Activation of Group I mGluRs Increases Spontaneous IPSC Frequency in Rat Frontal Cortex

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Chu, Zhiguo and John J. Hablitz. Activation of group I mGluRs increases spontaneous IPSC frequency in rat frontal cortex. J. Neurophysiol. 80: 621–627, 1998. The effect of metabotropic glutamate receptor (mGluR) activation on inhibitory synaptic transmission was examined by using whole cell patch-clamp recordings. Spontaneous (s) and miniature (m) inhibitory postsynaptic currents (IPSCs) were recorded from visually identified layer II/III pyramidal neurons in rat neocortex in vitro. Excitatory postsynaptic currents (EPSCs) were blocked by using bath application of 20 μM d(-)-2-amino-5-phosphonovaleric acid and 10 μM 6-cyano-7-nitroquinolinoxide-2,3-dione. In the presence of 1S,3R-1-aminoacyclopentane-1,3-dicarboxylic acid (30–100 μM), L-quisqualate (5 μM), and the group I selective mGluR agonist (S)-3,5-dihydroxyphenylglycine (100 μM), the frequency of sIPSCs was increased. Decay kinetics of sIPSCs were unaffected. No enhancement of mIPSCs was observed. Bath application of group II (2S,3S,4S-α-carboxycyclopropylglycine; 5 μM) and group III selective mGluR agonists (1S-2-amino-4-phosphonobutyric acid; 100 μM) had no detectable effects on the frequency or amplitude of sIPSCs. These findings indicate that activation of group I mGluRs (mGluR1 and/or mGluR5) enhances γ-aminobutyric acid–mediated synaptic inhibition in layer II/III pyramidal neurons in neocortex. The lack of effect on mIPSCs suggests a presynaptic action via excitation of inhibitory interneurons.

INTRODUCTION

Metabotropic glutamate receptors (mGluRs) are a heterogeneous family of G protein–coupled glutamate receptors that are linked to multiple second messenger systems. mGluRs are widely distributed in the brain. Activation of mGluRs can modulate a number of synaptic and membrane properties, producing both excitatory and inhibitory effects in many brain regions. Excitatory transmission has been reported to be both enhanced (Bortolotto and Collingridge 1993; Desai and Conn 1991; Hu and Storm 1992; Stratton et al. 1989) and reduced (Baskys and Malenka 1991; Burke and Hablitz 1994; Calabresi et al. 1992; Crepel et al. 1991; Desai et al. 1992; Glaum et al. 1992; Glaum and Miller 1992; Lohninger et al. 1993; Manzoni and Bockaert 1995) by mGluR activation. Similarly, inhibitory transmission can be depressed (Burke and Hablitz 1994; Calabresi et al. 1992; Desai and Conn 1991; Desai et al. 1992; Glaum and Miller 1992; Llano and Marty 1995) or enhanced (Llano and Marty 1995; Poncer et al. 1995; Sciancalepore et al. 1995). These disparate effects presumably reflect the diversity of mGluR subtypes and effector mechanisms, variable expression in different brain regions, and the use of an agonist 1S,3R-1-aminoacyclopentane-1,3-dicarboxylic acid (ACPD), which does not discriminate well between receptor mGluR subtypes.

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. GABA-mediated neurotransmission plays an important role in information processing in the neocortex (Luhmann and Prince 1991). Immunocytochemical studies indicate that GABA is localized in nonpyramidal aspiny or sparsely spiny interneurons that comprise ~20% of all neocortical neurons (Gabbott and Somogyi 1986; Hendry et al. 1987). We have shown previously that the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) in interneurons is increased by the mGluR agonists ACPD and quisqualic acid (Quis) (Zhou and Hablitz 1997). In current-clamp recordings, both Quis and ACPD induced a depolarization and action potential (AP) firing in neocortical interneurons. Because pyramidal neurons are the principal target of inhibitory interneurons, these findings predict that the frequency of sIPSCs should also be increased in pyramidal cells because of increased GABA release from inhibitory interneurons.

Because selective and potent agonists for specific groups of mGluRs recently have been developed, our goal was to determine if mGluR activation modulates inhibitory synaptic transmission in neocortical pyramidal neurons and to ascertain which group of mGluRs was involved. Some of these results were presented in abstract form (Chu et al. 1996).

