The Mechanism of Biphasic GABA Responses

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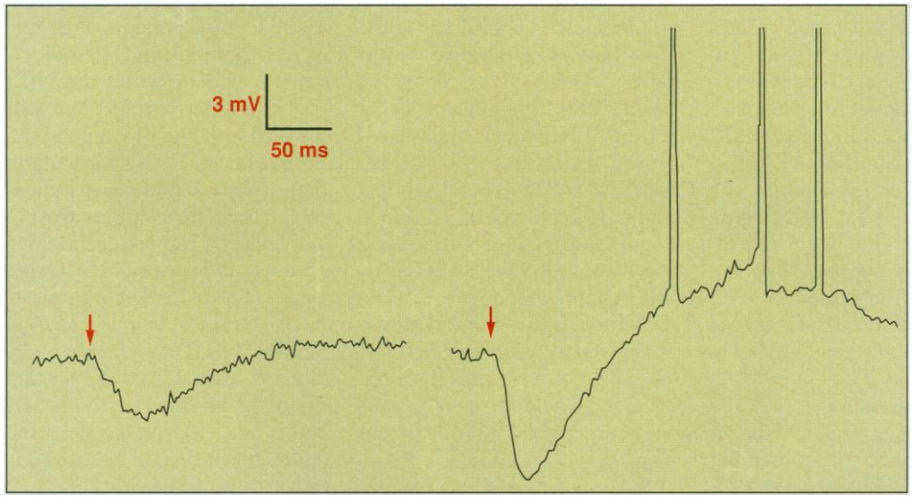
The neurotransmitter γ-aminobutyric acid (GABA) mediates much of the inhibitory synaptic transmission in the brain. When $GABA_A$ receptors are activated at inhibitory synapses, or when a small amount of exogenous GABA is applied from a pipette, many central neurons respond with a small hyperpolarization from their resting membrane potential (RMP) (see figure). This hyperpolarization and the underlying increase in membrane conductance are the basis of fast inhibitory synaptic transmission. However, when large amounts of GABA are applied, or when inhibitory synapses are activated at high frequencies, responses that start out as hyperpolarizing gradually decay back to, then overshoot, the RMP and become depolarizing. The depolarization induced by this inhibitory neurotransmitter can easily bring the membrane potential past threshold and trigger action potentials. Biphasic, hyperpolarizing-depolarizing GABA responses are seen under a variety of experimental conditions (*1*, *2*), but the ionic mechanism underlying the depolarizing component has been unclear. On page 977 of this issue, Staley and co-workers (*3*) propose a model to explain how activation of $GABA_A$ channels could produce these unusual responses.



The two sides of GABA neurotransmission. Brief application of GABA to the dendrites of a hippocampal pyramidal cell hyperpolarizes the cell (left). This response resembles the postsynaptic potential produced by a single action potential in a presynaptic inhibitory interneuron. Prolonging the duration of the GABA application (right) produces a biphasic response in the pyramidal cell, which evokes several action potentials. Similar biphasic responses occur when inhibitory interneurons fire bursts of action potentials.

$GABA_A$ channels are preferentially permeable to chloride ions. As a result, the GABA equilibrium potential (E_{GABA}; the membrane potential at which no net current flows and no voltage response is generated after GABA application) is close to the chloride equilibrium potential ($E_{chloride}$). In hippocampal pyramidal neurons, for example, E_{GABA} and $E_{chloride}$ are several millivolts negative to RMP. Thus, $GABA_A$ receptor–mediated synaptic responses and responses to small applications of GABA are hyperpolarizing at RMP and reverse polarity when cells are held negative to $E_{chloride}$. This is also the case for the hyperpolarizing component of biphasic responses to large GABA applications. However, the depolarizing component of such responses reverses polarity up to 30 mV positive to RMP, far from the predicted $E_{chloride}$.

The first explanation of depolarizing GABA responses to gain widespread acceptance was that the intracellular chloride concentration was higher in dendrites than in cell bodies, resulting in a more positive $E_{chloride}$ in dendrites than in cell bodies (*4*). This model was consistent with the common observation that responses to GABA applied to dendrites have more prominent depolarizing components than responses to somatically applied GABA. However, this model required that neurons maintain an intracellular chloride gradient by taking chloride up into their dendrites, while extruding chloride from their cell bodies. In addition, this model could not explain hyperpolarizing responses to dendritic GABA application (*5*) or activation of dendritic inhibitory synapses (*6*).

A more recent suggestion has been that an anion other than chloride, namely, bicarbonate (HCO_3^-) ions, might be responsible for depolarizing GABA responses (*6*, *7*). $GABA_A$ channels are significantly permeable to these ions (about one-fifth as permeable as to chloride) (*8*), HCO_3^- is present in millimolar concentrations in brain tissue, and $E_{HCO_3^-}$ is predicted to be very positive at physiological pH. Moreover, depolarizing $GABA_A$ responses similar to those in hippocampal neurons occur in crayfish muscle and are due to HCO_3^- current (*9*).

