GABA_A-Dependent Chloride Influx Modulates GABA_B-Mediated IPSPs in Hippocampal Pyramidal Cells

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Lopantsev, Valeri and Philip A. Schwartzkroin. GABA_A-dependent chloride influx modulates GABA_A-mediated IPSPs in hippocampal pyramidal cells. J. Neurophysiol. 82: 1218–1223, 1999. The relationship between postsynaptic inhibitory responses [the fast GABA_A-mediated inhibitory postsynaptic potential (IPSP) and the slow GABA_B-mediated IPSP] were investigated in hippocampal CA3 pyramidal cells. Mossy fiber-evoked GABA_A-mediated IPSPs were, paradoxically, of greater amplitude in cells with resting membrane potential of −62 mV (13.6 ± 0.5 mV; mean ± SE) as compared with cells with resting membrane potential of −54 mV (7.0 ± 0.8 mV). In addition, when a cell’s membrane potential was artificially manipulated, GABA_A-mediated IPSPs were reduced at relatively depolarized levels (−55 mV) and enhanced at relatively hyperpolarized potentials (at least −60 mV). In contrast, the preceding GABA_A-mediated IPSPs were larger at the more positive membrane potentials and smaller as the cell was hyperpolarized. Similar voltage dependency was obtained when monosynaptic GABA_A- and GABA_B-mediated IPSPs were isolated in the presence of glutamatergic receptor antagonists. However, monosynaptic GABA_B-mediated IPSPs isolated in the presence of glutamatergic and GABA_A receptor antagonists were not reduced at the more positive membrane potentials, and were significantly larger in amplitude than GABA_A-mediated IPSPs preceding by a monosynaptic GABA_A-mediated IPSP. The amplitude of the isolated monosynaptic GABA_B-mediated IPSPs recorded with potassium chloride-containing microelectrodes was significantly smaller than the comparable potential recorded with potassium acetate microelectrodes without chloride. We conclude that voltage-dependent chloride influx, via GABA_A receptor-gated channels, modulates postsynaptic GABA_A-mediated inhibition in hippocampal CA3 pyramidal cells.

INTRODUCTION

Inhibition mediated through postsynaptic γ-aminobutyric acid (GABA) receptors modulates neuronal excitability in the neocortex, hippocampus and others forebrain structures of the adult CNS. Under normal conditions, afferent activation of neurons in these regions induces short-lasting excitatory synaptic drive followed by two inhibitory postsynaptic potentials, mediated by GABA_A and GABA_B postsynaptic receptors. These fast and slow inhibitory postsynaptic potentials (IPSPs) are closely linked in time, but exhibit very different electrophysiological and pharmacological properties (for review, see Sivilotti and Nistri 1991). Chloride influx through GABA_A receptor-gated ion channels leads to an initial fast hyperpolarizing potential, which can be reversed by intracellular chloride injection; this action can be blocked by the GABA_A receptor antagonists bicuculline and picrotoxin (Ben-Ari et al. 1981; Knowles et al. 1984; Newberry and Nicoll 1984b). The metabotropic postsynaptic GABA_B receptors are linked to an increase in potassium permeability via activation of intracellular G proteins; the consequent slow, late hyperpolarizing potential can be imitated by microapplications of the GABA_B receptor agonist baclofen (Dutar and Nicoll 1988a,b; Gähwiler and Brown 1985; Newberry and Nicoll 1984a,b). The potassium current linked to GABA_B receptor activation exhibits inward rectification, reflected in a reduction in current amplitude as the cell’s membrane potential is moved in a positive direction, further from potassium equilibrium potential (Gähwiler and Brown 1985; Lüscher et al. 1997; Sodickson and Bean 1996, 1998).

Thus far, no clear evidence for the direct impact of GABA_A on GABA_B-mediated IPSPs has been documented. However, recent findings suggest that intracellular chloride may play an important role in the modulation of different G-protein-linked permeabilities, including that activated by GABA_B receptors (Lenz et al. 1997). Because GABA_A-mediated IPSPs precede GABA_B-mediated IPSPs in orthodromically activated neurons, we decided to investigate possible effects of chloride influx (via GABA_A receptor-gated channels) on GABA_B receptor-mediated inhibition. The results of the present study show that GABA_A receptor-mediated chloride influx, which depends sensitively on membrane potential, significantly affects GABA_B receptor-mediated inhibition in hippocampal pyramidal cells and thus may participate in the phenomenon of inward rectification.

