Biphasic Modulation of GABA Release From Stellate Cells by Glutamatergic Receptor Subtypes

Siqiong June Liu

Mueller Laboratory, Department of Biology, Penn State University, State College, Pennsylvania

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Liu SJ. Biphasic modulation of GABA release from stellate cells by glutamatergic receptor subtypes. J Neurophysiol 98: 550–556, 2007. First published May 30, 2007; doi:10.1152/jn.00352.2007. The release of inhibitory transmitters from CNS neurons can be modulated by ionotropic glutamate receptors that are present in the presynaptic terminals. In the cerebellum, glutamate released from climbing fibers (but not from parallel fibers) activates presynaptic AMPA receptors and suppresses the release of the inhibitory transmitter GABA from basket cells onto postsynaptic Purkinje cells. This input-specific modulation has been attributed to the close proximity of the climbing fibers to the axons of the basket cells. Our recent work indicates that glutamate released from parallel fibers can “spill over” and reach the axons of stellate cells. Here I test the possibility that this spillover glutamate can activate presynaptic AMPA receptors in stellate cells and in this way modulate their release of GABA. I find that stimulation of parallel fibers activates AMPA receptors and transiently suppresses autoreceptor and autaptic GABAergic currents in stellate cells. Activation of AMPA receptors reduces the release of GABA and the suppression occurs more frequently in immature cells that have a high release probability. By contrast the release of GABA from mature stellate cells that have a low release probability is potentiated by the activation of NMDA-type glutamate receptors on presynaptic terminals. Thus during development, the glutamatergic modulation of GABA release switches from an AMPA-receptor-mediated transient suppression to a NMDA-receptor–induced lasting potentiation.

INTRODUCTION

Inhibitory synapses control the timing and firing patterns of neurons by limiting the level of synaptic depolarization and by shunting excitatory currents. Excitatory transmitters such as glutamate can directly regulate inhibitory transmission by presynaptically changing the release of inhibitory transmitters from CNS neurons can be modulated by ionotropic glutamate receptors that are present in the presynaptic terminals (Belan and Kostyuk 2002; Engelman and MacDermott 2004). This modulation can change the balance between excitatory and inhibitory inputs and alter the output of a neuronal network.

One example is the GABAergic stellate and basket cells located in the molecular layer of the cerebellum. These interneurons form inhibitory synapses onto Purkinje cells and other stellate cells and suppress their activity (Hauser and Clark 1997). Glutamate can be released from one of two inputs. The release from climbing fibers activates presynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA receptors) on basket cells and transiently reduces the release of γ-aminobutyric acid (GABA) onto Purkinje cells (Satake et al. 2000). By contrast stimulation of the second glutamatergic input, the axons of granule cells (parallel fibers), does not decrease GABA release from the basket cells (Rusakov et al. 2005). This input-specific modulation of GABA release from basket cells has been attributed to the close proximity of the climbing fibers to the axons of basket cells. However, unlike basket cells that are located near the soma of Purkinje cells, stellate cells are distributed in the upper two thirds of the molecular layer, where the parallel fibers are abundant. Sensory stimulation evokes a burst of action potentials in cerebellar granule cells (Chadderton et al. 2004) and direct stimulation of these neurons evokes glutamate release, which activates extrasynaptic AMPARs on stellate cells (Carter and Regge 2000). In a recent study we found that stimulation of parallel fibers could activate N-methyl-D-aspartate (NMDA)–type glutamate receptors on the presynaptic terminals of stellate cells (Liu and Lachamp 2006). Thus the glutamate that spills over from the parallel fibers can reach the axons of stellate cells and may therefore also activate AMPARs and suppress their release of GABA.

The mechanism underlying the suppression of GABA release from basket cells involves the AMPA-induced reduction in Ca2+ entry through voltage-gated channels at the presynaptic terminal (Rusakov et al. 2005; Satake et al. 2006). Interestingly AMPA reduces the action potential–evoked Ca increase in only about 50% of basket cell terminals (Rusakov et al. 2005). What determines the sensitivity of an axon terminal to AMPAR-mediated modulation is not known.

