.00

are based on the Monod-Wyman-Changeux allosteric model adapted to describe ion channel currents [10-13]. In the first model (Model I), the inhibitor binds to an allosteric site, *i.e.*, a site not involved in the action of the agonist. By having a higher affinity to the resting (R) than the active (A) state, the inhibitor stabilizes the R-state and reduces P_A. Model II represents competitive inhibition, where the inert inhibitor competes with the agonist for a shared binding site. Models I and II contain two states, R and A. Model III is a three-state model containing, besides the R- and A-states, a state corresponding to the desensitized (D) receptor. In this model, the inhibitor stabilizes the D-state. The models are illustrated in Fig. (**1**).

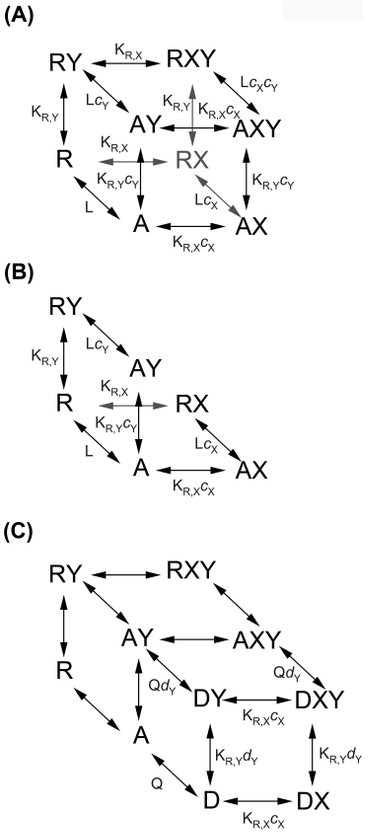

Fig. (1). The state diagrams of the activation/inhibition models. (**A**) Model I. In this model, the receptor is exposed to the agonist X and to the inhibitor Y. The two ligands bind to distinct sites. The receptor can be in a resting (R) or active (A) state. The equilibrium between the states is determined by the constants placed next to the arrows. L (=R/A) describes the equilibrium between the resting and active states. $K_{R,X}$ and $K_{R,Y}$ are the equilibrium dissociation constants for X and Y in the resting receptor. c_X and c_Y are the ratios of the equilibrium dissociation constants in the active and resting states. Y reduces occupancy of the A-state by having a higher affinity to the R-state. (**B**) Model II. In this Model, the agonist X and the inhibitor Y compete for the same set of sites. X has a higher affinity to the A-state thereby promoting activation while Y has identical affinities to the R- and A-states thereby acting as a competitive inhibitor of X. (**C**) Model III. In this model, the receptor can be in a resting, active, or desensitized (**D**) state. Q (=A/D) describes the equilibrium between the active and desensitized states. d_Y is the ratio of the equilibrium dissociation constants in the desensitized and active states. Other terms are as described above. For simplicity, a single binding step for X and Y is shown.

In all cases, the P_A of steady-state responses was calculated. For Model I, the P_A in the absence and presence of the inhibitor was calculated as follows [14]:

$$P_A = \frac{1}{1+L\left[\frac{1+[X]/K_{R,X}}{1+[X]/(K_{R,X}c_X)}\right]^{N_X}\left[\frac{1+[Y]/K_{R,Y}}{1+[Y]/(K_{R,Y}c_Y)}\right]^{N_Y}} \quad (1)$$

In this equation, X and Y stand for the agonist and inhibitor, respectively. $K_{R,i}$ is the equilibrium dissociation constant of drug i (X or Y) in the resting receptor, c_i is the ratio of the equilibrium dissociation constants in the active and resting states, and N_i is the number of binding sites. L (=R/A) expresses the level of activity in the absence of agonist or inhibitor.

For Model II, receptor activation was calculated as follows [15]:

$$P_A = \frac{1}{1+L\left[\frac{1+[X]/K_{R,X}+[Y]/K_{R,Y}}{1+[X]/(K_{R,X}c_X)+[Y]/(K_{R,Y}c_Y)}\right]^{N}} \quad (2)$$

where N is the number of shared binding sites for X and Y (constrained to 2). Other terms are as described above. Models I and II behave identically in the absence of inhibitor.