METHODS

Hippocampal slices were prepared from brains of 1- to 1.5-mo-old Sprague-Dawley male rats. Animals were decapitated under halothane anesthesia, and their brains were removed quickly into 2–4°C artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 3 KCl, 2 CaCl_2, 2 MgSO_4, 26 NaHCO_3, 1.25 NaH_2PO_4, and 10 dextrose, saturated with 95% O_2-5% CO_2 gas (pH 7.4). Horizontal hippocampal slices (400-μm thick) were cut using a Vibroslicer (Campden Instruments) and then transferred to a holding chamber containing ACSF at room temperature for ≥1 h before recording. In the recording chamber, slices were kept at 32°C at an interface between oxygenated ACSF and humidified gas. Rate of perfusion (0.8–1 ml/min) was kept constant throughout the experiment.

Intracellular recordings of CA3 pyramidal cells were performed with sharp glass microelectrodes (resistance = 70–100 MΩ) filled with one of the following solutions: 4 M potassium acetate; 3 M potassium acetate and 0.2 M ethylene-glycol-bis(β-aminoethyl ether)-N,N',N''-tetraacetic acid (EGTA); 3 M potassium acetate and...
1 M potassium chloride. All solutions were adjusted (with KOH) to pH 7.4. Only neurons with a resting membrane potential and synaptic responses stable for ≥30 min were included in our analysis. Signals were recorded using an Axoclamp-2A amplifier (Axon Instruments) in bridge mode. Bridge balance was monitored throughout the experiment. Cell resting membrane potential (RMP) was measured after withdrawal of the microelectrode from the cell; action potential amplitude was calculated from RMP; and cell input resistance was obtained from maximum voltage change in response to a hyperpolarizing current pulse (−0.4 nA, 100 ms). Data were digitized (Neuro-Corder, Neuro Data Instruments) and acquired using AxoScope software (Axon Instruments) on a 486-based computer.

A stimulating bipolar stainless steel electrode was placed in the stratum lucidum to activate the mossy fibers. To elicit monosynaptic IPSPs, the stimulating electrode was placed close (<1 mm) to the site of recording and glutamate receptor antagonists were added to the bathing medium. Stimuli (0.1-ms duration) were delivered at 0.1 Hz, at an intensity maximal for induction of a slow GABA B-mediated IPSP. Amplitudes of the GABA A- and GABA B-mediated IPSP were measured from the RMP, at latencies of 15 and 140 ms, respectively. Measurements were expressed as means ± SE, and compared across experimental conditions using Student’s t-test. Data were considered significantly different if P < 0.05.

Bicuculline methiodide (BMI, 20 μM, Sigma), 6-cyano-7-nitroquinolxaline-2,3-dione (CNQX, 20 μM, Research Biochemicals), 2-amino-5-phosphonovaleric acid (APV, 50 μM, Research Biochemicals), and P-(3-amino-propyl)-P-diethoxy methyl-phosphonic acid (CGP35348, 700 μM, Ciba Geigy) were applied via bath perfusion.

RESULTS

Intracellular recordings were obtained from 42 neurons in the pyramidal layer of CA3 region with microelectrodes filled with 4 M potassium acetate. The resting membrane potential of the recorded cells varied from −51 to −72 mV (mean ± SE = 60.9 ± 0.9 mV, n = 42), action potential amplitude from 80 to 104 mV (93.2 ± 1.1 mV, n = 42), membrane input resistance from 32 to 74 MΩ (51.3 ± 2.1 MΩ, n = 42). In normal ACSF, mossy fiber stimulation typically induced a sequence of postsynaptic potentials that included a fast initial excitatory postsynaptic potential (EPSP) (often capped by an action potential), a fast IPSP (termed “GABA A IPSP” because it was blocked by the GABA A receptor antagonist, BMI) and a subsequent slow IPSP (termed “GABA B IPSP” because it was blocked by the GABA B receptor antagonist, CGP35348) (Fig. 1A). At RMP, shape and size of the GABA A and GABA B IPSPs varied across cells, related largely to the RMP value for a given cell (Fig. 1A). At relatively positive RMPs (i.e., close to −50 mV), neurons had a pronounced GABA A IPSP, but the subsequent GABA B IPSP was small and of short-duration (see example in Fig. 1A). Neurons with the RMP more than or equal to −60 mV demonstrated a low-amplitude GABA A IPSP, but a high-amplitude and long-lasting GABA B IPSP (Fig. 1A).