Stellate cells not only innervate Purkinje cells but also innervate themselves by GABAergic autaptic connections. Additionally GABA released from stellate cells can activate axonal autoreceptors (Pouzat and Marty 1998, 1999). This autaptic synapse/autoreceptor current has proven to be very useful experimentally, to detect the evoked secretion of GABA from a single axon. Using this preparation I addressed two questions. First, could physiological-like stimulation of granule cells activate AMPARs and reduce the inhibitory autaptic/autoreceptor current in stellate cells? Second, if modulation was indeed present, did the AMPA-induced suppression of inhibitory postsynaptic currents (IPSCs) occur at all axonal terminals or was it limited to a distinctive subpopulation?

METHODS

Slice preparation

Sagittal or horizontal cerebellar slices (250 μm) were obtained from postnatal day (P) 13–P20 C57BL/6 mice with a Leica VT1000S vibrating microslicer in an ice-cold slicing solution as previously described (Liu and Cull-Candy 2005). The slicing solution contained (in mM) 125 NaCl, 2.5 KCl, 1 CaCl2, 7 MgCl2, 1.25 NaH2PO4, 26
![Image content]

**Electrophysiological recordings**

Voltage-clamp recordings were made using an Axopatch 700A amplifier (Axon Instruments, Foster City, CA) in an extracellular solution (in mM: 125 NaCl, 2.5 KCl, 2 CaCl₂, 1 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 25 glucose; pH 7.4) saturated with 95% O₂-5% CO₂. Recordings were made from visually identified neurons located in the outer two thirds of the molecular layer. Stellate cells were identified by their ability to fire spontaneous action potentials in the cell-attached configuration and by the presence of spontaneous excitatory and inhibitory synaptic currents in the perforated-patch configuration.

Autaptic and autoreceptor inhibitory postynaptic currents (aIPSCs) were evoked by a 1-ms depolarization to 0 mV from a holding potential of −70 mV in a voltage-clamp configuration at 0.3 Hz. aIPSCs were filtered at 2 kHz and digitized at 10 kHz. Electrodes with a resistance of 4–8 MΩ were filled with a pipette solution (in mM: 150 KCl, 4.6 MgCl₂, 0.1 CaCl₂, 10 HEPES, 1 EGTA, 4 Na-ATP, and 0.4 Na-GTP; pH 7.4) that included amphotericin B (300 μg/mL). Series resistance was monitored throughout the experiment. If this changed by >20%, the experiment was terminated.

With respect to stimulation of parallel fiber (PF) inputs, PFs in horizontal slices were stimulated by a train of four depolarizations at 100 Hz using a bipolar electrode (stimulation strength: 6–17 V; stimulation duration 20–140 μs) placed across the molecular layer about 200 μm from the recording electrode. An aIPSC was then evoked 100 ms after each burst. Because presynaptic AMPA receptors in stellate cells are activated by “spillover” glutamate released from PFs, activation of these AMPARs might depend strongly on temperature. Thus recordings were made at 36°C. Recordings at 36°C were stable for 10–15 min and therefore were not ideal for longer-term recordings.

**Application of AMPA**

aIPSCs were recorded at room temperature before and during the application of AMPA (0.3 μM AMPA was applied for 10 min). At room temperature perforated-patch recordings lasted for 20–60 min (series resistance was found to remain stable for 1 h in ~20% of the recorded cells). This was sufficient time to monitor the long-term change in aIPSC amplitude.

Recordings of spontaneous miniature IPSCs (mIPSCs) were made in the presence of 0.4 μM TTX (tetrodotoxin), 10 μM CPP [(±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid], 1 μM AM-251 [N-(piperidin-1-yl)-5-(4-iiodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide], and 10 μM SCH50911 [(--)-(R)-5,5-dimethylmorpholinyl-2-acetic acid ethyl ester hydrochloride] in the whole cell configuration. mIPSCs were recorded for 4 min before, during, and after AMPA application.

