GABA_B Receptor-Mediated Modulation of Presynaptic Currents and Excitatory Transmission at a Fast Central Synapse

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INTRODUCTION

At many central synapses, γ-aminobutyric acid-B (GABA_B) receptors modulate transmission by inhibitory effects on nerve terminals (Nicoll et al. 1990). Studies of somatic currents found that GABA_B receptors are coupled via G proteins to the inhibition of Ca^{2+} channels (Dolphin and Scott 1987), and inhibition of presynaptic Ca^{2+} channels was suggested to mediate the action of GABA_B receptors on synaptic transmission (Dittman and Regger 1996; Wu and Saggau 1997). However, GABA_B receptors hyperpolarize neurons through the activation of G protein-coupled inwardly rectifying potassium channels (GIRKs) (Andrade et al. 1986; Sodickson and Bean 1996), and it was reported that the K^+ channel blocker Ba^{2+} can reduce GABA_B-mediated presynaptic inhibition (Misgeld et al. 1989; Thompson and Gähwiler 1992). Therefore the mechanism of action of GABA_B receptors on channels in nerve terminals is unclear.

Studies of the giant synapse of the avian ciliary ganglion have described the modulation of presynaptic Ca^{2+} channels (Bennett and Ho 1991; Stanley and Mirozynick 1997; Yawo and Chuhma 1993). However, the small size of most synaptic structures in the mammalian brain made direct electrophysiological studies of presynaptic terminals and their modulation impossible. In mammals, single nerve fibers originating from bushy cells of the ventral cochlear nucleus make unusually large contacts (the calyces of Held) onto the cell bodies of principal cells in the medial nucleus of the trapezoid body (MNTB) (Barnes-Davies and Forsythe 1995; Borst and Sakmann 1996; Takahashi et al. 1996). In this study, I used patch-clamp techniques to examine directly the actions of GABA_B receptors on presynaptic channels in calyx nerve terminals of the mammalian auditory brain stem.

METHODS

Transverse slices (150–200 μm) of the brain stem containing the MNTB were prepared from 8- to 17-day-old Sprague-Dawley rats with the use of conventional methods (Isaacson and Walmsley 1995) and visualized with IR-DIC optics (Zeiss Axioskop). Slices were perfused with a Ringer solution containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgSO_4, 2.5 CaCl_2, 11 glucose, and 0.01 strychnine equilibrated with 95% O_2-5% CO_2. Patch pipettes (1.5–5 MΩ) were made from borosilicate glass capillaries. Neurons were impaled with a glass capillary filled with 3 M MgATP, 0.5 sodium guanosine 5'-triphosphate (NaGTP), and 0.2 or 10 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (pH 7.3). The internal solution was supplemented with Lucifer yellow (0.3 mg/ml; K^+ salt), and presynaptic recordings were confirmed at the end of each experiment with the use of epifluorescent illumination. Series resistance (<8 MΩ in postsynaptic recordings and <20 MΩ in presynaptic recordings) was routinely compensated by >80%. Synaptic currents were evoked via a Ringer-filled pipette (10-μm tip) placed at the midline. Presynaptic Ca^{2+} currents were isolated in the presence of tetrodotoxin (TTX, 1 μM), TEA (5 mM), and 4-aminopyridine (100 μM). Leak currents were subtracted on-line with a P/4 protocol. Ca^{2+} current amplitude was measured as the average value between 2 and 3 ms after the start of the voltage step. The activation time course of the Ca^{2+} current was determined by fitting a single exponential to the first 5 ms of the current traces (τ_{act}). Presynaptic facilitation was studied by using pairs of pulses (1-s interval) delivered every 10 s. The second pulse began 20 ms after a preceding 40-ms step to +120 mV. In experiments studying presynaptic K^+ currents, the superfusing solution was supplemented with TTX (1 μM), and KMeSO_4 replaced the Cs- and TEACl in the internal solution. Currents were recorded and filtered at 2–5 kHz with an Axopatch 1D amplifier (Axon Instruments) before being digitized at 4–10 kHz. Action potentials were recorded...
FIG. 1. Calyx excitatory postsynaptic currents (EPSCs) are blocked by baclofen. A1: stimulation at the midline evokes large amplitude dual-component EPSCs in principal cells of the medial nucleus of the trapezoid body (MNTB). EPSCs were evoked at holding potentials shown to the left of each trace, and the inset shows the I-V relationship of the fast (○) and slow component (●). A2: slow component of the EPSC is blocked by amino-5-phosphonovaleric acid (APV; 25 μM), and the remaining fast component is blocked by 6,7-dinitro-quinoxaline-2,3-dione (DNQX; 10 μM). B: baclofen (Bac, 50 μM) inhibits the dual-component EPSC. Traces are from the experiment plotted below.

with the same amplifier in current-clamp mode. Data were analyzed with the use of pClamp 6 (Axon Instruments). Unless stated otherwise, postsynaptic currents and presynaptic currents were recorded at holding potentials of −30 and −80 mV, respectively. All experiments were performed at room temperature (22–25°C). Results are shown as means ± SE. Significance was determined with the use of a paired t-test.

