Neuronal Discriminator Formed by Metabotropic γ-Aminobutyric Acid Receptors

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Zhang, Jian, Ning Tian, and Malcolm M. Slaughter. Neuronal discriminator formed by metabotropic γ-aminobutyric acid receptors. J. Neurophysiol. 80: 3365–3368, 1998. Neurotransmitters function in one of two modes, promoting either inhibition or excitation. However, the metabotropic γ-aminobutyric acid receptor (GABA_B) system can switch between these modes. In the presence of a small excitatory stimulus, the GABA_B mediates a shunting inhibition that suppresses excitation. However, in the presence of a strong excitatory stimulus, the GABA_B potentiates the response. This bipartite action is accomplished by linking the GABA_B to two electrogenic mechanisms; one activates an outward current and another reduces an outward current. As a consequence, the GABA_B serves as a discriminator that reduces the influence of weak signals while augmenting responses to strong signals. In retinal ganglion cells, this mechanism acts to promote the communication of phasic information.

INTRODUCTION

γ-Aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the brain, activates both ionotropic and metabotropic receptors (Dunlap 1981). The former gates a chloride conductance that provides a powerful shunting inhibition. However, the metabotropic GABA receptor (GABA_B) initiates subtler and more varied responses. Generally, GABA_B either increase a potassium conductance (Newberry and Nicoll 1984) or reduce a voltage-dependent calcium conductance (Dolphin and Scott 1986). Frequently, these two mechanisms are partitioned across the synapse. For example, in hippocampus postsynaptic GABA_Bs activate a potassium conductance responsible for a slow inhibitory postsynaptic potential (Dutar and Nicoll 1988). Presynaptically, GABA_Bs suppress calcium current and reduce transmitter release. Thus these two complementary responses combine to inhibit synaptic transmission. However, in the retina both GABA_B responses coexist on the ganglion cell. As a consequence, they produce a unique switching mechanism that filters synaptic signals based on the strength of the input.

METHODS

Whole cell recordings were obtained from neurons in the ganglion cell layer of the tiger salamander (Ambystoma tigrinum) retinal slice as previously described (Werblin 1978; Wu 1987). Experiments employed an Axoclamp 2A amplifier and PCLAMP software. Recording electrodes (~5 MΩ) contained (in mM) 106 K-glucuronate, 5 NaCl, 2 MgCl₂, 5 ethylene glycol-bis(β-aminoethyl ether)-N,N',N''-tetraacetic acid, and 5 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES, pH 7.4). Voltages were corrected for tip potential. The Ringer solution contained (in mM) 111 NaCl, 2.5 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 dextrose, and 5 HEPES (pH 7.8). Drugs were applied by superfusion. In calcium channel current experiments, the pipette solution also contained 4 mM ATP, 20 mM phosphocreatine, and 50 units/ml creatine phosphokinase, and the Ringer solution contained 10 mM barium and 40 mM tetraethylammonium (TEA; equimolar replacement of all calcium and some sodium).

RESULTS

The biophysical properties that generate a neuronal discriminator are illustrated in Fig. 1. Baclofen was applied to selectively activate GABA_Bs (Bowery et al. 1980). This agonist affected every cell tested in the ganglion cell layer. Two distinct GABA_B actions were observed. At voltages around the resting membrane potential (~100 to −50 mV), baclofen application produced a small current that reversed near −80 mV, close to the calculated potassium reversal potential (Fig. 1A). The GABA_B current shown in this figure is the difference current generated by voltage ramps in the presence and absence of 100 μM baclofen. The conductance produced by baclofen was 423 ± 95 pS (mean ± SE, n = 6). The resting conductance of these cells was 592 ± 101 pS. Thus the GABA_B shunting conductance represented a large fraction of the resting conductance, which indicates that it can elicit significant inhibition.

GABA_B activation also reduced a voltage-sensitive outward current that activated at voltages above −40 mV. Baclofen blocked only a portion of this outward current (Fig. 1B, trace 2). Potassium channel blockers inhibited this outward current (not shown). Calcium channel blockers also suppressed a portion of this outward current (Fig. 1C, trace 2) and occluded the action of baclofen (Fig. 1C, trace 3). This suggests that GABA_Bs suppressed calcium-dependent outward current. The inset in Fig. 1B demonstrates that baclofen reduced a voltage-activated calcium current in these ganglion cells (Bindokas and Ishida 1991; Zhang et al. 1997). To measure calcium channel current, the slice was pretreated with 40 mM TEA and 10 mM barium. Under these conditions, a step from −70 mV to +10 mV evoked an inward barium current (∞) that was partially suppressed by 100 μM baclofen (∗; mean reduction of 40 ± 3%, n = 22). In combination, these results indicate that GABA_Bs reduced a voltage-dependent calcium current, which in turn reduced a large, calcium-dependent potassium conductance.
**FIG. 1.** γ-Aminobutyric acid receptors (GABA$_B$ Rs) regulate 2 opposing conductances. **A:** difference current (baclofen − control) illustrates a baclofen-elicited, small current that reversed near −80 mV. This difference current was obtained from voltage-clamped ganglion cells that were ramped from −100 to −50 mV. A large outward current was evoked (1), which was partially suppressed by 100 μM baclofen (2). **B:** inward barium current (●) observed in a voltage-clamped ganglion cell that was stepped from −70 to 0 mV. This current was suppressed by baclofen (●). **C:** by using a protocol similar to that in **B,** an outward current was evoked (1), this was partially suppressed by 100 μM cadmium (2), and cadmium occluded the effect of 100 μM baclofen (3). The inset, following the paradigm in **A,** plots the difference current between traces 2 and 3 and illustrates that cadmium did not block the small, baclofen-elicited current observed at negative voltages. **D:** at less negative voltages, the net effect of baclofen is a large inward current. This net inward current is illustrated in Fig. 1D, which is the effect produced by baclofen in Fig. 1B (trace 2 minus trace 1).