**Data analysis**

Average aIPSCs were obtained from 10 consecutive sweeps using Clampfit (version 9.0, Axon Instruments). Each mean paired-pulse response was constructed from 50 to 70 events. The paired-pulse ratio (PPR) was calculated as mean aIPSC amplitude/mean aIPSC amplitude. The coefficient of variation (CV) of synaptic transmission was calculated from the peak amplitude of ≥70 events. Data are expressed as means ± SE. A two-tailed Student’s t-test was used to assess statistical significance.

**R E S U L T S**

The release of GABA from single axons of cerebellar stellate cells was examined by measuring the autaptic/autoreceptor inhibitory postsynaptic currents (aIPSCs) that were evoked by a 1-ms depolarization. This current was inhibited by SR95531 [2-(3-carboxypropyl)-3-amino-6-methoxyphenylpyridazinum bromide] and therefore is mediated by the activation of GABA_A receptors (Supplemental Fig. 1).†

**Burst stimulation of PF inputs transiently suppressed aIPSCs**

Autoreceptor currents were recorded at 36°C in a perforated-patch configuration from P12–P13 stellate cells. To determine the effects of glutamate released from PFs, an aIPSC was evoked 100 ms after a train of PF stimulation. The artificial cerebrospinal fluid contained 10 μM CPP, 1 μM AM-251, and 10 μM SCH50911 to block NMDA, CB1, and GABA_B receptors, respectively (Fig. 1, A and B). The aIPSC was compared with the control aIPSC without PF stimulation.

As shown in Fig. 1, PF stimulation reduced the amplitude of aIPSCs by 14.1 ± 2.9% (n = 5, P < 0.03, paired t-test). The suppression was transient because the aIPSC amplitude recovered within 1 min. This activity-dependent suppression was prevented by NBQX (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione), a non-NMDA-receptor (NMDAR) blocker (aIPSC_control: 36.2 ± 8.0 pA; aIPSC_stimulation: 35.8 ± 7.9 pA, n = 5, P = 0.39). No differences in the aIPSC amplitude and in the decay time constant were observed between controls with and without NBQX. Thus the PF stimulation-induced transient depression required the activation of non-NMDARs.

**Exogenous AMPA application suppressed aIPSCs in immature stellate cells by a presynaptic mechanism**

A recent study showed that activation of AMPARs suppressed Ca²⁺ entry in 50–60% of basket cells in the third postnatal week (Rusakov et al. 2006). This suggests that only a subset of presynaptic terminals are regulated by AMPARs. The probability of GABA release from basket/stellate cells is known to decrease during development (Liu and Lachamp 2006; Pouzet and Hestrin 1997). Thus one possibility is that the AMPAR-mediated inhibition of GABA release from stellate cells occurs preferentially in presynaptic terminals that have a high release probability. I therefore examined the effect of activating AMPARs on the aIPSCs recorded in P12–P20 stellate cells. To determine whether the extent of suppression was developmentally regulated and correlated with the release probability, I exogenously applied AMPA to obtain a maximal AMPAR-induced suppression of aIPSCs. The amplitude of aIPSCs recorded in the perforated-patch configuration was stable for ≥50 min (see Supplemental Fig. 2).