RESULTS

At a holding potential of −70 mV, stimulation at the midline of the brain stem evoked large (>2 nA peak amplitude) and fast (decay time constant ~1 ms) excitatory postsynaptic currents (EPSCs) in principal cells of the MNTB (Fig. 1A1). These responses were mediated by single nerve fibers because they occurred in an all-or-none fashion as the stimulus strength was increased (not shown). Membrane depolarization revealed a slowly decaying component of the EPSC that was most evident beyond the reversal potential (~0 mV) of the response (Fig. 1A1). The slow component was blocked by D-amino-5-phosphonovaleric acid (D-APV; 25 μM), and the fast component was abolished by 6,7-dinitro-quinoxaline-2,3-dione (DNQX; 10 μM, n = 3, Fig. 1A2). These results confirm that the calyx EPSC is mediated by both N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors (Barnes-Davies and Forsythe 1995).

Baclofen (50 μM) caused a rapid reduction in the amplitude of the evoked EPSC (Fig. 1B), in agreement with recent findings of GABA_B inhibition at the calyx synapse (Barnes-Davies and Forsythe 1995). The peak amplitude of the non-NMDA component was reduced by 88 ± 2% (SE, n = 6). Whole terminal voltage-clamp recordings were next used to determine the presynaptic action of baclofen (Fig. 2, A and B). Voltage steps from −80 mV evoked inward currents that peaked at approximately −10 mV (1.9 ± 0.2 nA, n = 11) and appeared to reverse at a potential of approximately +60 mV (Fig. 2C). Cadmium (100 μM) completely blocked these inward cur-
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FIG. 2. Voltage-clamp recordings of nerve terminal Ca^{2+} currents. A: IR-DIC video image of a principal cell and calyx. B: image of the same field under fluorescent illumination. The terminal was filled with Lucifer yellow via a patch electrode. Thin process is the axon. Scale bar is 5 μm in A and B. C: I-V relationship of currents evoked with the use of depolarizing steps from a holding potential of −80 mV. Amplitudes represent the early component of the current. D: Ca^{2+} currents are abolished by cadmium (100 μM).

Recordings from isolated cell bodies established that a major signaling pathway mediating the inhibition of Ca^{2+} channels by G protein-coupled receptors can be relieved by strong depolarization (Bean 1989; Hille 1994). A paired pulse protocol (Shapiro et al. 1994) was next used to look for a voltage dependence of GABA_B-mediated inhibition in the calyx nerve terminal (Fig. 3B). A prepulse to +120 mV reduced markedly (P < 0.01) the inhibition in response to baclofen (50 ± 6% vs. 34 ± 7% inhibition of the control and conditioned response, respectively, n = 5).

N-ethylmaleimide (NEM), a sulfhydryl alkylating agent, selectively uncouples pertussis toxin-sensitive G proteins from receptors (Jakobs et al. 1982; Shapiro et al. 1994). I next studied the action of NEM (50 μM) on the EPSC and presynaptic Ca^{2+} current. NEM caused a small and variable increase (36 ± 20%, n = 4) in the amplitude of the non-NMDA component of the EPSC. In the presence of NEM (5–10 min), baclofen caused only a weak inhibition of the EPSC (7 ± 2%, n = 6, Fig. 4A). Figure 4B illustrates the actions of NEM on presynaptic Ca^{2+} current in a representative recording. Baclofen caused a marked inhibition of the Ca^{2+} current under control conditions, but this effect was virtually abolished after NEM was added to the superfusing solution. This was not caused by a loss of responsiveness to agonist because baclofen caused robust inhibition when applied repeatedly to control slices (not shown). NEM caused a small increase in the amplitude of presynaptic Ca^{2+} currents (11 ± 6%, n = 4). In the presence of NEM, baclofen produced only a slight reduction of presynaptic Ca^{2+} currents (6 ± 1%). The actions of baclofen under control conditions and in the presence of NEM are summarized in Fig. 4C.