METHODS

Slices of frontal neocortex were prepared from Sprague-Dawley rats (16–24 days old) by using techniques similar to those previously described (Andreasen and Hablitz 1994; Zhou and Hablitz 1996). In brief, rats of either sex were anesthetized with ketamine (100 mg/kg) and decapitated. The brains were quickly removed and immersed in ice-cold oxygenated saline for 30–60 s. Coronal slices (200–250 μm) were cut by a Vibratome and stored at room temperature (21–23°C). After a recovery period of ≈1 h, individual slices were transferred to a recording chamber mounted on the stage of a Zeiss Axioskop FS microscope. The chamber was continuously perfused with oxygenated saline at a rate of 1–2 ml/min. All recordings were obtained at room temperature.

The normal extracellular solution contained the following (in mM): 125 NaCl, 3.5 KCl, 1.25 NaH2PO4, 2.5 CaCl2, 1.3 MgSO4, 2.6 CaCl2, 26.3 P10, 35.8 glucose, and 0.25 NaHCO3.
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26 NaHCO₃, and 10 d-glucose. The solution was bubbled continuously with a mixture of 95% O₂-5% CO₂ to maintain a pH of 7.4. The pipette solution contained the following (in mM): 135 CsCl, 0.5 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 10 N-2-hydroxyethylpiperazine-N,N',N'-tetraacetic acid (HEPES), 2 MgATP, and 0.2 Na guanosine 5'-triphosphate (GTP). The pH of the pipette solution was adjusted to 7.3 with 1 M NaOH and osmolarity was adjusted to 270 mosM.

Patch pipettes were pulled from KG-33 glass (Garner Glass) on a Narishige Model PP-83 puller and had resistances of 3–4 MΩ. Whole cell voltage-clamp recordings were made with an Axopatch-200 amplifier. Signals were stored on video tape using a Neurodata DR-384 recorder, filtered at 1–2 kHz and digitized at 10–20 kHz. SCAN software was used for analysis. Spontaneous IPSCs were detected automatically. The frequency, peak amplitude, and decay time constant (τ) of detected events were analyzed. Decay τ's were calculated from single or double exponential fits of IPSC decay by using a modified Levenberg-Marquardt least-squares algorithm. Fits to both single and averaged (after aligning responses on the rising phase) events were made. All values are expressed as means ± SE. Statistical analysis used paired t-tests, one-way analysis of variance (ANOVA), and the Kolmogorov-Smirnov

FIG. 1. ACPD increases the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) in neocortical pyramidal neurons. Whole cell patch recordings of sIPSCs from layer II/III pyramidal cells in rat neocortical slices are shown. A holding potential at −70 mV was used. A: 10 consecutive traces under control conditions (left), 3 min after bath application of 30 μM 1,3,3-R-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD; middle) and 5 min after washing (right). The ACPD-induced increase in the frequency of sIPSCs was reversible on washout. B: averaged synaptic currents from 161 sIPSCs under control condition and 1,743 sIPSCs after ACPD application, respectively. Averages were calculated from consecutive traces over equivalent time periods. Double exponential fits are superimposed on the averaged sIPSCs with the parameters listed. ACPD (30 μM) had no significant effect on sIPSC kinetics. C: cumulative fraction plot of sIPSC interevent interval before (Control) and after ACPD application. ACPD caused a significant shift in the interevent interval distributions toward shorter intervals, indicating a significant increase in the frequency of sIPSCs [Kolmogorov-Smirnov (K-S) statistic indicated P < 0.01]. D: cumulative fraction plot of sIPSCs' amplitude during the control period and after ACPD application. There was no significant change (K-S statistic) in the sIPSC amplitude distribution after ACPD application.
statistic to determine significance of difference in probability distributions.

6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), D(-)-2-amino-5-phosphonovaleric acid (D-APV), and Quis were purchased from Cambridge Research Biochemicals. ACPD, (S)-3,5-dihydroxyphenylglycine (DHPG), 2S,3S,4S-α-carboxycyclopropyl-glycine (1-CCG-I), and L-2-amino-4-phosphonobutyric acid (L-AP4) were purchased from Tocris Neuramin. Bicuculline methiodide and tetrodotoxin (TTX) were obtained from Sigma. All compounds were applied via the bath and each neuron served as its own control.

RESULTS

Recordings were obtained from 106 layer II/III pyramidal neurons, identified by their anatomic location and morphological characteristics. Neurons 200- to 400-μm below the pial surface with typical pyramidal-shaped cell bodies and prominent apical dendrites were selected for recording.

