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)

ALAIN DESTEXHE*†‡ and TERRENCE J. SEINOWSKI*§

*The Howard Hughes Medical Institute, The Salk Institute for Biological Studies, Computational Neurobiology Laboratory, 10010 North Torrey Pines Road, La Jolla, CA 92037; ‡Department of Physiology, Laval University School of Medicine, Québec, QC Canada G1K 7P4; and §Department of Biology, University of California at San Diego, La Jolla, CA 92037

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 (GABAergic) synaptic transmission mediated by GABA_A and GABA_B receptors, including cooperativity in the guanine nucleotide binding protein (G protein) cascade mediating the activation of K^+ channels by GABA_B receptors. If the binding of several G proteins is needed to activate the K^+ channels, then only a prolonged activation of GABA_B 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 GABA_B 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 GABA_A receptors in normal and epileptic discharges.

Two receptor types, GABA_A and GABA_B, 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); GABA_A-mediated currents have a relatively fast time course (time constant, 5–20 ms), whereas GABA_B 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 GABA_B responses, whereas GABA_A-mediated currents are evoked even for very low levels of presynaptic stimulation. Miniature GABA_A IPSPs also occur spontaneously and are thought to arise from the spontaneous release of GABA from a single vesicle, but they never have a GABA_B component (2–4).

Physiological data on GABAergic responses show marked differences between thalamic and hippocampal slices. In the thalamus, stimulation of the reticular (RE) nucleus or interneurons induces biphase GABAergic IPSPs in thalamocortical (TC) cells (5). The ratio between peak GABA_A and GABA_B currents evoked by RE neurons is insensitive to the intensity of the stimulation (6), but it changes markedly if the discharge of RE cells is enhanced by pharmacological means (6–9).

In hippocampal slices, GABAergic currents can be elicited in the dendrites of pyramidal cells by stimulating interneurons in the stratum radiatum. Unlike the situation in thalamic cells, the GABA_A/GABA_B ratio depends critically on the intensity of the stimulation (10, 11) and on the presence of uptake (3, 12, 13).

In this paper, we focus on the activation kinetics of GABA_B 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

$$\frac{\partial T(x,t)}{\partial t} = f_{\text{release}}(x,t) - \frac{V_{\text{max}} T(x,t)}{T(x,t) + K_m} + D \nabla^2 T(x,t),$$

where $T(x,t)$ is the concentration of GABA at point $x$ 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. 1A 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 concentration of GABA by 1 mM in the corresponding compartment when the presynaptic voltage crossed a threshold value of 0 mV. For a cleft width of 200–500 Å, a peak transmitter concentration of $\approx 1$ mM (18) would correspond to 3000–7500 molecules of transmitter released (19).

Uptake, present in both interneurons and astrocytes (20), was modeled by a standard Michaelis–Menten equation, with a $K_m$ value of 4 μM, estimated from kinetic studies of GABA transporters (21). The value of $V_{\text{max}}$ could be only roughly estimated from the literature and was taken to be $V_{\text{max}} = 0.1$ μM s\(^{-1}\) in all compartments unless uptake was blocked.

In the absence of uptake, we modeled the slow decay of GABA in two ways: we simulated a large patch of postsynaptic membrane (900 μm\(^2\)) from which the transmitter leaked out.

Abbreviations: GABA, γ-aminobutyric acid; IPSP, inhibitory postsynaptic potential; IPSC, inhibitory postsynaptic current; RE, thalamic reticular; TC, thalamocortical; G protein, guanine nucleotide binding protein.
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 the membrane area (Dl). (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 (Dl = 10^-8 cm^2/s; see Fig. 1A). Both methods gave slow decay times comparable to that estimated from experiments (3, 13), but they were not 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)

\[
\frac{dr}{dt} = α[T]^2(1 - r) - βr \]

\[ I_{\text{GABA}_\text{A}} = \bar{g}_{\text{GABA}_\text{A}}(V - E_{\text{Cl}}), \]  

where the binding of two molecules of transmitter T leads to the opening of the channel with rate constants of α = 2 × 10^10 M^-2s^-1 and β = 162 s^-1 (obtained by fitting the model to whole-cell recorded GABA_A current; Fig. 2 Top Left); the maximal conductance is \(g_{\text{GABA}_\text{A}} = 1\) nS, r is the fraction of receptor in the open state, and \(E_{\text{Cl}} = -80\) mV is the chloride reversal 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 simplex procedure to optimize the parameters (solid traces). Whole-cell-recorded GABAergic IPSCs were obtained from granule cells of the dentate gyrus (25, 26) (noisy traces; provided by T. Otis, Y. Dekeninck, and I. Mody). Traces below show GABAergic IPSCs at a single synapse 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 the G protein is in excess, and quasi-stationarity of the fast reactions

\[ \frac{d[R]}{dt} = K_r[T](1 - [R] - [D]) - K_d[R] + K_d[D] \]

