PERSPECTIVE

Perspective on the Relationship between GABA\textsubscript{A} Receptor Activity and the Apparent Potency of an Inhibitor

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Abstract: Background: In electrophysiological experiments, inhibition of a receptor-channel, such as the GABA\textsubscript{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\textsubscript{50}) of the inhibition curve.

Objective: We sought to estimate sensitivity of the fitted IC\textsubscript{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\textsubscript{50} of the inhibition curve is sensitive to the degree of activity of the control response. In Models I and II, the IC\textsubscript{50} of inhibition was increased as the probability of being in the active state (P\textsubscript{A}) of the control response increased. In Model III, the IC\textsubscript{50} of inhibition was reduced at higher P\textsubscript{A}.

Conclusion: We infer that the apparent potency of an inhibitor depends on the P\textsubscript{A} of the control response. While the calculations were carried out using the activation and inhibition properties that are representative of the GABA\textsubscript{A} receptor, the principles and conclusions apply to a wide variety of receptor-channels.

Keywords: GABA\textsubscript{A} receptor, activation, inhibition, modeling, IC\textsubscript{50}.

1. INTRODUCTION

Binding of the transmitter \(\gamma\)-aminobutyric acid (GABA) to the \(\gamma\)-aminobutyric acid type A receptor (GABA\textsubscript{A}R) 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\textsubscript{A}) rises. In electrophysiological recordings, the increase in P\textsubscript{A} manifests as higher whole-cell peak current. At saturating GABA concentrations, the peak P\textsubscript{A} of the GABA\textsubscript{A} receptor varies between \(-0.4\) (\(\alpha_4\beta_2\gamma;\) [1]) and \(-0.9\) (\(\alpha_1\beta_2\gamma_2;\) [2, 3]). The maximal P\textsubscript{A} can vary considerably when the receptor is activated by other agonists. For example, the peak P\textsubscript{A} of the \(\alpha_1\beta_2\gamma_2\) GABA\textsubscript{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\textsubscript{50}) and slope (n\textsubscript{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\textsubscript{50} (midpoint of the inhibition curve) and n\textsubscript{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\textsubscript{50} value [5-9]. Statistical approaches can be employed to determine if a change is statistically significant.

Here, we show that the fitted IC\textsubscript{50} of an inhibitor is sensitive to the level of the control response. As a result, IC\textsubscript{50}s measured at different activity levels cannot be meaningfully compared and statistical analysis is not appropriate. While EC and P\textsubscript{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\textsubscript{A} rather than a constant EC value, because the latter may not equivalently correlate with P\textsubscript{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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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.](image)

(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 active 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 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]:

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

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]:

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

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, $L = 8000$, $K_{R,X} = 10 \mu M$, $c_X = 0.004$ 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 $5.5 \mu M$ 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 inhibitor had a ten-fold higher affinity to $X$ or $Y$) in the resting receptor, $c_1$ is the ratio of the equilibrium dissociation constants in the active and resting states, and $N_Y$ was 2. In Model II, the $K_{R,Y}$ was 4.3 $\mu M$, 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 $\mu M$, $d_Y$ was $1 \times 10^{-5}$, and $N_Y$ was set to 1.

Next, we altered the concentration of the agonist to generate control responses with $P_X$ 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
agonist concentration, leading to an increase in $P_A$, is associ-
ated with an increase in $P_A$ of 0.85. In Model I, the
inhibitor had a $K_a$ (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 $K_a$ of 4.3, a $c$ of 1, and an $N$ of 2.

In Model III, the inhibitor had a $K_a$ 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 $IC_{50}$ 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. (A higher resolution/colour version of this figure is available in the electronic copy of the article).

$IC_{50}$ is reduced at higher agonist concentrations [16].

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.

Previous studies of competitive antagonists (our Model I) 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].

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.

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

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

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

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