The amplitudes of the GABA A and GABA B IPSPs were compared in two groups of cells with RMP = −54 and −62 mV (Fig. 1B). The mean amplitude of the GABA B IPSPs was significantly larger at −54 mV (11.3 ± 0.3 mV, n = 4) than at −62 mV (6.6 ± 0.3 mV, n = 4). In contrast the mean amplitude of the GABA B was significantly smaller at −54 mV (7.0 ± 0.8 mV, n = 4) than at −62 mV (13.6 ± 0.3 mV, n = 4). In 18 neurons, positive or negative DC current was passed through the intracellular microelectrode, and postsynaptic responses were induced at the different membrane potential levels (Fig. 2A). The GABA A IPSPs had a monotonic linear dependence on membrane potential and usually reversed in polarity between −65 and −70 mV (Fig. 2B). Dependency of the GABA B IPSPs on membrane potential was monotonic in the range −60 to −95 mV; these potentials had maximal amplitude at −60 mV and were reduced as the membrane potential was hyperpolarized. However, this monotonic relationship was lost as the cell was depolarized from −60 mV. Indeed, in 15 of 18 recorded neurons at −55 mV (where the fast GABA A IPSPs were large), the GABA B IPSPs were smaller than at −60 mV (Fig. 2, A and B). In three remaining cells, GABA B IPSPs had an equal amplitude at −55 and −60 mV.

The smaller amplitude of the GABA A IPSP at more negative membrane potentials is due to reduction in the driving force for chloride ion influx. Surprisingly, GABA B IPSPs, which are a result of increased potassium permeability and have a reversal potential close to −100 mV (Alger 1984; Hablitz and Thalmann 1987; Otis et al. 1993), had a smaller amplitude at relatively positive membrane potentials, where the driving force for potassium ion efflux should be maximal. Similar voltage-dependent reduction in the GABA B IPSP amplitude has been reported previously (Knowles et al. 1984; Newberry and Nicoll 1985).

We did not investigate postsynaptic responses at membrane potentials more positive than −55 mV because afterhyperpolarizations associated with spontaneous action potential firing occurred at more positive potentials and overlapped with small amplitude GABA B IPSPs. Furthermore, because the calcium-dependent potassium conductance activated by action potentials (Alger and Nicoll 1980b; Hotson and Prince 1980; Schwartzkroin and Stafstrom 1980) or glutamatergic excitatory synaptic transmission (Nicoll and Alger 1981) may reshape postsynaptic responses, we examined GABA-mediated
postsynaptic potentials in neurons loaded with EGTA diffused from the intracellular microelectrode (0.2 M). EGTA sufficiently buffered intracellular calcium because short pulses of the depolarizing current induced long-lasting burst discharges in these cells with no afterhyperpolarization (a potential attributable to a calcium-dependent potassium current) (data not shown). Under these conditions, both GABA_A and GABA_B IPSPs demonstrated voltage dependencies similar to those seen in experiments performed with potassium-acetate-filled microelectrodes (n = 5; data not shown).

Inhibitory effect of the GABA_B IPSPs on depolarization-induced action potential generation was measured in six neurons. Pulses of depolarizing current were injected through the intracellular microelectrode, and current intensity was adjusted to threshold for spike discharge with the cell membrane potential maintained at either −55 or −60 mV. Current injection (latency = 140 ms) then was paired with a stimulus-evoked synaptic response, such that current-evoked spiking would start at the peak of the GABA_B IPSP. Current amplitude was adjusted so that current-evoked action potential discharges induced at membrane potentials of −55 and −60 mV had the same latency (4.5 ± 0.8 and 4.7 ± 0.9 ms, respectively; n = 6; Fig. 3, A and B). When current injection was paired with the GABA_B IPSP, spiking was delayed significantly longer when the membrane potential was −60 mV (142.3 ± 25.6, n = 6) compared with a membrane potential of −55 mV (29.7 ± 7.2 ms, n = 6; Fig. 3, A and B). This longer spike delay was consistent with the larger, long duration of the GABA_B IPSP at −60 mV compared with −55 mV.

