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GROUP II METABOTROPIC GLUTAMATE RECEPTORS INHIBIT GLUTAMATE RELEASE AT THALAMOCORTICAL SYNAPSES IN THE DEVELOPING SOMATOSENSORY CORTEX

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Abstract—Thalamocortical synapses provide a strong glutamatergic excitation to cortical neurons that is critical for processing sensory information. Unit recordings *in vivo* indicate that metabotropic glutamate receptors (mGluRs) reduce the effect of thalamocortical input on cortical circuits. However, it is not known whether this reduction is due to a reduction in glutamate release from thalamocortical terminals or from a decrease in cortical neuron excitability. To directly determine whether mGluRs act as autoreceptors on thalamocortical terminals, we examined the effect of mGluR agonists on thalamocortical synapses in slices. Thalamocortical excitatory postsynaptic currents (EPSCs) were recorded in layer IV cortical neurons in developing mouse brain slices. The activation of group II mGluRs with (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG IV) reduced thalamocortical EPSCs in both excitatory and inhibitory neurons, while the stimulation of group I or group III mGluRs had no effect on thalamocortical EPSCs. Consistent with a reduction in glutamate release, DCG IV increased the paired pulse ratio and the coefficient of variation of the EPSCs. The reduction induced by DCG IV was reversed by the group II mGluR antagonist, LY341495, and mimicked by another selective group II agonist, (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylic acid (APDC). The mGluR2 subtype appears to mediate the reduction of thalamocortical EPSCs, since the selective mGluR3 agonist, N-acetylaspartylglutamate (NAAG), had no effect on the EPSCs. Consistent with this, we showed that mGluR2 is expressed in the barrels. Furthermore, blocking group II mGluRs with LY341495 reduced the synaptic depression induced by a short stimulus train, indicating that synaptically released glutamate activates these receptors. These results indicate that group II mGluRs modulate thalamocortical processing by inhibiting glutamate release from thalamocortical synapses. This inhibition provides a feedback mechanism for preventing excessive excitation of cortical neurons that could play a role in the plasticity and refinement of thalamocortical connections during this early developmental period.

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Abbreviations: ACSF, artificial cerebrospinal fluid; APDC, (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylic acid; AP5, DL-2-amino-5-phosphonopentanoic acid; BSA, bovine serum albumin; CV, coefficient of variation; DCG IV, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine; DHPG, 3,5-dihydroxyphenylglycine; EPSCs, excitatory postsynaptic currents; GCP II, glutamate carboxypeptidase II; L-AP4, L-(+)-2-amino-4-phosphonobutyric acid; mGluRs, metabotropic glutamate receptors; NAAG, N-acetylaspartylglutamate; NIH, National Institutes of Health; NMDA, *N*-methyl-D-aspartate; PMPA, phosphonomethyl-pentanedioic acid; PPR, paired pulse ratio; TBS, tris-buffered saline.

Key words: glutamate, interneurons, barrel cortex.

Thalamic stimulation of cortical circuits is the initial step in the cortical processing of sensory information. Thalamocortical terminals excite cortical neurons via the release of glutamate which activates AMPA and *N*-methyl-D-aspartate (NMDA) receptors on the cortical neurons (Castro-Alamancos and Connors, 1997). To monitor and regulate the amount of glutamate released, many glutamatergic terminals express presynaptic metabotropic glutamate receptors (mGluRs), which are divided into three groups based on sequence homology, pharmacological profiles, and biochemical signaling (Conn and Pin, 1997; Anwyl, 1999). Group II and III mGluRs decrease the release of glutamate (Pisani et al., 1997; Poisik et al., 2005), while group I mGluRs either enhance (Reid et al., 1999; Schwartz and Alford, 2000) or reduce the release of glutamate (White et al., 2003).