Although GABA_B receptors activate GIRKs in the dendrites and cell bodies of central neurons (Nicoll et al. 1990; Sodickson and Bean 1996), it is unclear if GIRKs are coupled to GABA_B receptors in nerve terminals. I next tested for the presence of baclofen-induced K^{+} currents with the use of a voltage-ramp protocol (−170 to −40 mV, 50-ms duration). Baclofen did not induce a current over this voltage range (Fig. 4D) nor did it cause a change in the properties of presynaptic action potentials recorded in current clamp (Fig. 4D, inset) in all cells examined (n = 4). These results indicate that GABA_B receptors do not activate K^{+} channels in the calyx nerve terminal.
FIG. 3. Baclofen causes voltage-dependent inhibition of presynaptic Ca$^{2+}$ channels. 

A1: baclofen (50 μM) reversibly reduces Ca$^{2+}$ currents in the nerve terminal. 

A2: Baclofen (50 μM) reversibly reduces Ca$^{2+}$ currents in the nerve terminal. 

B1: strong depolarization relieves γ-aminobutyric acid-B (GABA$_B$) receptor-mediated inhibition of nerve terminal Ca$^{2+}$ currents. 

B2: bar graph summarizes the effects of baclofen on Ca$^{2+}$ current of control responses and those preceded by strong depolarization (n = 5).

**DISCUSSION**

Whereas many G protein-coupled receptors were shown to inhibit Ca$^{2+}$ currents in the cell bodies of mammalian central neurons (Hille 1994; Nicoll et al. 1990), their actions at nerve endings are less clear. At the calyx nerve terminal, baclofen caused a rapid inhibition of presynaptic Ca$^{2+}$ current. This was associated with a slowing in the activation kinetics of the Ca$^{2+}$ channels. Furthermore, strong depolarization relieved GABA$_B$-mediated inhibition. These actions are hallmarks of membrane-delimited, G protein-mediated inhibition (Hille 1994) in which channels undergo a shift in their voltage dependence of gating (Bean 1989). The G proteins involved in the inhibition of synaptic transmission and presynaptic Ca$^{2+}$ channels are presumably of the G$_o$/G$_i$ class because NEM abolished the actions of baclofen. The small facilitating action of NEM by itself suggests a relief of tonic inhibition that may reflect agonist-independent G protein activation or, alternatively, the activation of receptors by ambient levels of neuromodulators in the slice.

Although GABA$_B$ receptors mediate postsynaptic inhibition via the activation of GIRKs in central neurons (Nicoll et al. 1990), baclofen did not induce a K$^+$ conductance in the calyx nerve terminal. These results are consistent with a recent study of transgenic mice lacking the GIRK2 gene (Lüscher et al. 1997) and suggest that the activation of presynaptic K$^+$ channels does not contribute to GABA$_B$-mediated inhibition of synaptic transmission.

Given that transmitter release at the calyx synapse has a highly nonlinear dependence on presynaptic Ca$^{2+}$ influx (Borst and Sakmann 1996; Takahashi et al. 1996), the 50% reduction in presynaptic Ca$^{2+}$ current is likely to account for the large (90%) reduction in EPSC amplitude. Although these findings do not rule out a direct effect on the release machinery itself (Dittman and Regehr 1996; Scanziani et al. 1992), these results are consistent with Ca$^{2+}$ channels play-
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FIG. 4. GABA_B-mediated inhibition of the EPSC, and presynaptic Ca^{2+} channels is N-ethylmaleimide (NEM) sensitive. 
A: NEM (50 μM) blocks the ability of baclofen (Bac, 50 μM) to inhibit the EPSC. Sweeps represent time points indicated in the graph. 
B: NEM (50 μM) blocks the ability of baclofen (Bac, 50 μM) to inhibit presynaptic Ca^{2+} current. Voltage steps were evoked to 0 mV from a holding potential of ±80 mV. 
C: summary of baclofen-induced inhibition of the EPSC and presynaptic Ca^{2+} current (I_{Ca}) under control conditions and in the presence of NEM. Numbers in parentheses are the number of observations. 
D: baclofen (50 μM) has no effect on currents elicited by a voltage ramp with the use of a K^+-based internal solution. Inset: baclofen does not alter the action potential properties of a terminal recorded in current clamp. V_{rest}, ±75 mV; current step, 100 pA.

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