The aIPSC amplitude was determined in stellate cells before and during the application of a low concentration of AMPA. The amplitude of aIPSCs in P12–P15 cells decreased by 25.1 ± 4.0% during AMPA application (from −46.5 ± 8.2 to −33.6 ± 5.1 pA, n = 16; P < 0.005; paired t-test; Fig. 2, A and B) and returned to 43.2 ± 9.7 pA after the removal of AMPA (5-min washout). Of the 16 P12–P15 cells recorded, 14 showed a depression of >10% (Supplemental Fig. 3). AMPA application in the presence of CB1 and GABA_B receptor blockers also suppressed the aIPSC amplitude by 26.5 ± 4.3% (P12–P13, n = 9; ranging from 11 to 49%). Thus AMPA application indeed produced a greater reduction in aIPSC

† The online version of this article contains supplemental data.
amplitude than did PF stimulation (Fig. 1). The activation of AMPARs appeared to cause this inhibition of aIPSCs because NMDAR blockers did not prevent the AMPA-induced suppression of aIPSCs (Fig. 2A). By contrast, the average current amplitude in P17–P19 cells (n/110056) did not change during AMPA application. Thus exogenous AMPA application suppressed aIPSCs in immature but not mature stellate cells.

I next determined whether the AMPA-induced suppression of aIPSCs appeared to cause this inhibition of aIPSCs because NMDAR blockers did not prevent the AMPA-induced suppression of aIPSCs (Fig. 2A). By contrast, the average current amplitude in P17–P19 cells (n/110056) did not change during AMPA application. Thus exogenous AMPA application suppressed aIPSCs in immature but not mature stellate cells.

I next determined whether the AMPA-induced suppression of aIPSCs amplitude was the result of a decrease in the release of GABA. If this were the case one would expect that the suppression would be associated with an increase in the PPR and a decrease in 1/CV^2. The PPR was determined before and during the application of AMPA. In P12–P15 cells there was an increase in the PPR during the application of AMPA (from 0.30 ± 0.08 to 0.41 ± 0.10; n = 13, P < 0.04; by paired t-test; Fig. 2C), indicating a reduction in GABA release. However, no significant change in PPR was found in P17–P19 cells. Thus an increase in PPR (i.e., a decrease in release probability) occurred predominantly in immature cells that displayed the depression. The 1/CV^2 of aIPSCs was also found to decrease during the AMPA-induced suppression of aIPSC amplitude. This was illustrated by the correlation between 1/CV^2 and the change in the current amplitude (Fig. 2D; R^2 = 0.43, n = 26, slope = 1.04 ± 0.24; P < 0.0004). Together these results suggest that AMPA application transiently reduced the secretion of GABA from stellate cells.

In further support of a presynaptic mechanism, mIPSC frequency in P12–P13 stellate cells increased during AMPA application.
Application by about 11-fold (from 0.4 ± 0.2 to 4.3 ± 1.5 Hz; n = 6; P < 0.05) and returned to the control level after the removal of AMPA (Fig. 2E). The amplitude of mIPSCs did not change, a result that is consistent with a previous report by Bureau and Mulle (1998) and supports the idea that the expression site of this plasticity is presynaptic.

**Modulation of GABA release by glutamate depends on developmental age and the initial synaptic release probability**

To confirm that the probability of GABA release from basket/stellate cells decreased during development, I examined the aIPSCs in response to two depolarizations separated by 20 ms in P12–P20 stellate cells. Consistent with previous observations (Liu and Lachamp 2006; Pouzat and Hestrin 1997), the PPR increased with age (R² = 0.53; n = 37; slope = 0.12 ± 0.02; P < 0.0001), indicating that the GABA release probability decreased during development (Fig. 3A).

To address the question of why AMPA application suppressed aIPSCs mainly in immature neurons, I tested the hypothesis that the depression of GABA release occurred preferentially at those synapses that had a high release probability (i.e., a low PPR). As shown in Fig. 3B, depression was more frequently observed in cells with a low PPR than in those with a higher PPR (low release probability). Thus the AMPA-induced change in the aIPSC amplitude correlated with the initial PPR (R² = 0.34, n = 27, slope = 33.5 ± 9.5, P < 0.002; Fig. 3B). I then determined whether reducing the release probability in immature P12–P13 stellate cells by lowering the extracellular Ca concentration from 2 to 1 mM prevented the AMPA-induced suppression of aIPSCs. In low Ca the PPR increased from 0.06 ± 0.04 to 0.47 ± 0.15 (n = 6; P < 0.05) and AMPA application no longer suppressed aIPSC amplitude (Fig. 3C). These results are consistent with the idea that activation of AMPA receptors is more likely to induce a transient depression of GABA release at synapses that have a high release probability.

