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Potentiation of $GABA_A$ receptors by neurosteroids: Mechanisms and sites

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Abstract. Neurosteroids increase the strength of neuronal inhibition by enhancing the activity of GABA-A receptors. The response to lower concentrations of GABA is increased in the presence of these drugs. One question is the nature of the basis for the potentiation — is there a change in the affinity of the receptor for GABA or an increase in the efficacy of activation? The answer for these drugs is that the major change occurs in the efficacy of activation, with no change in channel opening but a decrease in rates for entering states with a closed channel. There are at least 2 sites of action, which can be distinguished by the activity of steroid analogues with different structures. There are at least 3 mechanisms of action. Mutations of $GABA_A$ receptor subunits indicate that at least one site for steroid action is likely to be at the extreme carboxyl end of the γ2 subunit. Analysis of data obtained at a low concentration of GABA suggests that the ability of steroids to potentiate the $GABA_A$ receptor may be affected by the extent of ligation with GABA.

Keywords: GABA-A receptor; Neurosteroid; Anesthetic mechanism; Potentiation

1. Introduction

We have been studying the actions of steroids and steroid analogues, with an emphasis on understanding their effects on ion channels which may underlie the clinically important effects producing anesthesia. Many anesthetics appear to act on the major inhibitory transmitter-activated ion channel in the brain, the γ-aminobutyric acid type A ($GABA_A$) receptor, to enhance the response to a given concentration of GABA ("potentiate" the receptor). To understand the mechanism of action, it is first necessary to have some picture

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of how the $GABA_A$ receptor functions in the absence of anesthetic. We then studied the changes produced by neuroactive steroids and, finally, examined the effects of a limited series of mutations on the actions of neuroactive steroids. All the experimental data were obtained using cloned subunits from rat brain, transiently expressed in HEK cells and studied using cell-attached recordings of single channel currents [1,2].

2. Results and discussion

2.1. $GABA_A$ receptor activation

We have focussed on studies of "clusters" of openings produced by concentrations of GABA which are high enough to desensitize most receptors. By analyzing the distributions of the durations of the open- and closed-channel periods *within* a cluster we are able to infer the minimal numbers of ways in which receptors may have open or closed channels ("open channel" and "closed channel" states), and the rates for moving from one state to another. The data [1,2] indicate that there are at least 3 open channel states, and 4 closed channel states within a cluster (Table 1).

An analysis of the duration of open channel states indicates that all 3 types of openings are produced by activation of receptors with 2 bound GABA molecules. Only 1 of the closed channel states reflects channel activation (CTact in Table 1), defined as a closed state in the pathway connecting the resting, unliganded state of the receptor to the liganded, open channel state (see Fig. 1). A possible kinetic scheme incorporating these observations is shown in Fig. 1.

2.2. The action of neuroactive steroids on activity within clusters

We examined [2] the actions of 2 synthetic neuroactive steroids, ACN ((3α,5α,17β)-3-hydroxyandrostane-17-carbonitrile) and B285 ((3α,5β,17β)-3-hydroxy-18-norandrostane-17-carbonitrile). These steroids have at least 2 sites of action, and 3 distinguishable functional effects on $GABA_A$ receptors. It is important to note that all of these actions would, separately, produce potentiation of activation.

What functional effects are seen? Two effects are seen in terms of the durations of open times within clusters. The fraction of openings which are in the long duration component increases (from about 10% of the total to about 45%). Also, the mean duration of the openings in the long duration

Table 1
Measured parameters for duration and relative frequencies for dwells in open and closed channel states within clusters elicited by 50 μM GABA, in the absence of any modulators

Open state	Duration (msec)	Total (%)	Note
There are three ways to be open within clusters			
OT1	0.4	27	"Brief duration openings"
OT2	2.4	61	"Intermediate duration openings"
OT3	6.3	13	"Long duration openings"
There are 4 ways to be open within clusters			
CT1	0.3	60	"Brief glitch"
CT2	2.3	12	"Slow glitch"
CTsd	20	2	"Short lived desensitized state"
CTact	–	25	"Activation related closure"

Entry into the CTact component corresponds to channel closing (see Fig. 1).