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

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Lopantsev, Valeri and Philip A. Schwartzkroin. GABA_A-dependent chloride influx modulates reversal potential of GABA_B-mediated IPSPs in hippocampal pyramidal cells. J Neurophysiol 85: 2381–2387, 2001. Changes in intracellular chloride concentration, mediated by chloride influx through GABA_A receptor–gated channels, may modulate GABA_B receptor–mediated inhibitory postsynaptic potentials (GABA_B IPSPs) via unknown mechanisms. Recording from CA3 pyramidal cells in hippocampal slices, we investigated the impact of chloride influx during GABA_A receptor–mediated IPSPs (GABA_A IPSPs) on the properties of GABA_B IPSPs. At relatively positive membrane potentials (near −55 mV), mossy fiber–evoked GABA_A IPSPs were reduced (compared with their magnitude at −60 mV) when preceded by GABA_A receptor–mediated chloride influx. This effect was not associated with a correlated reduction in membrane permeability during the GABA_B IPSP. The mossy fiber–evoked GABA_A IPSP showed a positive shift in reversal potential (from −99 to −93 mV) when it was preceded by a GABA_A IPSP evoked at cell membrane potential of −55 mV as compared with −60 mV. Similarly, when intracellular chloride concentration was raised via chloride diffusion from an intracellular microelectrode, there was a reduction of the pharmacologically isolated monosynaptic GABA_A IPSP and a concurrent shift of GABA_A IPSP reversal potential from −98 to −90 mV. We conclude that in hippocampal pyramidal cells, in which “resting” membrane potential is near action potential threshold, chloride influx via GABA_A IPSPs shifts the reversal potential of subsequent GABA_A receptor–mediated postsynaptic responses in a positive direction and reduces their magnitude.

INTRODUCTION

The inhibitory effect of γ-aminobutyric acid (GABA) is mediated in forebrain primarily by two different types of postsynaptic receptors, termed GABA_A and GABA_B (for review see Sivilotti and Nistri 1991). Stimulation of afferents to hippocampal pyramidal cells results in short-lasting glutamatergic excitation followed by a fast inhibitory postsynaptic potential (IPSP) generated by chloride influx through GABA_A receptor–gated ion channels (Ben-Ari et al. 1981; Knowles et al. 1984; Newberry and Nicoll 1984b). A longer latency long-lasting IPSP often follows the GABA_A receptor–mediated IPSP and is mediated by postsynaptic GABA_B receptors linked to potassium channels via intracellular G-proteins (Alger 1984; Andrade et al. 1986; Dutar and Nicoll 1988; Hablitz and Thalmann 1987; Newberry and Nicoll 1984a,b; Thalmann 1988). Functional properties of this GABA_B-mediated potential are not completely understood. For instance, current evoked by the GABA_B receptor agonist, baclofen, exhibits inward rectification; i.e., a reduction in current amplitude as the cell’s membrane potential is depolarized. This feature has been attributed to the properties of the potassium channels coupled to GABA_B receptors (Gähwiler and Brown 1985; Lüscher et al. 1997; Sodickson and Bean 1996). However, both inward rectification (Knowles et al. 1984; Newberry and Nicoll 1985) and linear voltage dependency (Hablitz and Thalmann 1987; Otis et al. 1993) have been reported for synaptically activated GABA_B-mediated potentials/currents in hippocampal slices.

Artificial changes in intracellular chloride concentration may modulate G-protein–linked potassium permeability, including that activated by GABA_A receptors (Lenz et al. 1997). Therefore recently we investigated possible effects of GABA_A receptor–mediated chloride influx on GABA_B-mediated IPSPs in hippocampal pyramidal cells (Lopantsev and Schwartzkroin 1999). We showed that reduction of GABA_B-mediated IPSPs, at relatively positive membrane potentials (close to −55 mV), is induced by GABA_B-mediated chloride influx; inward rectifying properties of the potassium channels did not contribute in this effect over the range of membrane potentials investigated in our study.