Previous experiments suggest that mGluRs could be modulating thalamocortical inputs within the rodent somatosensory system. In rodents, fibers arising from the ventrobasal nucleus of the thalamus project in a well-organized somatotopic pattern to specialized structures in layer IV known as barrels (Woolsey and Van der Loos, 1970). Each barrel receives a dense thalamic input conveying sensory information from a single whisker on the rodent snout. Immunohistochemical studies have demonstrated that group I and group II mGluRs are densely expressed in the barrel hollows in layer IV of the somatosensory cortex where the thalamocortical terminals are located (Ohishi et al., 1998; Muñoz et al., 1999). These studies indicate that mGluRs are expressed in the vicinity of thalamocortical synapses, but they did not determine whether the mGluRs were expressed on the thalamocortical terminals or on the cortical neurons. In addition, the stimulation of mGluRs *in vivo* reduces cortical activation induced by both somatosensory and visual stimuli (Cahusac, 1994; Taylor and Cahusac, 1994; Beaver et al., 1999), indicating that mGluRs modulate the cortical processing of sensory information. These experiments demonstrate that mGluRs decrease thalamocortical excitation; however, they did not determine whether the reduction is mediated by a presynaptic decrease in the release of glutamate from thalamocortical terminals or by a postsynaptic hyperpolarization of the cortical neurons.

To directly determine whether mGluRs inhibit glutamate release from thalamocortical synapses, we used the thalamocortical slice preparation (Agmon and Connors,

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1991; Porter and Nieves, 2004) which provides better access to thalamocortical circuits.

EXPERIMENTAL PROCEDURES

Thalamocortical slice preparation

Young mice from postnatal days 6–12 were obtained from our breeding facility at the Ponce School of Medicine. In compliance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (Publication DHHS NIH 86-23), the Institutional Animal Care and Use Committee of the Ponce School of Medicine approved all procedures involving animals. All efforts were made to minimize the number of animals used and their suffering. The animals were anesthetized with halothane, decapitated, and the brains were removed and placed in ice-cold oxygenated artificial cerebrospinal fluid (ACSF) containing 126 mM $NaCl_2$, 3 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 26 mM $NaHCO_3$, and 20 mM glucose, bubbled with carbogen (95% O_2 and 5% CO_2). Brains slices of 300–400 μm thickness were prepared with a Vibratome 1000 Plus (Vibratome, St. Louis, MO, USA) by standard procedures to preserve thalamocortical connectivity (Agmon and Connors, 1991; Porter et al., 2001). Slices containing both the ventrobasal thalamic nucleus and the barrel cortex were saved and incubated for 1 h at room temperature in ACSF.

Electrophysiology

After the 1 h incubation, individual slices were transferred to a submersion recording chamber mounted on a E600 upright microscope (Nikon Instruments, Melville, NY, USA), and perfused with room temperature, carbogenated ACSF at a rate of 2–3 ml/min. To stimulate thalamocortical axons, we placed a bipolar tungsten microelectrode in the ventrobasal nucleus or internal capsule under brightfield illumination to view the anatomical landmarks of the thalamocortical pathway. Excitatory postsynaptic currents (EPSCs) were recorded with a patch-clamp amplifier (MultiClamp 700A, Axon Instruments, Union City, CA, USA) using glass micropipettes that were pulled on a Flaming/Brown micropipette puller (Sutter Instruments, Novato, CA, USA). The positions of the stimulating electrode and micropipettes were adjusted to maximize EPSC amplitude. Paired stimuli separated by 50 ms were given every 10–15 ms.

Single neurons were visually identified using a video camera (Dage MTI, Michigan City, IN, USA) connected to the Nikon E600 microscope. All neurons included in this study were located in layer IV of the barrel cortex. Whole-cell current-clamp and voltage-clamp recordings were done with patch pipettes filled with an internal solution containing 12 mM KCl, 140 mM K-gluconate, 0.2 mM EGTA, 10 mM Hepes, 0.3 mM GTP and 0.4 mM ATP (pH 7.3, 285 mOsm). Resting membrane potential was measured after achieving whole cell configuration and cells with a membrane potential more depolarized than −50 mV were discarded. To obtain an intrinsic firing pattern an initial hyperpolarizing square current step was given followed by depolarizing current steps in current-clamp mode. Recordings were filtered at 4 kHz, digitized at 10 kHz, and saved to a computer using pCLAMP9 software (Axon Instruments). Membrane potentials were not corrected for the junction potential. Recordings were not compensated for series resistance, but changes in series resistance were continuously monitored and recordings were eliminated from analysis if the series resistance changed by more than 15%. As previously described (Agmon et al., 1996; Beierlein and Connors, 2002), evoked EPSCs which exhibited latencies that varied by less than 1 ms were considered monosynaptic thalamocortical EPSCs and were included in the analysis. The latencies of the evoked EPSCs showed an average latency from the beginning of the stimulation artifact of 6.2±0.03 ms in slices from mice 6–12 postnatal days old (n=44). These latencies are consistent with the latencies of thalamocortical EPSCs evoked by stimulation of either the ventrobasal nucleus or the internal capsule and measured in slices from similarly aged animals (Agmon and O'Dowd, 1992; Crair and Malenka, 1995; Agmon et al., 1996; Lu et al., 2001; Laurent et al., 2002). Since a previous study indicated that corticothalamic EPSCs exhibit paired pulse facilitation while thalamocortical EPSCs exhibit paired pulse depression (Beierlein and Connors, 2002), we only included inputs which displayed paired pulse depression in our analysis. To examine the effect of mGluR activation on EPSCs, we diluted the drugs in ACSF containing 100 μM DL-2-amino-5-phosphonopentanoic acid (AP5) and 10 μM bicuculline to block NMDA and $GABA_A$ currents, respectively.