IPSCs were recorded in isolation by using 10 μM CNQX and 20 μM APV to block ionotropic glutamate receptors. Under the ionic conditions employed, IPSCs recorded at the holding potential (−70 mV) were inward currents. Bath application of bicuculline methiodide (10 μM) blocked all synaptic currents recorded under these conditions indicating that they were mediated by GABA<sub>α</sub> receptors (n = 4).

1S,3R-ACPD increases spontaneous IPSC frequency

Figure 1A shows examples of sIPSCs recorded under control conditions, during bath application of 30 μM ACPD, and on drug washout. Application of the mGluR agonist ACPD (30–100 μM) increased the frequency of sIPSCs recorded in this and all cells tested (n = 12). These effects were dose dependent and reversible on drug washout. The decay kinetics of sIPSCs were not affected, as shown in Fig. 1A. ACPD produced a leftward shift in the distribution of sIPSC intervals, indicating an increase in frequency. The mean interevent interval was 3.6 ± 0.6 (SE) s in control and 1.0 ± 0.2 s after ACPD application (P < 0.01, paired t-test; n = 12). In contrast, sIPSC amplitudes were not significantly changed, as shown in Fig. 1D. The mean sIPSC amplitude was 38.3 ± 3.9 pA

![Figure 1](http://jn.physiology.org/)

**FIG. 1.** Effects of ACPD on miniature IPSCs (mIPSCs) recorded in the presence of tetrodotoxin (TTX; 0.5 μM). A: 10 consecutive traces under control conditions and 5 min after bath application of 30 μM ACPD. The metabotropic glutamate receptors (mGluRs) agonist ACPD (30 μM) had no effect on the frequency or amplitude of mIPSCs. B: averaged synaptic currents under control conditions (top) and after ACPD application (bottom). Double exponential fits are superimposed on the averaged sIPSCs with the parameters listed. ACPD (30 μM) has no significant effect on mIPSC kinetics. C and D: cumulative fraction plots of mIPSC interevent intervals and amplitudes under control conditions and during ACPD application, respectively. ACPD had no detectable effect on either frequency or amplitude of mIPSCs (K-S statistic was nonsignificant).
was reversible on washing. Figure 4B shows recordings of mIPSCs under control conditions and after application of 5 μM Quis. Similar to the findings with ACPD, no detectable change in mIPSC frequency was observed. Results obtained from 11 pyramidal neurons are summarized in Fig. 4C.

The above results suggest that group I mGluRs mediate the observed increase in sIPSC frequency. To verify this and determine whether group II and III receptors were involved, the effect of group specific agonists was examined. As shown in Fig. 5A, the group I selective agonist DHPG (100 μM) (Pin and Duvoisin 1995) significantly increased the frequency of sIPSCs. The results with DHPG are summarized in Fig. 5B; although sIPSC interevent intervals were significantly reduced, amplitudes were not changed. DHPG did not significantly affect mIPSC frequency (Fig. 5, C and D) or amplitude (Fig. 5D).

L-CCG-I (5 μM), an agonist for group II mGluRs (Pin and Duvoisin 1995), had no detectable effect on the frequency (Fig. 6A, left) or amplitude (Fig. 6A, right) of sIPSCs (n = 10). L-AP4 (100–500 μM), a selective activator of group III mGluRs (Pin and Duvoisin 1995), did not alter sIPSC frequency (Fig. 6B, left) or amplitude (Fig. 6B, right; n = 9).

DISCUSSION

The principal finding of this study was that the frequency of sIPSCs in layer II/III neocortical pyramidal cells was enhanced by activation of group I mGluRs. This increase in sIPSC frequency was presumably due to a direct depolarization of GABAergic interneurons and an increase in AP-dependent release of neurotransmitter (Zhou and Hablitz 1997). The lack of effect of mGluR activation on mIPSCs is consistent with such a mechanism.

In the present study, DHPG and Quis reliably increased the frequency of sIPSCs, suggesting the involvement of group I mGluRs. The largest increase in sIPSC frequency as observed with Quis, a more potent agonist of group I receptors. This finding is consistent with the results obtained in the CA1 region of the hippocampus showing that the rank order of agonist potency for group I mGluR-mediated inhibition of afterhyperpolarizations was Quis > glutamate > ACPD ≈ DHPG (Gereau and Conn 1995). The lack of effect of L-CCG-I (agonist for group II mGluRs) and L-AP4 (selective activator of group III mGluRs) on sIPSC frequency also supports the involvement of group I receptors.