The mechanism underlying chloride-dependent depression of the GABA_B-mediated IPSP is still unknown. Here we have further investigated how GABA_A receptor–mediated chloride influx affects the properties of the GABA_B-mediated IPSP. We tested two main hypotheses: 1) an increase in intracellular chloride concentration reduces membrane permeability associated with GABA_B-mediated IPSPs, and 2) enhanced intracellular chloride influences the properties of the current through potassium channels coupled to GABA_B receptors without changing membrane permeability. We found that the GABA_A receptor–mediated, chloride-dependent, reduction of GABA_B-mediated IPSPs is not associated with a decrease in membrane permeability, but is attributable to an alteration in GABA_B-mediated IPSP reversal potential.

METHODS

Male Sprague-Dawley rats 1–1.5 mo old, were used in our experiments. After decapitation under halothane anesthesia, the brain was
quickly removed into 2–4°C artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 3 KCl, 2 CaCl₂, 2 MgSO₄, 26 NaHCO₃, 1.25 NaH₂PO₄, and 10 dextrose, saturated with 95% O₂-5% CO₂ gas (pH 7.4). Transverse hippocampal slices (400 µm thick) were cut using a Vibroslicer (Campden Instruments, Sileby, UK) and then transferred to a holding chamber containing gas-saturated ACSF at room temperature (22–24°C) for at least 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 obtained with glass microelectrodes filled with 3 M potassium acetate (resistance, 80–110 MΩ) or with 3 M potassium chloride (resistance, 50–70 MΩ). Pipette solutions were adjusted to pH 7.4 with KOH. Only neurons with a resting membrane potential and synaptic responses stable for at least 20 min were included in our analysis. Signals were recorded using an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA) in bridge mode. Bridge balance was monitored throughout the experiment. Cell resting membrane potential (RMP) was verified after withdrawal of the microelectrode from the cell; action potential amplitude was calculated from RMP; and cell apparent input resistance was obtained from maximum voltage change in response to a hyperpolarizing current pulse (duration 200 ms, amplitude −0.4 nA). Data were digitized (Neuro-Corder, Neuro Data Instruments, New York, NY) and acquired using AxoScope software (Axon Instruments, Foster City, CA) on a pentium-based computer.

A bipolar stainless steel stimulating electrode was placed in the stratum lucidum to activate the mossy fibers. Stimuli (0.1 ms duration) were delivered at 0.1 Hz, at an intensity maximal for induction of GABA-mediated IPSPs. 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. Amplitude of the GABA₄-mediated IPSP was measured from the resting membrane potential, at a latency of 140 ms unless otherwise stated. Changes in membrane resistance during IPSPs were tested with brief hyperpolarizing current pulses (duration, 4–10 ms; amplitude, −0.2 to −0.4 nA), while apparent input resistance was tested at the peak of GABA₄-mediated IPSP with longer hyperpolarizing pulses (duration, 70 ms; amplitude, −0.15 to −0.2 nA). The conductance changes (ΔG) associated with the GABA₄-mediated IPSP were calculated according to the relation: ΔG = 1/R_{IPSP} − 1/R_{rest}, where R_{IPSP} is the membrane resistance measured during GABA₄-mediated IPSP at 200 ms after mossy fiber stimulation and R_{rest} is the resting membrane resistance (Hablitz and Thalmann 1987). Reversal potentials were obtained from the regression lines plotted for every cell. Latency of pharmacologically isolated, monosynaptic GABA₄-mediated IPSPs was measured between artifact of stimulation and the time point on response curve corresponding to the resting membrane potential. Measurements were expressed as means ± SE, and compared using Student’s t-test. Data were considered significantly different if P < 0.05.

Bicuculline methiodide (BMI, 20 µM, Sigma, St. Louis, MO), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 µM, Research Biochemicals International, Natick, MA), (±)2-amino-5-phosphonopentanoic acid (AP-5, 50 µM, Research Biochemicals International, Natick, MA), P-n3-aminopropyl-P-diethylaminoethyl-phosphonic acid (CGP35348, 700 µM, Ciba Geigy, Basel), and cesium chloride (1 mM) were applied via bath perfusion.