For Model III, the P_A was calculated as follows [16]:

$$P_A = \frac{1}{1+L\left[\frac{1+[X]/K_{R,X}}{1+[X]/(K_{R,X}c_X)}\right]^{N_X}+\frac{1}{Q}\left(\frac{1+[Y]/K_{R,Y}d_Y}{1+[Y]/K_{R,Y}}\right)^{N_Y}} \quad (3)$$

where Q (=A/D) is a measure of desensitization in the absence of active drugs. Q was constrained to 100 to minimize desensitization in the absence of an inhibitor. d_Y is the ratio of equilibrium dissociation constants of the inhibitor in the desensitized and active states. Other terms have been defined above. It is assumed in Model III that the agonist (X) does not desensitize and the inhibitor (Y) does not activate.

For all simulations, L = 8000, $K_{R,X}$ = 10 μM, c_X = 0.004 and N_X = 2. Initial calculations of inhibition were conducted at a control P_A of 0.5 (Fig. **2A**). The concentration of agonists producing a response with P_A of 0.5 was 5.5 μM in Models I and II. The presence of the term Q in eq. 3 (Model III) slightly affects the activation in the absence of Y (the concentration of X producing the steady-state P_A of 0.5 is 5.55 μM). The specific properties, *i.e.*, $K_{R,Y}$, c_Y, and d_Y of the inhibitor within a model were adjusted to generate curves with similar IC_{50}s (~5 μM). In Model I, the $K_{R,Y}$ was 5.8 μM, c_Y was 10, *i.e.*, the inhibitor had a ten-fold higher affinity to the resting than the active state, and N_Y was 2. In Model II, the $K_{R,Y}$ was 4.3 μM, and c_Y was 1. Model II simulates Y-mediated competitive inhibition of receptor activation by X. In Model III, $K_{R,Y}$ was 250 μM, d_Y was 1×10^{-4}, and N_Y was set to 1.

Next, we altered the concentration of the agonist to generate control responses with P_A ranging from 0.05 to 0.85, and calculated the effect of the inhibitor in the framework of each model. In Models I and II, the concentration of the