Monosynaptic GABA-mediated IPSPs were investigated in seven neurons in the presence of the non-N-methyl-D-aspartate (NMDA) receptor antagonist CNQX (20 μM) and the NMDA receptor antagonist APV (50 μM). The stimulating electrode was placed close (<1 mm) to the site of recording, so that inhibitory interneurons located near the recorded pyramidal cell could be directly stimulated. Under these conditions, both GABA_A and GABA_B IPSPs behaved in a manner similar to that seen when the mossy fibers were stimulated in normal ACSF (Fig. 4, A and B). GABA_A IPSPs reversed at a slightly more negative membrane potential (between −70 and −75 mV), probably due to blockade of the initial EPSP which overlaps slightly with the initial phase of the GABA_B IPSP. GABA_B IPSPs had a maximal amplitude at a membrane potential of −60 mV and still showed an amplitude reduction at more depolarized (e.g., −55 mV) levels (Fig. 4, A and B). When plotted against membrane potential, the GABA_B IPSP showed a monotonic dependency as long as the preceding GABA_A IPSP (amplitude measured at peak) was depolarized or of relatively small hyperpolarizing amplitude (between membrane potentials of −95 and −60 mV; Fig. 4C). However, the GABA_B IPSP was significantly reduced when the monosynaptic GABA_A IPSP exceeded some threshold level.
Application of the GABA$_A$ receptor antagonist BMI (20 μM) concomitantly with CNQX (20 μM) and APV (50 μM) blocked fast monosynaptic IPSPs and isolated the monosynaptic GABA$_B$ IPSPs. Pharmacologically isolated GABA$_B$ IPSPs had a maximal amplitude at −55 mV and showed a monotonic change in amplitude as the membrane was hyperpolarized (n = 8; Fig. 5, A and B). The amplitude of the isolated monosynaptic GABA$_B$ IPSP at a membrane potential of −55 mV was 13.7 ± 0.6 mV (n = 5), significantly larger than the amplitude of the comparable GABA$_B$ IPSP preceded by a GABA$_A$ IPSP (8.6 ± 0.8 mV, n = 5) (i.e., recorded in the presence of only the glutamatergic receptor antagonists; Fig. 5C). No differences in GABA$_B$ IPSP amplitude were found between these conditions when cell membrane potential was varied between −60 and −95 mV. Bath application of the GABA$_B$ receptor antagonist CGP35348 (700 μM) blocked the isolated slow IPSP (n = 6; Fig. 5A).

In eight neurons, isolated monosynaptic GABA$_B$ IPSPs were recorded with microelectrodes filled with 3 M potassium acetate plus 1 M potassium chloride. IPSP amplitudes were measured 15 min after penetrating the cell, thus allowing chloride ions to diffuse from the micropipette into the cell. The average input resistance of cells recorded with chloride-containing electrodes was 60.4 ± 4.7 MΩ (comparable to 51.3 ± 2.1 MΩ in cells recorded with potassium acetate-filled microelectrodes). The amplitude of IPSPs recorded under these conditions, at a membrane potential of −55 mV was 7.7 ± 0.9 mV (n = 6), significantly smaller than the amplitude of isolated monosynaptic GABA$_B$ IPSPs recorded with microelectrodes containing no chloride (13.7 ± 0.6 mV, n = 5) (Fig. 6, A and B). However, this isolated GABA$_B$ potential was comparable in amplitude to that of GABA$_B$ IPSPs preceded by monosynaptic GABA$_A$ IPSPs (recorded in the presence of the glutamatergic receptor antagonists; 8.6 ± 0.8 mV, n = 5) and to the amplitude of the GABA$_B$ IPSPs recorded in normal ACSF (7.0 ± 0.8 mV, n = 4).