R E S U L T S

Intracellular recordings were obtained from 68 neurons in the pyramidal layer of CA3 region under different pharmacological conditions using different intracellular electrolytes. The resting membrane potential of the recorded cells varied from −52 to −70 mV, action potential amplitude from 82 to 103 mV, and membrane input resistance from 37 to 77 MΩ. Recordings from 10 of 68 neurons were made in normal ACSF with microelectrodes filled with 3 M potassium acetate. Mossy fiber stimulation induced an initial excitatory postsynaptic potential (often capped by an action potential) followed by a fast IPSP (termed “GABA₄ IPSP” since it was blocked by the GABA₄ receptor antagonist, BMI) and a subsequent slow IPSP (termed “GABA₅ IPSP” since it was blocked by the GABA₅ receptor antagonist, CGP35348). Postsynaptic responses were induced at different membrane potential levels, established by passing positive or negative steady current through the intracellular microelectrode (Fig. 1A). Dependency of the GABA₅ IPSPs on membrane potential was monotonic in the range −60 to −95 mV; these potentials had a maximal amplitude at −60 mV and were reduced as the membrane potential was hyperpolarized (Fig. 1B). However, at a membrane potential of −55 mV, the amplitude of the GABA₅ IPSP was smaller than that recorded at membrane potentials between −60 and −70 mV, and the monotonic relationship was lost.

Recently we have shown that reduction of the GABA₅ IPSP at membrane potentials close to −55 mV is due to chloride influx mediated by the preceding GABA₆ IPSP (Lopantsev and Schwartzkroin 1999). Decline in membrane permeability could be responsible for chloride-dependent reduction of the GABA₅ IPSP. Therefore membrane resistance was measured during GABA₅ IPSPs evoked at membrane potentials of −55 and −60 mV, i.e., at membrane potentials where the GABA₅ IPSP amplitude lost its monotonic voltage dependency. Pulses of negative current (duration, 70 ms; amplitude, −0.15 to −0.2 nA) were passed through the intracellular microelectrode at 130 ms after mossy fiber stimulation (Fig. 1C). Membrane resistance measured during GABA₅ IPSPs at these membrane potentials (35.4 ± 1.9 MΩ at −55 mV, mean ± SE, n = 7 and 37.7 ± 2.3 MΩ at −60 mV, n = 7) was not significantly different (Fig. 1D). The calculated conductance changes associated with GABA₅ IPSPs evoked at −55 mV (7.8 ± 1.2 nS, n = 7) and −60 mV (9.9 ± 1.1 nS, n = 7) were not significantly different. Also changes in membrane resistance were monitored during mossy fiber–evoked GABA₄-mediated IPSPs by passing brief pulses of negative current (duration, 10 ms; amplitude, −0.2 to −0.4 nA) through the recording electrode (not shown). Resistance measures were compared during GABA-mediated IPSPs evoked at membrane potentials of −55 and −60 mV in eight neurons. Membrane resistance was not different during GABA₅ IPSPs at membrane potentials of −55 and −60 mV, except at the time point of 50 ms after mossy fiber stimulation, when the GABA₅ IPSP contributes significantly to the hyperpolarization (asterisk in Fig. 1E). At this time point, membrane resistance was reduced by 54% during GABA-mediated IPSPs evoked at a membrane potential of −55 mV and was significantly lower than in the cell held at −60 mV (resistance reduced by 40%). Thus in spite of the reduction of GABA₅ IPSP amplitude at a membrane potential of −55 mV (as compared with −60 mV), corresponding membrane conductance was not affected at peak and throughout most of the time course of the GABA₅ IPSP. Significant differences in membrane resistance during GABA-mediated IPSPs, evoked at the membrane potentials of −55 versus −60 mV, were detected only at 50 ms after mossy fiber stimulation, when GABA₄ and GABA₅ IPSPs overlap.
We therefore compared the changes in membrane resistance during pharmacologically isolated monosynaptic GABA$_A$ IPSPs evoked at membrane potentials of $-55$ and $-60$ mV and measured up to 500 ms after stimulation. However, at 50 ms after stimulation, membrane resistance decreased by 28% at a membrane potential of $-55$ mV and by only 15% (significantly less) at $-60$ mV (asterisk in Fig. 1F). Overall, reduction in membrane resistance during the pharmacologically isolated monosynaptic GABA$_A$ IPSP was smaller than the reduction recorded in response to mossy fiber stimulation (in normal ACSF). This difference may be explained by the likelihood that fewer inhibitory synapses were activated under the direct stimulation protocol (used to isolate monosynaptic GABA-mediated potentials in the presence of glutamate receptor antagonists) than...
under conditions of normal synaptic activation of inhibitory interneurons.

