G protein activation kinetics and spillover of γ-aminobutyric acid may account for differences between inhibitory responses in the hippocampus and thalamus

(synaptic transmission/neuromodulation/epilepsy)

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Communicated by Stephen Heinemann, The Salk Institute for Biological Studies, La Jolla, CA, June 14, 1995

ABSTRACT We have developed a model of γ-aminobutyric acid (GABA)ergic synaptic transmission mediated by GABAA and GABAB receptors, including cooperativity in the guanine nucleotide binding protein (G protein) cascade mediating the activation of K+ channels by GABAB receptors. If the binding of several G proteins is needed to activate the K+ channels, then only a prolonged activation of GABAB receptors evoked detectable currents. This could occur if strong stimuli evoked release in adjacent terminals and the spillover resulted in prolonged activation of the receptors, leading to inhibitory responses similar to those observed in hippocampal slices. The same model also reproduced thalamic GABAB responses to high-frequency bursts of stimuli. In this case, prolonged activation of the receptors was due to high-frequency release conditions. This model provides insights into the function of GABAB receptors in normal and epileptic discharges.

Two receptor types, GABAA and GABAB, are responsible for most inhibitory postsynaptic potentials (IPSPs) mediated by the release of γ-aminobutyric acid (GABA) from presynaptic terminals. These receptors have characteristic differences in their kinetics (reviewed in refs. 1 and 2); GABAA-mediated currents have a relatively fast time course (time constant, 5–20 ms), whereas GABAB receptors induce much slower changes in the excitability of the cell (time constant, 150–200 ms).

They differ as well in their activation. Typically, relatively strong stimulation is needed to evoke GABAB responses, whereas GABAA-mediated currents are evoked even for very low levels of presynaptic stimulation. Miniature GABAA IPSPs also occur spontaneously and are thought to arise from the spontaneous release of GABA from a single vesicle, but they never have a GABAB component (2–4).

In this paper, we focus on the activation kinetics of GABAB responses, in which K+ channels are activated through a guanine nucleotide binding protein (G protein) cascade (14, 15). We assume that this activation shows some “cooperativity,” in the sense that independent binding of several G-protein subunits is needed to open the K+ channels.

METHODS

Release, Diffusion, and Uptake of GABA. The equation for the diffusion of GABA in the synaptic cleft is

$$\frac{\partial T(\mathbf{x}, t)}{\partial t} = \frac{f_{\text{release}}(\mathbf{x}, t)}{T(\mathbf{x}, t)} - \frac{V_{\text{max}}T(\mathbf{x}, t)}{T(\mathbf{x}, t) + K_m} + DV^2T(\mathbf{x}, t),$$  \[1\]

where $T(\mathbf{x}, t)$ is the concentration of GABA at point $\mathbf{x}$ and time $t$, and the three terms on the right represent, respectively, release, uptake, and diffusion of GABA. The diffusion coefficient was $D = 8 \times 10^{-6}$ cm$^2$/s, based on values of compounds of similar molecular weight (16).

We simulated a two-dimensional array of square ($0.5 \times 0.5$ μm) compartments (Fig. 1 A and B), representing the thin extracellular space between the postsynaptic neuron and processes emanating from other cells, either neurons or astrocytes. The area of each compartment was that of a typical single synaptic terminal (17); the array therefore represents many interleaved synaptic and glial terminals. The underlying assumptions are that (i) the width of the synaptic cleft ($\approx 200$ Å (17)) and extracellular space is less than the typical size of the synaptic terminal, allowing a two-dimensional approximation; (ii) the diffusion outside the area of terminals is negligible; (iii) the diffusion is instantaneous inside each compartment.