**DISCUSSION**

For both fast GABA$_A$ and slow GABA$_B$ IPSPs, the reversal potential is negative to the usual CA3 resting membrane potential of −60 mV. Thus depolarizing the cell should increase IPSP amplitudes because there is an increased driving force for both chloride ($E_{Cl^-} \approx -70$ mV) and potassium ($E_{K^+} \approx -90$ mV).
with GABAB receptor-mediated inhibition, can be depressed positive membrane potentials. In our experiments (also see Otis et al. 1993), pharmacologically isolated GABA B IPSPs have been reported previously (Knowles et al. 1993; Sodickson and Bean 1996, 1998) identified an IPSPs (i.e., in the presence of the glutamatergic receptor antagonist) showed the same relationship as afferent-evoked IPSPs, which are sufficient to modulate GABA B-mediated inhibition. In fact, GABA B IPSP-mediated inhibition of action potential discharge was more efficient at a cell membrane potential of −60 mV than at −55 mV.

It is noteworthy, however, that high-amplitude GABA B IPSPs were recorded at membrane potentials between −60 and −70 mV—membrane potentials at which the GABA A IPSP was still hyperpolarizing and at which GABA A receptors still mediated chloride influx. These chloride currents apparently did not lead to an intracellular chloride concentration sufficient to interfere with GABA A-mediated IPSPs. Indeed, our measurements have shown that monosynaptic GABA B-mediated IPSPs were reduced significantly only when the preceding monosynaptic GABA A IPSP exceeded some threshold level. This result suggests that chloride influx (associated with high-amplitude GABA A IPSPs) must establish some minimal level of intracellular chloride concentration, perhaps at sites remote from the region of chloride influx, to affect GABA A-mediated inhibition. Lenz et al. (1997) suggest that intracellular chloride reduces GABA B-mediated inhibition by targeting either potassium channels or G proteins (to which the channels are tightly coupled) (Andrade et al. 1986). The need for a relatively high intracellular chloride concentration to produce effects on GABA B-mediated inhibition may be a function of the low sensitivity of these targets to chloride. Alternatively, potassium channel-linked G proteins may be spatially distant from chloride influx, thus requiring diffusion of chloride to a cell site “remote” from the GABA A receptor-gated channels.

That such a separation might exist is suggested by the finding that distinct types of inhibitory interneurons, with separate synaptic target sites, may be responsible for GABA A and GABA B-mediated responses in the hippocampus (Nurse and Lacaille 1997). However, distal GABA B-mediated responses, even those in CA1 dendrites induced by activation of interneurons in stratum lacunosum-molecular (Williams and Lacaille 1982), may still be subject to chloride-dependent modulation. These neurons receive a high level of spontaneous inhibitory synaptic input, mediated primarily by postsynaptic GABA A receptors (Alger and Nicoll 1980a; Collingridge et al. 1984). The chloride flux associated with this spontaneous GABA A-mediated input may help explain the “anomalous” behavior of GABA B-mediated responses induced by baclofen microapplications to CA1 cells at relatively positive membrane potentials (Newberry and Nicoll 1985a); these GABA B-mediated hyperpolarizations were reduced in amplitude, much as seen with afferent stimulation.

Chloride-dependent modulation of GABA B-mediated inhibition may play a significant role in some physiological and pathological phenomena. For instance, GABA B-mediated...
IPSPs have not been detected at early postnatal periods of development (Gaiarsa et al. 1995; McLean et al. 1996), when intracellular chloride concentrations are high (Owens et al. 1996). The absence of GABA_B-mediated IPSPs at this age may be due to strong chloride-dependent inhibition of GABA_A-mediated events rather than to the absence of GABA_B receptors. Indeed, high levels of GABA_B receptors may be seen at early postnatal times; binding experiments suggest that these receptors peak at postnatal day 3 and then decline into adulthood (Turgon and Albin 1994). Also, it has been shown, that GABA_B-mediated IPSPs may control the duration of interictal epileptiform discharges (deCurtis et al. 1999; Karlsson et al. 1992) as well as the transitions from interictal to ictal-like activity in the hippocampus (Malouf et al. 1990; Scanziani et al. 1991). If GABA_B-mediated inhibition is sensitive to intracellular chloride concentration, it is possible that intracellular chloride oscillations (mediated by GABA_A receptors or other mechanisms) may modulate the strength of GABA_B-mediated inhibition and thus help determine the pattern of epileptiform activity.

This study was supported by National Institute of Neurological Disorders and Stroke Grant NS-18895.

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Received 12 February 1999; accepted in final form 12 May 1999.

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