Our recent work showed that burst stimulation of PFs also activates presynaptic NMDA receptors and induces a lasting increase in GABA release from stellate cells (Liu and Lachamp 2006). Thus glutamate can exert two opposing effects on...
GABA release: 1) a transient suppression of GABA release resulting from the activation of AMPARs and 2) a lasting enhancement of GABA release that is induced by NMDAR activation. Can a single stellate cell show both responses? We have previously shown that the application of AMPA can induce a lasting increase in GABA release from mature stellate cells and that paradoxically this potentiation can be blocked by NMDAR blockers (Liu and Lachamp 2006). Thus AMPA application presumably increases the release of glutamate and glycine from other cerebellar cells by activating AMPARs on these cells. Whereas glutamate could be released from cerebellar granule cells, glycine could originate from Bergmann glial cells, Golgi cells, or Lugaro cells (Huang et al. 2004; Zeilhofer et al. 2005). Endogenously released glutamate and glycine could then activate NMDARs on stellate cells. Because AMPA application can produce both a transient suppression and a lasting enhancement of GABA release, we measured the AMPAR-mediated suppression (during AMPA application) and NMDAR-induced lasting potentiation (15–30 min after AMPA application) in the same stellate cells (Supplemental Fig. 4).

As shown in Fig. 3D, cells that exhibited an AMPAR-mediated depression did not show long-term potentiation. On the contrary, a lasting potentiation occurred only in cells that did not display a transient suppression of GABA release. This is illustrated by the correlation between the change in aIPSC amplitude during AMPA application and the lasting change in aIPSCs observed 15–30 min after AMPA application ($R^2 = 0.66, n = 11, slope = 1.55 \pm 0.38, n = 11, P < 0.003$). These results indicate that GABA release from a stellate cell can be either transiently depressed or persistently enhanced by the activation of glutamate receptors.

I then tested the idea that whether a stellate cell undergoes a transient depression or an enduring potentiation is correlated with the initial release probability. Cells were divided into two groups based on their initial PPR (PPR: 0–0.5 and 0.6–1.6). As predicted, the long-term potentiation (but not the transient suppression) was found only in cells with a low release probability (i.e., that exhibited a high PPR; Fig. 3E). In contrast, a transient depression (but not a lasting potentiation) was observed in cells that had a high release probability (i.e., a low PPR). Thus the glutamatergic modulation
of GABA secretion from stellate cells depends on the initial release probability.

The observation that the probability of GABA release decreased during synaptic maturation raises the possibility that the AMPAR-mediated transient suppression is more likely to occur in immature stellate cells, whereas the NMDAR-induced lasting potentiation of GABA release will occur preferentially in mature neurons. During AMPA application aIPSCs in cells from P13–P16 mice displayed depression, whereas P17–P19 cells showed no change (P13–P16: −15 ± 5%, n = 6 vs. P17–P19: 7 ± 8%, n = 5; P < 0.05; Fig. 3F). By contrast, a lasting increase in aIPSCs was observed only in P17–P19 cells, but not in cells from P13–P16 mice (P17–P19: 59 ± 10% vs. P13–P16: 13 ± 13%, P < 0.03). Thus during synaptic maturation the glutamatergic modulation of GABA release switches from an AMPA receptor–mediated transient suppression to a NMDA receptor–induced lasting potentiation.