The release of GABA was simulated by increasing the
Fig. 1. Model of the release of transmitter at adjacent synapses in two-dimensional geometry. (A) Schematic representation of the model used with an array of adjacent processes (0.5 × 0.5 μm) representing interleaved synaptic terminals and astrocytes. Lateral diffusion (D) occurred in the extracellular space, with leakage to outside the membrane area (D_L). (B) Representation of three typical configurations: release in a single site (Isolated), release at a few sites simultaneously (Sparse), and high density of simultaneously releasing sites (Dense). (C) Time course of transmitter concentration at the release site represented with and without uptake (solid and dashed lines, respectively). (D) Time course of the transmitter represented for seven adjacent sites along a horizontal line in B in the presence of uptake.

only through the borders, neglecting diffusion in the third dimension. Alternatively, we introduced a leak in each compartment with a smaller diffusion coefficient (D_L = 10^-8 cm^2/s; see Fig. 1A). Both methods gave slow decay times comparable to that estimated from experiments (3, 13), but the latter was more convenient.

Integration of the reaction–diffusion equation (Eq. 1) was performed using a first-order explicit integration method with a discretization step of Δt = 0.5 μm. The von Neumann criterion (see ref. 22) gives a minimal time step of Δt = Δx^2/2D = 150 μs for numerical stability. We used Δt = 10–100 μs.

Binding of GABA on Postsynaptic Receptors. GABA_A receptors have at least two binding sites for GABA and show desensitization (23, 24). However, blocking uptake reveals prolonged GABA_A currents (3, 13), suggesting that desensitization was minimal. We neglected desensitization and modeled these receptors by using a simple first-order kinetic scheme (see ref. 27)

\[
\frac{dr}{dt} = \alpha[T]^r(1-r) - \beta r
\]

\[I_{\text{GABA}} = g_{\text{GABA}}[G]^r(V-E_K),\]  

where the binding of two molecules of transmitter T leads to the opening of the channel with rate constants of \(\alpha = 2 \times 10^{10} \text{ M}^{-2}\text{s}^{-1}\) and \(\beta = 162 \text{ s}^{-1}\) (obtained by fitting the model to whole-cell recorded GABA_A current; Fig. 2 Top Left); the maximal conductance is \(g_{\text{GABA}} = 1 \text{ nS}\), \(r\) is the fraction of receptors in the open state, and \(E_K = -95 \text{ mV}\) is the potassium potential.

Fig. 2. Time course of GABAergic synaptic currents under different conditions. For each type of GABA receptor, GABA_A (Left) and GABA_B (Right), a schematic diagram is shown (Top) as well as the time course of the current under different conditions. Best fit: traces indicate the best fit obtained after running a simple procedure to optimize the parameters (solid traces). Whole-cell-recorded GABA-ergic IPSCs were obtained from granule cells of the dentate gyrus (25, 26) (noisy traces; provided by T. Otis, Y. Dekerninck, and I. Mody). Traces below show GABAergic IPSCs at a single site for the three densities indicated in Fig. 1. Model IPSCs are shown in the presence (solid trace) and absence (dashed lines) of uptake.

The model of GABA_B receptors was based on a model introduced previously (27), including a desensitized state of the receptor, several G-protein binding sites, assuming a G protein is in excess, and quasi-stationarity of the fast reactions

\[
\frac{d[R]}{dt} = K_d[R] - K_d[R] - K_d[R] + K_d[D]
\]

\[
\frac{d[D]}{dt} = K_d[R] - K_d[D]
\]

\[
\frac{d[G]}{dt} = K_d[R] - K_d[G]
\]

\[
I_{\text{GABA}} = g_{\text{GABA}}[G]^r(V-E_K),\]