Since the experiments described above indicate that the chloride-mediated reduction of the GABA<sub>B</sub> IPSP at −55 mV was not associated with reduction in membrane permeability, we explored an alternative possibility: that GABA<sub>A</sub> receptor-mediated chloride influx affects the properties of the current through potassium channels coupled to GABA<sub>A</sub> receptors and induces a shift of the GABA<sub>B</sub> IPSP reversal potential. To evaluate this possibility, monosynaptic GABA<sub>B</sub> IPSPs were isolated pharmacologically in the presence of glutamate receptor antagonists (CNQX, 20 μM and AP-5, 50 μM) and the GABA<sub>A</sub> receptor antagonist BMI (20 μM), in eight neurons recorded with potassium acetate-filled microelectrodes and in seven neurons recorded with potassium chloride-filled microelectrodes. Cesium (Cs<sup>+</sup> 1 mM) was added to the medium to block voltage-dependent potassium conductances, and particularly inward rectification in the hyperpolarizing direction, that might interfere with evaluation of the GABA<sub>B</sub> IPSP (Hablitz and Thalman 1987). Extracellular cesium does not block outward GABA<sub>B</sub>-mediated currents (Jarolimek et al. 1994). Hyperpolarizing steady current was passed through the intracellular microelectrode, and the GABA<sub>B</sub> IPSP amplitude was measured (at 140 ms after stimulation) at different membrane potentials. Reversal potential of the monosynaptic GABA<sub>B</sub> IPSP was then calculated from the regression lines for each cell. IPSPs recorded with potassium acetate- and potassium chloride-filled microelectrodes had similar latencies (36.9 ± 1.9 ms and 37.1 ± 2.7 ms, respectively), but hyperpolarizing IPSPs recorded with potassium chloride-filled microelectrodes had smaller amplitudes (compare examples in Fig. 2, A and C). IPSPs recorded with potassium acetate-filled microelectrodes had a reversal potential of −98.1 ± 1.2 mV (n = 8), while potentials recorded with potassium chloride-filled microelectrodes reversed at significantly more positive level of −90.3 ± 2.0 mV (n = 7; Fig. 2, B and D). Similar values were obtained when the reversal potential of these IPSPs was measured at a latency of 200 ms after mossy fiber stimulation (−98.3 ± 1.1 mV with potassium acetate- and −90.0 ± 2.4 mV with potassium chloride–filled microelectrodes). These data show that elevation of intracellular chloride shifts the monosynaptic GABA<sub>B</sub> IPSP reversal potential in a positive direction—an effect that could cause the observed reduction of these potentials at membrane potentials more positive than GABA<sub>B</sub> IPSP reversal potential.