Although calcium influx was responsible for the large outward current seen in Figs. 1, B and C, it did not generate the small, shunting potassium current illustrated in Fig. 1A. This is demonstrated by the inset in Fig. 1C, which shows that cadmium did not occlude the effect of baclofen at voltages between −90 and −50 mV (trace 3 minus trace 2 of Fig. 1C).

In summary, baclofen regulates two potassium currents, a small outward current that is not calcium-dependent and a large outward current initiated by voltage-activated calcium influx. GABA$_B$R generation of the small outward current produces shunting inhibition. However, when GABA$_B$Rs reduce the calcium-dependent outward potassium current, the net effect is a large inward current. This net inward current is illustrated in Fig. 1D, which is the effect produced by baclofen in Fig. 1B (trace 2 minus trace 1).

These opposing conductances allow the ganglion cell to function as a discriminator, filtering synaptic inputs based on signal strength. To illustrate this effect, ganglion cells were current clamped and currents of variable amplitude were injected (Fig. 2). The voltage response to a small current (+15 or −10 pA) was suppressed by baclofen. However, baclofen augmented the voltage response to stronger depolarizing currents (+30 and +45 pA, Fig. 2A). Baclofen also produced a 20% average increase in spiking during a +45-pA current step (control = 22.6/ s spikes, 50 μM baclofen = 27.1/ s, n = 9, Wilcoxin’s paired signed-ranks test P < 0.05). This differential effect can be explained by the discrete actions of the two GABA$_B$R conductances. A small depolarization was shunted by the baclofen-activated, calcium-independent potassium conductance. However, a larger amplitude depolarization moved the cell voltage to a region where baclofen produced a potentiating net inward current. Cadmium application replicated the latter effect of baclofen (Fig. 2B). This supports the hypothesis that enhancement was due to reduction of calcium-dependent potassium cur-
rents. Cadmium did not suppress the response to small currents (+15 pA) (data not shown), consistent with the calcium independence of this component of GABA$_B$R action.

Thus GABA$_B$R action can be described as a discriminator, rather than as an inhibitor or excitor. Its physiological significance is that it will suppress signals below threshold, while potentiating signals above threshold. In the retina, it permits discrimination between tonic and phasic synaptic signals. The basis for this phenomenon is illustrated by comparing the synaptic voltages and currents observed during the light response of a sustained ON ganglion cell (Fig. 3A).

Surprisingly, the voltage response to a light driven synaptic input is relatively tonic (Fig. 3, left trace), but the underlying synaptic current in the same cell is phasic (right trace). The only evidence of phasic synaptic current in the voltage record is the higher spike frequency at the beginning of the light response. The disjunction between excitatory postsynaptic current (EPSC) and potential (EPSP) is due to a "ceiling" created by the large voltage-activated potassium current (Coleman and Miller 1989; Lukasiewicz and Werblin 1988). Correspondingly, ganglion cell EPSCs correlate with spike frequency but not with EPSPs (Diamond and Copenhagen 1995).

However, GABA$_B$R activation relieved this voltage ceiling by reducing the outward current. Consequently, large transient EPSCs encountered less outward current and produced larger depolarizations (Fig. 3B, a vs. a'). At the same time, the small outward current induced by baclofen suppressed the weak, delayed synaptic inputs (Fig. 3, b vs. b'). The result is that the EPSP was more phasic. Several laboratories observed that baclofen caused ganglion cell responses to switch from sustained to transient (Ikeda et al. 1990; Müller et al. 1992; Slaughter and Bai 1989). Although part of this effect may be due to presynaptic actions of baclofen, at least part is due to the direct, dual effects of baclofen on the ganglion cell.

In conclusion, the GABA$_B$R is able to produce a biphasic response that confers the properties of a discriminator. The low conductance outward current is well matched to the high resting input resistance of the cell. It effectively shunts weak EPSCs yet is too small to significantly reduce responses to large EPSCs. Large EPSPs bring ganglion cells into the voltage range where GABA$_B$Rs augment the response. This potentiation is associated with a much larger conductance, consonant with an influence on large excitatory currents. A discriminator provides an important new mechanism for information processing in the CNS.

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