where \([R]\) and \([D]\) are, respectively, the fraction of activated and desensitized receptor, \([G]\) (μM) is the concentration of activated G protein, \(g_{\text{GABA}} = 1 \text{ nS}\) is the maximal conductance of K^+ channels, \(E_K = -95 \text{ mV}\) is the potassium reversal potential, and \(K_d\) is the dissociation constant of the binding of G to the K^+ channels. The G-protein cascade occurs in the following steps: (i) the transmitter binds to the receptor, leading to its activated form; (ii) the activated receptor catalyzes the activation of G proteins; (iii) G proteins bind to open K^+ channel, with n independent binding sites. Direct fitting of the model to whole-cell recorded GABA_B currents gave the following values (Fig. 2 Top Right): \(K_d = 100 \mu\text{M}\), \(K_1 = 6.6 \times 10^5 \text{ M}^{-1}\text{s}^{-1}\), \(K_2 = 20 \text{ s}^{-1}\), \(K_3 = 5.3 \text{ s}^{-1}\), \(K_4 = 17 \text{ s}^{-1}\), \(K_5 = 8.3 \times 10^{-5} \text{ M}^{-1}\text{s}^{-1}\), and \(K_6 = 7.9 \text{ s}^{-1}\) with n = 4 binding sites (see Supporting Information).
Estimation of Parameters. All simulations were run using NEURON (28). The values of parameters were obtained by fitting the entire model, including release, uptake, diffusion, and receptor kinetics, directly to experimental recordings with a simplex algorithm (22). At each iteration of the simplex algorithm, the model was run and the least-squares error was estimated between the experimental recording and the model. This procedure was repeated from different initial conditions to find robust values for the parameters, which were consistent with values estimated from the literature.

response could be revealed for a relatively narrow range of densities of releasing terminals. Finally, for high densities of simultaneously releasing sites, both GABA_A and GABA_B IPSCs occurred and their time courses were prolonged in the absence of uptake (“Dense” in Fig. 2).

Because of receptor saturation, GABA_A-mediated currents were relatively insensitive to the density of terminals and the exact time course of GABA_A decay was dominated by the low value of the unbinding constant $\beta$. In comparison, the amplitude of GABA_A-mediated currents was highly sensitive to the
cooperativity \((n = 1)\) was optimized identically as described above. In this case, \(\text{GABA}_B\) responses were proportional to the stimulus (compare solid and open circles in Fig. 3 Bottom).

We simulated the properties of \(\text{GABA}_B\) responses in thalamic slices by using bursting models of \(\text{RE}\) cells based on the presence of a low-threshold calcium current \((30)\) (Fig. 4A). Under normal conditions, stimulation in the \(\text{RE}\) nucleus evoked biphasic IPSPs in \(\text{TC}\) cells with a rather small \(\text{GABA}_B\) component (Fig. 4B). We mimicked an increase of intensity by increasing the number of \(\text{RE}\) cells discharging. The ratio between \(\text{GABA}_A\) and \(\text{GABA}_B\) IPSPs was independent of the intensity of stimulation (Fig. 4D) but only if the density of \(\text{GABA}_A\) synapses on \(\text{TC}\) cells was low. Blocking \(\text{GABA}_A\) receptors locally in the \(\text{RE}\) nucleus enhanced the burst discharge of these cells and evoked a more prominent \(\text{GABA}_B\) component in \(\text{TC}\) cells (Fig. 4C).

Results similar to those shown in Fig. 4 were obtained in models where we assumed that each \(\text{RE}\) cell establishes a dense aggregate of four \(\text{GABA}_B\) terminals on \(\text{TC}\) cells, as suggested by morphological studies \((31)\). However, terminals from different \(\text{RE}\) cells had to be located sufficiently distant from each other, so that there was minimal spillover between them.

**DISCUSSION**

Several hypotheses have been proposed for explaining the properties of \(\text{GABA}_B\) responses \((1, 2, 32)\): (i) a co-released factor is needed to activate \(\text{GABA}_B\) receptors; (ii) \(\text{GABA}_B\) receptors are located extrajunctionally; (iii) different populations of interneurons mediate \(\text{GABA}_A\) and \(\text{GABA}_B\) responses.

We have proposed and tested an alternative hypothesis that

**Time Course of GABA.** Our model of the release of GABA included spillover from adjacent terminals and uptake in a two-dimensional extracellular space. Diffusion dominated the initial time course of transmitter decay, and uptake strongly limited the spillover to adjacent terminals, as proposed earlier \((13)\). A more complete three-dimensional model would be needed to investigate these points in more detail.