What type of response would be predicted for $GABA_A$ channels carrying a mixed chloride/HCO_3^- current? For channels that are permeant to more than one ion (as most are), the equilibrium potential is roughly a weighted average of the equilibrium potentials of the various permeant ions and can be predicted by the Goldman-Hodgkin-Katz (GHK) equation. With estimates of intracellular and extracellular chloride and HCO_3^- concentrations and a permeability ratio of 0.2, E_{GABA} (~75 mV) calculated by the GHK equation is between $E_{chloride}$ (~85 mV) and $E_{HCO_3^-}$ (~20 mV), but is much closer to $E_{chloride}$. The HCO_3^- permeability of $GABA_A$ channels easily explains the small positive deviation of E_{GABA} from $E_{chloride}$ (*10*). However, simply adding HCO_3^- permeability to the equation does not predict responses that are both hyperpolarizing and depolarizing, but instead predicts monophasic responses that reverse at a membrane potential slightly positive to $E_{chloride}$, as is the case for crayfish muscle (*9*). How, then, are biphasic responses generated by activation of $GABA_A$ receptors? In particular, how does activation of these channels depolarize the membrane potential far above the calculated E_{GABA}?

Answers to these questions are provided by the model proposed in the report by Staley *et al.* (*3*). The model contends that intense activation of $GABA_A$ channels, as during application of large amounts of GABA or rapid activation of inhibitory synapses, results in an activity-dependent

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breakdown in the chloride gradient. Chloride accumulates in the intracellular space and is depleted from the extracellular space, resulting in a positive shift in $E_{chloride}$. At the same time, HCO_3^- is depleted from the intracellular space and accumulates in the extracellular space, resulting in a negative shift in $E_{HCO_3^-}$. The important feature of the model is the prediction that the HCO_3^- gradient will collapse more slowly than the chloride gradient, due to regeneration of intracellular HCO_3^- (from CO_2 and H_2O) and removal of extracellular HCO_3^-, reactions catalyzed by intracellular and extracellular carbonic anhydrase. The result of this differential gradient breakdown would be a large positive shift in $E_{chloride}$, a smaller negative shift in $E_{HCO_3^-}$, and thus a net positive shift in the weighted average, E_{GABA}.

What experimental evidence is consistent with this model? The most important underlying assumption, that the chloride gradient breaks down faster than the HCO_3^- gradient, has not yet been directly tested. The chloride gradient breaks down during prolonged application of GABA (in preparations where possible contamination by HCO_3^- is minimized) (*11*, *12*), but it is not clear that it breaks down enough to allow the necessary shift in E_{GABA} (*7*). The rate of breakdown of the HCO_3^- gradient has not been estimated, although a large redistribution of HCO_3^- would produce severe intracellular acidosis. Estimates of the relative rates of breakdown of the chloride and HCO_3^- gradients might be provided by voltage-clamp experiments in HCO_3^- and chloride-depleted media. Two other predictions of the model are confirmed by experimental results (*3*, *7*). The first is that depleting HCO_3^- will inhibit depolarizing GABA responses and shift E_{GABA} toward $E_{chloride}$. The second is that increasing the rate at which the HCO_3^- gradient collapses by inhibiting carbonic anhydrase should minimize the positive shift in E_{GABA}, truncating depolarizing responses.

The model also accounts for much of the phenomenology surrounding depolarizing GABA responses, especially if it is assumed that the breakdown in the HCO_3^- gradient is negligible. For example, the model predicts that depolarizing responses would be more prominent in dendrites, where the small intracellular volume would hasten the chloride gradient breakdown. The model accounts for the late appearance of depolarizing responses (it takes time for the chloride gradient to collapse), the facilitation of depolarizing responses by increasing channel open time (the chloride gradient collapses faster), and selective block of the depolarizing components by low concentrations of $GABA_A$ receptor antagonists (the chloride gradient collapses more slowly). Finally, the model explains the absence of depolarizing GABA responses in a number of preparations (including slice cultures) that contain buffers other than HCO_3^- and CO_2 (*12*).

Do biphasic, hyperpolarizing-depolarizing responses ever occur in vivo? If so, what is their physiological significance? In hippocampal slices in vitro, depolarizing GABA responses can be elicited by high-frequency (100 to 200 Hz) activation of inhibitory neurons (*7*). Recordings from hippocampal inhibitory neurons in vivo have shown that these cells can fire synchronously at similar frequencies, for example, during hippocampal sharp waves (*13*). If biphasic GABA responses occur during such activity, what might the consequences be? Hyperpolarization followed by depolarization is the perfect sequence for deinactivation then activation of low-threshold calcium channels in pyramidal neuron dendrites (*14*). Therefore, the intradendritic calcium concentration might increase. The depolarization would also alleviate magnesium block of *N*-methyl-D-aspartate receptor channels (*7*) as shown by Staley *et al.* (*3*), resulting in a further increase in dendritic calcium. Increases in dendritic calcium concentration are required for various forms of synaptic plasticity (*15*), and therefore activation of dendritic $GABA_A$ receptors could enhance these processes. This is an interesting possibility, because synaptic inhibition usually inhibits synaptic plasticity (*16*). Thus, depending on the circumstances, activation of dendritic $GABA_A$ receptors could either prevent or enhance synaptic plasticity.

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