LY 341495, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG IV), 3,5-dihydroxyphenylglycine (DHPG), L-(+)-2-amino-4-phoshonobutyric acid (L-AP4), (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylic acid (APDC), and N-acetylaspartylglutamate (NAAG) were purchased from Tocris (Ellisville, MO, USA). Bicuculline and AP5 were purchased from Sigma (St. Louis, MO, USA).

Data analysis and statistics

Experiments were accepted for analysis if the effect of the mGluR agonist on the EPSCs reversed upon removal of the drug and there was no confounding di- or polysynaptic activity in the EPSCs. Data were analyzed using Clampfit (Axon Instruments). Average EPSCs were taken from 10 consecutive traces including failures. The paired pulse ratio (PPR) was calculated from EPSCs evoked by paired stimuli as the mean amplitude of the EPSC evoked by the second stimulus (EPSC2) divided by the mean amplitude of the EPSC evoked by the first stimulus (EPSC1). The coefficient of variation (CV) was calculated as the standard deviation of the EPSC amplitude divided by the mean EPSC amplitude from 10 consecutive traces including failures. Data were analyzed using Student's t-test or repeated measures ANOVA (Statistica, Statsoft, Tulsa, OK, USA). After a significant main effect, post hoc comparisons were done using the Tukey HSD test. Statistical significance was set at $P<0.05$. Values are reported as the mean±the standard error of the mean (S.E.M.).

mGluR2 immunohistochemistry

Mice (P8-9) were anesthetized with halothane, decapitated, and the brains were removed and fixed in a 4% paraformaldehyde and sucrose solution overnight. After fixing overnight, slices (80 μm in thickness) were prepared with a freezing microtome (Microm HM 400, Fisher Scientific, Pittsburgh, PA, USA) and rinsed with tris-buffered saline (TBS) for an hour. Endogenous peroxidases were inhibited by incubating slices with 0.3% H_2O_2 in TBS for 30 min. To enhance epitope exposure the slices were incubated for 30 min in TBS preheated to 80 °C in a water bath (Jiao et al., 1999). Nonspecific binding was prevented by incubating the slices in a blocking solution containing 0.25% Triton X-100 and 5% bovine serum albumin (BSA) in TBS for 1 h. A mouse anti-mGluR2 antibody (Advance Targeting System Inc., San Diego, CA, USA) was diluted (1 μg/ml) in TBS containing 1% BSA and 0.25% Triton X-100 and incubated overnight. The next day, the slices were washed with TBS and incubated overnight with a biotinylated anti-mouse antibody (Vector Laboratories, Burlingame, CA, USA) diluted (10 μg/ml) in TBS containing 1% BSA and 0.25% Triton X-100. Next, slices were washed with PBS and revealed with a standard avidin–biotin peroxidase procedure (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA) as previously described (Porter et al., 2001). In control slices the same protocol was followed except the primary antibody against mGluR2 was omitted. In the absence of the primary antibody no staining was observed. Images of the immunoreactivity were taken with a cooled CCD camera (CoolSNAPcf, Roper Scientific, Trenton, NJ, USA) using MetaMorph software version 6.3r7 (Molecular Devices Co., Downingtown, PA, USA).