The concentration of GABA was significantly influenced by the density of co-releasing terminals. We found a prolonged presence of GABA when many adjacent sites co-released, which was critical for \(\text{GABA}_B\) responses.

**Cooperativity of GABA\(_B\) Responses.** The multiplicity of G-protein binding sites assumed here had previously been suggested to explain the multieponential time course of the \(\text{GABA}_B\) current \((26)\). In this paper, we showed that this hypothesis can also explain the characteristic properties of \(\text{GABA}_B\) responses.

With several G-protein binding sites, a sufficient level of G protein must be activated intracellularly in order to produce a detectable K\(^+\) current. This implies that prolonged activation of the receptors must occur to evoke \(\text{GABA}_B\) responses. This property can account for the following observations: (i) \(\text{GABA}_B\) currents can be revealed by facilitating transmitter release with sucrose \((33)\). (ii) There is no \(\text{GABA}_B\) component in miniature IPSCs \((3, 4)\), in unitary IPSPs recorded from dual impalements \((34)\), or in IPSPs obtained from very weak stimulation \((13)\). These situations were simulated by the present model assuming that release occurred at single or distantly located sites. (iii) \(\text{GABA}_B\) currents show multieponential decay, a 10- to 20-ms delay of onset and a sigmoidal rising phase \((26)\) (Fig. 2B). The delay was needed here for the active G protein to build up intracellularly to reach a level sufficient to activate the K\(^+\) channels. Other potential morph...
Our model suggests that GABA<sub>B</sub> currents could help switch the thalamus from tonic to bursting mode. In awake animals, RE cells discharge single spikes tonically at a rate of 10–40 Hz, which evoke only fast IPSPs, in contrast to the biphasic IPSPs seen during sleep (37). In our model, RE cells elicited GABA<sub>B</sub> currents only when they were bursting. As GABA<sub>B</sub> IPSPs can powerfully promote bursting activity in TC cells (5), and TC bursts effectively evoke RE bursts (6, 9, 36, 37), GABA<sub>B</sub> currents may act as a “filter,” transparent to tonic activity but strongly activated by bursting activity, serving to maintain the thalamus in a bursting mode. Petal mal epileptic discharges may be a perversion of this natural phenomenon through disinhibition in the RE nucleus (6, 7, 9, 36).

**Testing the Hypothesis of G-Protein Cooperativity.** The present model explains the differences between thalamic and hippocampal inhibitory responses, but it is also possible that there are regional differences in the distribution of GABAergic receptors or that different receptor subtypes are expressed in different regions.

The model makes several testable predictions. First, the predicted multiplicity of G-protein binding sites can be tested by applying activated G proteins on membrane patches (38) or by voltage-clamp experiments. In other systems, a tetrameric structure was demonstrated for the K<sup>+</sup> channels (15), and the kinetics of G-protein action were shown to involve several G-protein binding sites on the channel (39–41).

The second prediction is that GABA<sub>B</sub> responses are highly nonlinear (Fig. 3B). The sharp dependence of GABA<sub>B</sub> currents with an increasing number of presynaptic spikes could be verified by using dual impalements.

The third prediction is that there should be a higher density of dendritic GABAergic terminals in the hippocampus compared to the thalamus. GABAergic terminals are relatively dense on the dendrites of hippocampal cells (42), but precise measurements have not been made. In the thalamus, dense aggregates of a few inhibitory terminals have been observed on the dendrites of TC cells (31), but these aggregates were sparse and might originate from different presynaptic RE cells (E. G. Jones, personal communication), consistent with the present model.

We acknowledge Drs. T. Otis, Y. Dekoninck, and I. Mody for kindly providing access to their data; Drs. J. Clements and J. Huguenard for comments on the manuscript; and Dr. T. Bartol for insightful discussions. This research was supported by the Howard Hughes Medical Institute and the National Institutes of Health.