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

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

\[ I_{\text{GABA}_\text{B}} = \bar{g}_{\text{GABA}_\text{B}}(G)^n(V - E_{\text{K}}), \]  

where [R] and [D] are, respectively, the fraction of activated and desensitized receptor, [G] (μM) is the concentration of activated G protein, \(g_{\text{GABA}_\text{B}} = 1\) nS is the maximal conductance of K^+ channels, \(E_{\text{K}} = -95\) mV is the potassium reversal potential, and \(K_d\) is the dissociation constant of the binding of G on the K^+ channel. 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): \(K_d = 100\) μM, \(K_1 = 6.6 \times 10^6\) M^-1s^-1, \(K_2 = 20\) s^-1, \(K_3 = 5.3\) s^-1, \(K_4 = 17\) s^-1, \(K_5 = 8.3 \times 10^{-5}\) M^-1s^-1, and \(K_6 = 7.9\) s^-1 with n = 4 binding sites (see Results). Some simulations were performed with n = 1 using different values of the parameters (not shown).
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 minimal assumptions about the underlying physiological conditions. A detailed description of the model and parameter estimation procedure can be found in the original article. A clear 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
cooperativity (n = 1) was optimized identically as described above. In this case, GABA_B responses were proportional to the stimulus (compare solid and open circles in Fig. 3 Bottom).

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

Results similar to those shown in Fig. 4 were obtained in models where we assumed that each RE cell establishes a dense aggregate of four GABAergic terminals on TC cells, as suggested by morphological studies (31). However, terminals from different 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 GABA_B responses (1, 2, 32): (i) a co-released factor is needed to activate GABA_B receptors; (ii) GABA_B receptors are located extrajunctionally; (iii) different populations of interneurons mediate GABA_A and GABA_B responses. We have proposed and tested an alternative hypothesis that this effect is due to properties of the receptors and second messengers involved in generating these responses.

![Fig. 4. Enhancement of the GABA_B response in TC cells through disinhibition in the RE nucleus.](image)

**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 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 GABA_B current (26). In this paper, we showed that this hypothesis can also explain the characteristic properties of GABA_B responses.

With several G-protein binding sites, a sufficient level of G protein must be activated intracellullarly in order to produce a detectable K⁺ current. This implies that prolonged activation of the receptors must occur to evoke GABA_B responses. This property can account for the following observations: (i) GABA_B currents can be revealed by facilitating transmitter release with sucrose (33). (ii) There is no 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) 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 mechanisms may also contribute (see ref. 15). (iv) Other models of GABA_B transduction, including a more detailed model (27) and a simplified model with pulses of transmitter (unpublished data), produced very similar results only if there were multiple G-protein binding sites. The same conclusion has been reached independently in another model (D. Golomb, X. J. Wang, and J. Rinzel, personal communication).

**Evoking GABA_B Responses.** We simulated the intensity dependence of GABAergic responses assuming that increasing stimulus intensities recruited more presynaptic neurons. If the terminals emanating from these neurons were densely packed, then significant spillover occurred. In this case, GABA_A and GABA_B components were evoked with a relative amplitude that depended on intensity, similar to observations in hippocampal slices (10, 11). In contrast, if the terminals were sparse, the GABA_B component could be evoked only with high-frequency release. Such conditions arise when presynaptic neurons produce bursts of action potentials. In this case, the GABA_B/GABA_A ratio was independent of the number of presynaptic neurons discharging, similar to observations in thalamic slices (6). Two factors were critical in determining GABA_B responses: the density of co-release terminals and the number and frequency of presynaptic action potentials.

In hippocampal (35) and thalamic slices (5–7, 9), GABA_B responses are often enhanced after the application of GABA_A antagonists such as bicuculline. Several explanations have been proposed—for example, different populations of interneurons may mediate GABA_A and GABA_B responses (5) or...