To evaluate whether voltage-dependent GABA<sub>A</sub> receptor-mediated chloride influx is sufficient to affect the mossy fiber–induced GABA<sub>A</sub> IPSP reversal potential, we measured the GABA<sub>B</sub> IPSP reversal potential directly after GABA<sub>A</sub> IPSPs in normal ACSF (in the presence of 1 mM Cs<sup>+</sup>) with microelectrodes filled with potassium acetate. Slices were exposed to Cs<sup>+</sup> only for a brief period of time (no longer than 10 min) after establishing stable intracellular recording in normal ACSF to avoid development of Cs<sup>+</sup>–induced epileptiform discharges (Janigro et al. 1997). Mossy fiber–evoked synaptic responses were paired with pulses of hyperpolarizing current (latency, 60 ms after stimulation; duration, 1200 ms) injected through the intracellular microelectrode. With this protocol, the GABA<sub>A</sub> IPSP was evoked at membrane potentials of −55 and −60 mV (determined by steady current control), while the following GABA<sub>B</sub> IPSP was examined at different levels of hyperpolarization (determined by the current pulses; Fig. 3, A and C). The amplitude of GABA<sub>A</sub> IPSPs was measured at 200 ms after mossy fiber stimulation to avoid interference with the charging curve evoked by the hyperpolarizing current pulse. Voltage responses evoked by current pulses alone (at membrane potentials of −55 and −60 mV) were measured at 140 and 1200 ms after onset of the current pulse (not shown); measurements at these time points were not different, indicating that at 140 ms the membrane had reached a steady-state level and maintained this level throughout the pulse. GABA<sub>B</sub> IPSPs (measured at a latency of 200 ms) superimposed on these current-induced hyperpolarizations had a reversal potential of −92.6 ± 2.1 mV (n = 7) when they were preceded by GABA<sub>A</sub> IPSPs evoked at −55 mV (Fig. 3B). GABA<sub>B</sub> IPSPs preceded by GABA<sub>A</sub> IPSPs at −60 mV had a significantly more negative reversal potential of −99.1 ± 1.4 mV (n = 7; Fig. 3D). Similar results were obtained when reversal potential

![Figure 2](https://example.com/figure2.png)

**FIG. 2.** Increase in intracellular chloride concentration, due to diffusion from the intracellular microelectrode, shifted the reversal potential of the pharmacologically isolated monosynaptic GABA<sub>B</sub> IPSPs in a positive direction. A: monosynaptic GABA<sub>B</sub> IPSPs isolated in the presence of glutamate receptor antagonists (CNQX, 20 μM and AP-5, 50 μM) and the GABA<sub>A</sub> receptor antagonist BMI (20 μM), recorded at different membrane potentials with potassium acetate–filled microelectrode, in the presence of Cs<sup>+</sup> (1 mM), recorded at different membrane potentials with potassium acetate–filled microelectrode, in the presence of Cs<sup>+</sup> (1 mM). B: voltage dependency of monosynaptic GABA<sub>B</sub> IPSPs recorded with potassium acetate–filled microelectrodes. The reversal potential was close to −98 mV. C: isolated monosynaptic GABA<sub>A</sub> IPSPs, recorded at different membrane potentials with potassium chloride–filled microelectrode in the presence of Cs<sup>+</sup> (1 mM). These potentials had smaller amplitude than those recorded with potassium chloride–filled microelectrodes. The reversal potential was close to −90 mV.
of monosynaptic GABA_B IPSPs was measured (at a latency of 200 ms) following monosynaptic GABA_A IPSPs (not shown). In these latter experiments, GABA_B IPSPs reversed at $-93.0 \pm 1.8$ mV ($n = 9$) when preceding GABA_A IPSPs were evoked at $-55$ mV, but had a significantly more negative reversal potential of $-98.9 \pm 1.4$ mV ($n = 9$) when preceded by GABA_A IPSPs evoked at $-60$ mV.

Mossy fiber–evoked GABA_A IPSPs could persist beyond 140 (or 200) ms after mossy fiber stimulation, and thus interfere with our measurements of GABA_A IPSP reversal potential. To evaluate this possibility, we measured the duration of the mossy fiber–evoked GABA_A IPSPs in five neurons, at membrane potentials close to the GABA_A IPSP reversal potential, in the presence of the GABA_B receptor antagonist CGP35348 (700 μM) and Cs$^+$ (1 mM; Fig. 3E). Our recordings showed that the duration of GABA_A IPSPs, at membrane potentials between $-90$ and $-100$ mV, did not exceed 130 ms (Fig. 3F). Therefore voltage excursions associated with GABA_A IPSPs do not interfere with measurements of the GABA_B IPSP reversal potential.