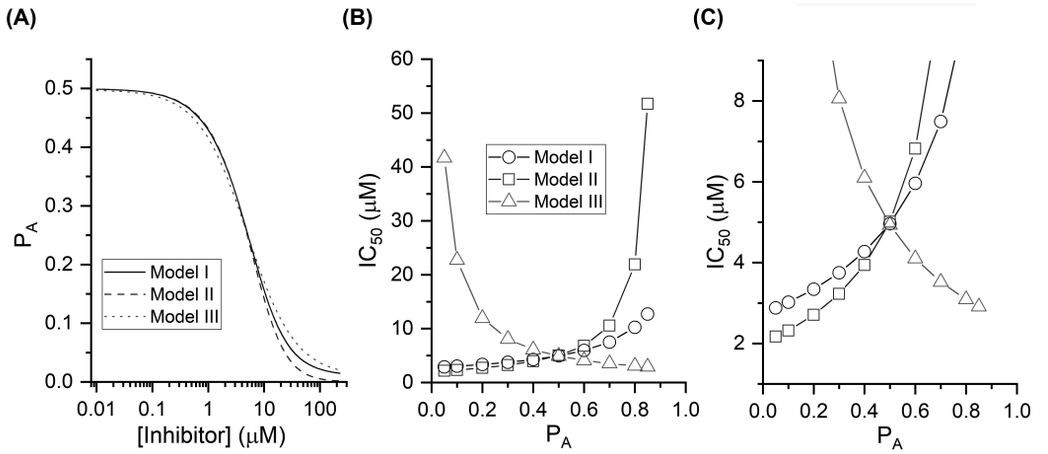

Fig. (2). The effect of the P_A of the control response on inhibition. (**A**) The inhibition concentration-response relationships were calculated using eq. 1 (Model I), eq. 2 (Model II), or eq. 3 (Model III). The control response (*i.e.*, no inhibitor present) had a P_A of 0.5. In Model I, the inhibitor had a K_R (equilibrium dissociation constant in the resting receptor) of 5.8 μM, a *c* (ratio of equilibrium dissociation constants in the active and resting receptors) of 10, and an N (number of binding sites) of 2. In Model II, the inhibitor had a K_R of 4.3, a *c* of 1, and an N of 2. In Model III, the inhibitor had a K_R of 250 μM, a *d* (ratio of equilibrium dissociation constants in the desensitized and active receptors) of 0.0001, and an N of 1. The curves were fitted to the Hill equation, yielding IC_{50}s of 5.0 μM (Model I), 5.0 μM (Model II), and 4.9 μM (Model III). With these parameters inhibition is essentially complete at high inhibitor concentrations. (**B**) The relationships between the P_A of the control response and the associated IC_{50} for the inhibitor. Inhibition concentration-response relationships were calculated using eqs. 1-3, and fitted to the Hill equation. The data indicate that in Models I and II, the inhibitor becomes less potent (higher IC_{50}) when the P_A of the control response is increased. In Model III, higher P_A of the control response is associated with higher potency of the inhibitor. (**C**) The panel illustrates the relationship between P_A and IC_{50} at a higher resolution of the ordinate. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

agonist X was varied between 0.85 μM (P_A = 0.05) and 57 μM (P_A = 0.85). In Model III, [X] varied between 0.85 μM and 71 μM. The inhibition concentration-response curves were fitted with the Hill equation, and the relationships between P_A of the control response and the IC_{50} of the inhibitor are given in Fig. (**2B**-**C**).

The data indicate that in Models I and II, an increase in agonist concentration, leading to an increase in P_A, is associated with an increase in the IC_{50} of the inhibitor. For example, in Model I, the IC_{50} of the inhibition curve is 2.9 μM when measured at P_A of 0.05, and 12.7 μM when measured at P_A of 0.85. In Model II, that simulates competitive inhibition between the agonist and the inhibitor, the IC_{50} of the inhibition curve is 2.2 μM when measured at P_A of 0.05, and 52 μM when measured at P_A of 0.85. In contrast, Model III predicts lower IC_{50} of inhibition at a higher P_A of the control response. When inhibition is measured at P_A of 0.05, the IC_{50} is 42 μM. At P_A of 0.85, the IC_{50} is 2.9 μM.

Previous studies of competitive antagonists (our Model II) and partial agonists at the muscle nicotinic receptor have demonstrated that IC_{50} values increase when determined at higher levels of activation [17, 18]. The analysis of inhibition using the Schild equation [19, 20] also relies on the underlying concept that the IC_{50} for a competitive antagonist will be larger when tested against a higher concentration of agonist. Open-channel blocking drugs are well-known to inhibit responses with high P_A more efficaciously than those of low P_A [21], consistent with a reduction in IC_{50} in Model III. Similarly, for the inhibitory steroid pregnenolone sulfate that acts by stabilizing a desensitized state (our Model III), the IC_{50} is reduced at higher agonist concentrations [16].

CONCLUSION

In sum, we have shown here that the IC_{50} of an inhibitor is sensitive to the P_A of the control response to the agonist in the absence of inhibitor. As the P_A of the control response increases, the IC_{50} can decrease or increase, depending on the mechanism of action of the inhibitor. In models where the inhibitor acts allosterically to stabilize the resting state, or competes with the agonist for a shared binding site (competitive inhibition), the IC_{50} is increased at higher P_A of the control response. In a model where the inhibitor stabilizes the desensitized state or another non-conducting, post-active state, the IC_{50} is decreased at higher control P_A. For example, a change in control P_A from 0.2 to 0.3 increases the calculated IC_{50} for our hypothetical inhibitor by 10% (Model I) to 20% (Model II), or decreases the IC_{50} by 30% (Model III). Our simulations also indicate that the IC_{50} is most sensitive to changes in control P_A over different ranges, depending on the model (high P_A for models I and II, low P_A for model III).

A corollary of the data presented in Fig. (**2**) is that comparison of inhibition among mutated or different subtypes of a receptor requires the determination of P_A of the control response; measurement of inhibition at a constant EC value is inadequate because a change in receptor structure may modify the relationship between P_A and EC values. An approach to estimate P_A of the macroscopic current response has been described previously [22, 23].

Our simulations were conducted using control P_A values and the activation and inhibition properties that are representative of the mammalian $GABA_A$ receptor. The underly-

ing principles, however, likely apply to a wide variety of receptor-channels.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

The study was supported by the National Institutes of Health National Institute of General Medical Sciences (grants R01GM108580, R35GM140947, R01GM108799) and funds from the Taylor Family Institute for Innovative Psychiatric Research.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

Declared none.

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