Our previous studies of the effects of mGluR activation on neocortical interneurons demonstrated a direct depolarizing action of Quis and ACPD (Zhou and Hablitz 1997). APs resulting from this depolarization would be expected to increase the frequency of spontaneous, presumably AP-dependent, mIPSCs. Alternatively, the increased frequency of IPSCs could result from a direct effect of mGluRs on the presynaptic terminal. Activation of group I mGluRs (mGluR1 and mGluR5) can activate phospholipase C and release Ca^{2+} from intracellular stores through the inositol(1,4,5)trisphosphate (InsP_{3}) pathway (Abe et al. 1992; Aramori and Nakanishi 1992; Masu et al. 1991; Nakanishi 1992; Schoepp and Conn 1993). Protein kinase C (PKC) phosphorylation
FIG. 4. Effects of quisqualic acid (Quis) on sIPSCs and mIPSCs. A: Quis reversibly increases sIPSC frequency in neocortical pyramidal neurons. Continuous records of sIPSCs under control conditions (left), 2 min after bath application of 5 μM Quis (middle), and 5 min after washout (right). Increases were observed in all cells tested (n = 22). B: effects of Quis on mIPSCs in neocortical pyramidal cells. Eight consecutive traces are shown under control condition and 2 min after bath application of Quis (5 μM). C: summary histograms showing effects of Quis on mIPSC interevent interval and amplitude (n = 11).

of mGlurRs can produce oscillations in intracellular Ca^{2+} (Kawabata et al. 1996). Because transmitter release from GABAergic synapses is thought to be Ca^{2+} dependent, mGlurR modulation of intracellular Ca^{2+} could affect GABA release (Stefani et al. 1994). However, the lack of effect of mGlurR activation on IPSC amplitude or frequency of mIPSCs makes an action at the presynaptic terminal seem unlikely. The most likely mechanism for the observed increase in sIPSC frequency is an increase in the firing of inhibitory interneurons.

L-AP4 preferentially activates group III mGlurRs (Pin and Duvoisin 1995). These receptors are generally located presynaptically and reduce synaptic transmission in a variety of brain regions (Burke and Hablitz 1994; Macek et al. 1996; Salt and Eaton 1995). The failure of L-AP4 to increase sIPSC frequency in the present study argues against a contribution of group III receptors to the observed effects of Quis and ACPD. L-AP4 has been reported to presynaptically depress evoked inhibitory synaptic transmission in neocortex (Burke and Hablitz 1994). However, no effect of L-AP4 on sIPSC amplitude was observed in the present experiments. It is possible that L-AP4 reduced evoked transmission via a reduction of the excitatory input to interneurons. Group II mGlurRs are preferentially activated by low concentrations of L-CCG-I (Pin and Duvoisin 1995). Application of this drug did not affect sIPSC frequency or amplitude, suggesting that group II receptors are not involved. The observed pharmacological profile is therefore consistent with an action of group I receptors.

Although excitation of inhibitory interneurons appears to be involved, the exact effector mechanism whereby activation of group I mGlurRs results in an increase of sIPSC frequency is unclear. It has been suggested that, in hippocampal CA1 neurons, the effect is mediated through a adeno-
FIG. 5. Effects of the group I mGluR agonist DHPG on sIPSCs and mIPSCs in layer II/III pyramidal neurons. A: activation of group I mGluR by DHPG produces an increase in sIPSC frequency. Left panel: control recordings; middle panel: records were obtained 2 min after application of 100 μM DHPG; right panel: records taken 5 min after drug removal. B: summary of DHPG effects on interevent intervals and amplitudes (n = 11). C: records showing that 100 μM DHPG does not increase mIPSC frequency (n = 9). D: summary data showing the lack of effect of DHPG on mIPSC interval or amplitude.

FIG. 6. Lack of effect of group II and III mGluR agonists on sIPSCs. A: histograms showing average sIPSC interevent intervals and amplitudes under control conditions and after 2S,3S,4S-α-carboxycyclopropyl-glycine (L-CCG-I). B: similar plots from experiments with L-2-amino-4-phosphonobutyric acid (L-AP4).
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