Pharmacologically isolated monosynaptic GABA_B IPSPs have a long latency (onset at about 37 ms) in response to stimulation that directly activates interneurons. At this time point during the mossy fiber response, membrane potential is governed primarily by the GABA_A IPSP and is shifted in a negative direction from resting membrane potential. We estimated a membrane potential established by GABA_A IPSPs at the time point corresponding to the onset of the GABA_B IPSP so as to better describe its voltage dependency associated with aberrant behavior. Membrane potential was measured at 37 ms after mossy fiber stimulation, in responses evoked with the cell resting potential at $-55$ and $-60$ mV (Fig. 4A), i.e., at membrane potentials where the GABA_A IPSP had different properties. The membrane potential values (at a latency of 37 ms, dictated by the GABA_A IPSP) were $-64.2 \pm 0.4$ mV ($n = 10$) and $-67.4 \pm 0.3$ mV ($n = 10$), at $-55$ and $-60$ mV, respectively (Fig. 4B). Therefore onset of the GABA_B IPSP evoked by mossy fiber stimulation, in a cell with resting membrane potential of $-55$ mV, occurred at a membrane potential close to $-64$ mV.

**FIG. 3.** Reversal potential of GABA_A IPSPs was more positive when preceded by GABA_A IPSPs evoked at relatively positive membrane potentials: A: mossy fiber–evoked postsynaptic responses were paired with pulses of hyperpolarizing current (latency, 60 ms; duration, 1200 ms) such that GABA_A IPSPs were evoked at a membrane potential of $-55$ mV, while subsequent GABA_B IPSPs developed at different hyperpolarizing levels. GABA_B IPSPs were measured at a latency of 200 ms after mossy fiber stimulation (140 ms after hyperpolarizing pulse onset). Responses were recorded in the presence of Cs$^+$ (1 mM). Action potentials are truncated. B: GABA_A IPSP amplitudes, plotted against membrane potential level, when preceded by GABA_A IPSPs evoked at a membrane potential of $-55$ mV. The reversal potential was close to $-93$ mV. C: mossy fiber–evoked postsynaptic responses were paired with the pulses of hyperpolarizing current such that GABA_A IPSPs were evoked at a membrane potential of $-60$ mV, while subsequent GABA_B IPSPs developed at different hyperpolarizing levels. Responses were recorded in the presence of Cs$^+$ (1 mM). Action potentials are truncated. D: GABA_A IPSP amplitudes, plotted against membrane potential level, when preceded by GABA_A IPSPs evoked at a membrane potential of $-60$ mV. The reversal potential was close to $-99$ mV. E: mossy fiber–evoked GABA_A IPSPs were recorded at different membrane potential levels in the presence of the GABA_A receptor antagonist CGP35348 (700 μM) and Cs$^+$ (1 mM). Action potentials are truncated. F: GABA_B IPSP duration, plotted against membrane potential level. Note that GABA_A IPSP duration did not exceed 130 ms when measured at membrane potentials between $-90$ and $-100$ mV.

**FIG. 4.** Mossy fiber–evoked GABA_A IPSP is generated at a membrane potential more negative than resting membrane potential. A: membrane potential set by GABA_A IPSP was $8-10$ mV more negative than resting membrane potential of $-60$ and $-55$ mV at the onset of mossy fiber–evoked GABA_A IPSP (37 ms after mossy fiber stimulation). B: membrane potential recorded at a latency of 37 ms after mossy fiber stimulation in responses evoked at membrane potentials of $-55$ and $-60$ mV.
DISCUSSION