**DISCUSSION**

In the cerebellar cortex, stellate cells are present in the upper two thirds of the molecular layer, whereas basket cells are found close to the somata of Purkinje cells. Here I show that stimulation of the glutamatergic parallel fibers in a manner that mimics physiological activity can transiently reduce the amplitude of autoreceptor/autaptic currents in cerebellar stellate cells by activating AMPA receptors. Thus the glutamate that is released from PFs can activate AMPARs on stellate cells and reduce GABA release. By contrast, glutamate released from climbing fibers (but not from PFs) activates AMPARs on the presynaptic terminals of basket cells and suppresses the release of GABA from cerebellar basket cell onto Purkinje cells (Rusakov et al. 2005; Satake et al. 2004, 2006). One unusual feature of the AMPAR-mediated suppression of aIPSCs was that it occurred in only a subset of cells. A similar heterogeneous response to AMPAR activation was also seen at the axon terminals of basket cells, where activation of AMPARs suppressed Ca\(^{2+}\) entry in 50–60% of terminals (Rusakov et al. 2005). In the present study, P12–P20 mice were used. During this period there was a decrease in the probability of GABA release (Liu and Lachamp 2006; Puozat and Hestrin 1997), although the underlying mechanism is not clear. One possibility is that it is explained by an increase in the expression of parvalbumin in stellate cells, leading to a change in presynaptic Ca signaling (Collin et al. 2005). I found that the terminals of stellate cells that have a low release probability cannot undergo a further reduction in GABA release (because a correlation between the AMPA-induced reduction in transmitter secretion and the initial release probability was observed). This suggests that release probability is one of the factors that determines whether AMPAR activation is effective. However, the possibility that AMPARs are absent at terminals with a low release probability cannot be ruled out.

The developmental switch in AMPAR-mediated regulation of evoked GABA release is consistent with the results of Bureau and Mulle (1998). In their study AMPAR activation was shown to enhance mIPSC frequency only in immature stellate cells, but not in mature cells. However, in contrast to the AMPA-induced suppression of evoked GABA release, AMPA application potentiated the spontaneous release of GABA from stellate cells. This difference between the regulation of spontaneous and evoked release did not arise from the activation of distinct subtypes of AMPARs because both responses involve activation of Ca-impermeable AMPARs (but not Ca-permeable AMPARs; Satake et al. 2006). Although the mechanism underlying the potentiation of spontaneous release is not known, AMPAR activation can reduce Ca entry through voltage-gated Ca channels by a G-protein–coupled signaling pathway, leading to the suppression of evoked GABA release (Rusakov et al. 2005; Satake et al. 2004). Similar contrasting actions of presynaptic AMPARs on evoked and spontaneous release of GABA from stellate cells.
release of inhibitory transmitters have been observed in the spinal cord dorsal horn (Engelman et al. 2006).

Glutamate can presynaptically activate AMPARs and NMDARs, producing opposing effects on the release of GABA from stellate/basket cells with distinct temporal dynamics. In basket cells, stimulation of climbing fibers activates AMPARs and transiently suppresses GABA release (Satake et al. 2000). Burst activity of climbing fibers also activates NMDARs and enhances GABA release for tens of minutes (Duguid and Smart 2004). By contrast, presynaptic glutamate receptors on stellate cells can be activated by burst stimulation of parallel fibers. Activation of NMDARs induces a long-lasting enhancement of GABA release (Liu and Lachamp 2006), whereas activation of AMPARs transiently suppresses the presynaptic release of GABA. How glutamate modulates GABA release appears to depend, in part, on the initial release probability of the particular synapse. The presynaptic release of GABA can be either transiently suppressed by the activation of AMPARs at synapses that have a high release probability or persistently enhanced by the activation of NMDARs if the initial release probability is low (Liu and Lachamp 2006). Thus the glutamatergic modulation of GABA release from stellate cells switches from a transient suppression to a lasting potentiation as the probability is low (Liu and Lachamp 2006). Thus the glutamatergic modulation of GABA release from stellate cells switches from a transient suppression to a lasting potentiation as the probability is low (Liu and Lachamp 2006). This work was supported by National Science Foundation Grant IBN-0344559.

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G R A N T S

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