In hippocampal pyramidal cells recorded in vitro, stimulus-evoked GABA<sub>B</sub> IPSPs are reduced when the cell is depolarized positive to ~60 mV (Knowles et al. 1984; Newberry and Nicoll 1985). This behavior has been explained by inwardly rectifying properties of the potassium channels coupled to GABA<sub>A</sub> receptors (Gähwiler and Brown 1985; Lüscher et al. 1997; Sodickson and Bean 1996). However, this explanation is not entirely satisfying, since GABA<sub>B</sub> receptor–mediated potentials/currents pharmacologically isolated from GABA<sub>A</sub> receptor–mediated potentials/currents show monotonic voltage dependency (Hablitz and Thalmann 1987; Otis et al. 1993).

Further, monosynaptic GABA<sub>B</sub> IPSPs demonstrate long latency — close to 37 ms in our experiments. At this time point, membrane potential is primarily governed by the preceding GABA<sub>A</sub> IPSP evoked by mossy fiber activation. Our calculations show that GABA<sub>B</sub> receptor–coupled potassium channels are activated when the GABA<sub>A</sub> IPSP sets the membrane potential at ~7–9 mV below resting membrane potential for CA3 pyramidal cell. These results indicate that at the resting membrane potential at which GABA<sub>B</sub> IPSPs were reduced (i.e., at ~55 mV), the onset of GABA<sub>B</sub> IPSPs occurred at approximately ~64 mV. Rectification of GABA<sub>B</sub> receptor–mediated responses at this membrane potential has not been reported.

Recently we found that reduction of the GABA<sub>B</sub> IPSPs at relatively positive membrane potentials (close to ~65 mV) is due to chloride influx associated with the preceding GABA<sub>A</sub> IPSP (Lopantsev and Schwartzkroin 1999). In the present study, we have found that membrane depolarization (from ~60 to ~55 mV) enhances the GABA<sub>A</sub> receptor–mediated conductance during mossy fiber–evoked IPSPs or during monosynaptically evoked GABA<sub>A</sub> IPSPs. This observation suggests that a more intensive chloride influx occurs during GABA<sub>A</sub> receptor–mediated events evoked at more positive membrane potentials.

Changes in intracellular chloride concentrations also could be mediated by a voltage-activated chloride conductance described in hippocampal pyramidal cells (Madison et al. 1986; Staley 1994). However, we may rule out its possible impact on GABA<sub>B</sub> IPSPs for a number of reasons. First, this conductivity operates at membrane potentials close to (or more negative than) resting level, while we described chloride sensitivity of the GABA<sub>B</sub> IPSP during membrane depolarization. Second, chloride-dependent modulation of GABA<sub>B</sub> IPSPs was completely blocked by application of a GABA<sub>A</sub> receptor antagonist (Lopantsev and Schwartzkroin 1999). Also, measurements of membrane resistance during pharmacologically isolated monosynaptic GABA<sub>A</sub> IPSPs have shown that at a membrane potential of ~55 mV, enhanced chloride influx does not activate any additional long-lasting membrane permeability, which could overlap with GABA<sub>B</sub> IPSPs and affect their magnitude.

One possible explanation for chloride-dependent reduction of the GABA<sub>B</sub> IPSP is that higher intracellular chloride interacts with the intracellular G-protein signaling mechanism and/or the coupled potassium channel to reduce membrane permeability. However, we found that membrane permeability was not affected during reduced GABA<sub>B</sub> IPSPs at positive membrane potentials. Another possibility is that a rise in intracellular chloride modulates properties of the current through the potassium channels coupled to GABA<sub>B</sub> receptors. This latter explanation is consistent with the results of our experiments. First, we found that the reversal potential of the pharmacologically isolated monosynaptic GABA<sub>B</sub> IPSP shifted in a positive direction (from ~98 to ~90 mV) in cells loaded with chloride ions from the intracellular microelectrode. This finding is in line with previous studies showing that GABA– and baclofen-induced currents, recorded in cultured hippocampal neurons with low resistance chloride-filled intracellular microelectrodes, reversed at relatively positive membrane potentials (close to ~72 mV) (Gähwiler and Brown 1985). Second, direct measurements of the mossy fiber–evoked GABA<sub>B</sub> IPSP revealed a more positive reversal potential when the preceding GABA<sub>A</sub> IPSP was evoked at ~55 mV than at ~60 mV.

Interestingly, GABA<sub>A</sub> receptor–mediated chloride influx still exists in the cell soma at membrane potentials between ~60 and ~70 mV. However, this level of chloride influx was not sufficient to affect GABA<sub>B</sub> IPSP reversal potential. Only at membrane potentials more positive than ~60 mV was the GABA<sub>A</sub> receptor–mediated chloride influx sufficient to induce the positive shift in reversal potential of the GABA<sub>B</sub> IPSP—i.e., to alter the properties of the current through GABA<sub>B</sub> receptor–coupled potassium channels. This apparent paradox may be due to a spatial separation of chloride channels mediating GABA<sub>A</sub> IPSPs and the GABA<sub>B</sub> receptor–linked channels. Predominant dendritic location of GABA<sub>B</sub> receptors (Newberry and Nicoll 1985), where transmembrane distribution of chloride may be different from that in the cell soma (Jarolimk et al. 1999; Misgeld et al. 1986), makes it difficult to estimate exactly what direction and strength of transmembrane chloride flow is able to affect significantly the functioning of GABA<sub>A</sub> receptor–linked potassium channels.

It is interesting to note that spontaneous GABA<sub>A</sub> but not GABA<sub>B</sub>-mediated events have been recorded in hippocampal slices in normal ACSF (Alger and Nicoll 1980; Collingridge et al. 1984; Miles and Wong 1984; Otis and Mody 1992). However, recent study has revealed mixed fast/slow IPSPs, evoked in CA1 pyramidal cell when a single presynaptic interneuron generated a burst of action potentials (Thomson and Destexhe 1999). Blockade of the fast GABA<sub>A</sub>-mediated IPSP (by bicuculline) was necessary to uncover the GABA<sub>B</sub> receptor–mediated response. This result suggests that the same interneuron can activate both GABA<sub>A</sub> and GABA<sub>B</sub> postsynaptic receptors, but that concomitant activation of these receptors may evoke a "pure" GABA<sub>A</sub> IPSP; i.e., the GABA<sub>B</sub> IPSP is suppressed. This scenario can be explained by the interaction demonstrated in our study, involving a GABA<sub>A</sub>-mediated chloride–dependent reduction of the GABA<sub>B</sub> receptor–mediated component.

Does this experimentally identified chloride modulation of GABA<sub>B</sub> IPSPs have any real physiological consequences? It is perhaps relevant that GABA<sub>B</sub> receptor–mediated currents evoked by activity of inhibitory interneurons contribute to the rhythmic activity (8–15 Hz) induced by a muscarinic receptor agonist in hippocampal slice culture (Scanziani 1999). Similar rhythmic activities in hippocampus in vivo (e.g., theta-rhythm) are also characterized by intense discharges of the inhibitory interneurons (Freund and Buzsaki 1996; Ylinen et al. 1995) that may provide a significant drive for evoking both GABA<sub>A</sub>– and GABA<sub>B</sub> receptor–mediated events in postsynaptic pyramidal cells. It seems likely that under these conditions, interaction between spontaneously occurring GABA<sub>A</sub>– and GABA<sub>B</sub> IPSPs may involve chloride-dependent modulation of GABA<sub>B</sub>
CHLORIDE MODULATES REVERSAL POTENTIAL OF GABA_B IPSP


IPSps since resting membrane potential of pyramidal cells in vivo is close to values investigated in our experiments (–55 and –60 mV). We can speculate that GABA_A receptor–mediated fluctuations in intracellular chloride concentration may affect GABA_B IPSP reversal potential, and thus modulate strength of GABA_B-mediated postsynaptic potentials or even mask their appearance.

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