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ACTIVATION OF BOTH GROUP I AND GROUP II METABOTROPIC GLUTAMATERGIC RECEPTORS SUPPRESS RETINOGENICULATE TRANSMISSION

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Abstract—Relay cells of dorsal lateral geniculate nucleus (LGN) receive a Class 1 glutamatergic input from the retina and a Class 2 input from cortical layer 6. Among the properties of Class 2 synapses is the ability to activate metabotropic glutamate receptors (mGluRs), and mGluR activation is known to affect thalamocortical transmission via regulating retinogeniculate and thalamocortical synapses. Using brain slices, we studied the effects of Group I (dihydroxyphenylglycine) and Group II ((2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine) mGluR agonists on retinogeniculate synapses. We showed that both agonists inhibit retinogeniculate excitatory postsynaptic currents (EPSCs) through presynaptic mechanisms, and their effects are additive and independent. We also found high-frequency stimulation of the layer 6 corticothalamic input produced a similar suppression of retinogeniculate EPSCs, suggesting layer 6 projection to LGN as a plausible source of activating these presynaptic mGluRs.

Key words: lateral geniculate nucleus, corticothalamic, thalamus.

INTRODUCTION

Glutamatergic inputs in the thalamus and cortex have been classified into two types: Class 1 and Class 2. Class 1 inputs are thought to provide the main route for information transfer, whereas Class 2 inputs are thought to serve a generally modulatory function (reviewed in Sherman and Guillery (2006) and Sherman (2012)). One of the modulatory properties of these Class 2 inputs is their ability to activate metabotropic glutamate receptors (mGluRs). Several studies of cortical circuitry indicate that Class 2 inputs there can activate mGluRs that act to reduce the amplitude of synaptic transmission from Class 1 inputs (Lee and Sherman, 2009, 2012; DePasquale and Sherman, 2012). Since one of the first defined Class 2 pathways is the layer 6 corticothalamic input to thalamic relay cells (Reichova and Sherman, 2004), and since there is recent evidence that activation of presynaptic mGluRs on retinal terminals can suppress retinogeniculate transmission (Govindaiah et al., 2012; Hauser et al., 2013), we sought to expand on this observation in brain slices from mice by further characterizing the role of mGluRs on retinogeniculate transmission and determining the role layer 6 input might have in this process. A preliminary report of these studies has been made (Lam and Sherman, 2011b).

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Abbreviations: ACSF, artificial cerebrospinal fluid; DCG IV, (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine; DHPG, dihydroxy phenylglycine; EGTA, ethylene glycol tetraacetic acid; EPSCs, excitatory postsynaptic currents; GDP-β-S, guanosine 5′-[β-thio]diphosphate; GTP, guanosine triphosphate; HFS, high-frequency stimulation; LGN, lateral geniculate nucleus; mGluRs, metabotropic glutamate receptors.

EXPERIMENTAL PROCEDURES

Preparation of brain slices

Our procedures followed the animal care guidelines of the University of Chicago and closely followed our previously published methodology (Lam and Sherman, 2005, 2012; DePasquale and Sherman, 2012). BALB/c mice (Harlan) of ages 12–21 days postnatal were deeply anaesthetized by inhalation of isoflurane, and their brains were quickly removed and chilled in ice-cold artificial cerebrospinal fluid (ACSF), which contained (in mM): 125 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$, 25 glucose. Their brains were sliced at 500 μm using a vibrating tissue slicer (Campden Instruments, Loughborough, UK). The slices were cut either coronally (Fig. 1A) or parasagittally at an angle that preserved both corticothalamic and retinogeniculate inputs to the dorsal lateral geniculate nucleus (LGN, Fig. 1B; Turner and Salt, 1998). These slices were then transferred to a holding chamber containing oxygenated ACSF and incubated at 30 °C for at least 1 h before each experiment.

Physiological recording

A few threads of nylon filaments, attached to a platinum wire slice holder, were used to secure the slices in the bath during the experiment. The slice was carefully placed during the experiment so that the nylon threads did not interfere with electrophysiological recording and electrical stimulation.

The LGN was identified in the slice by its location and the presence of the optic tract at its lateral edge.

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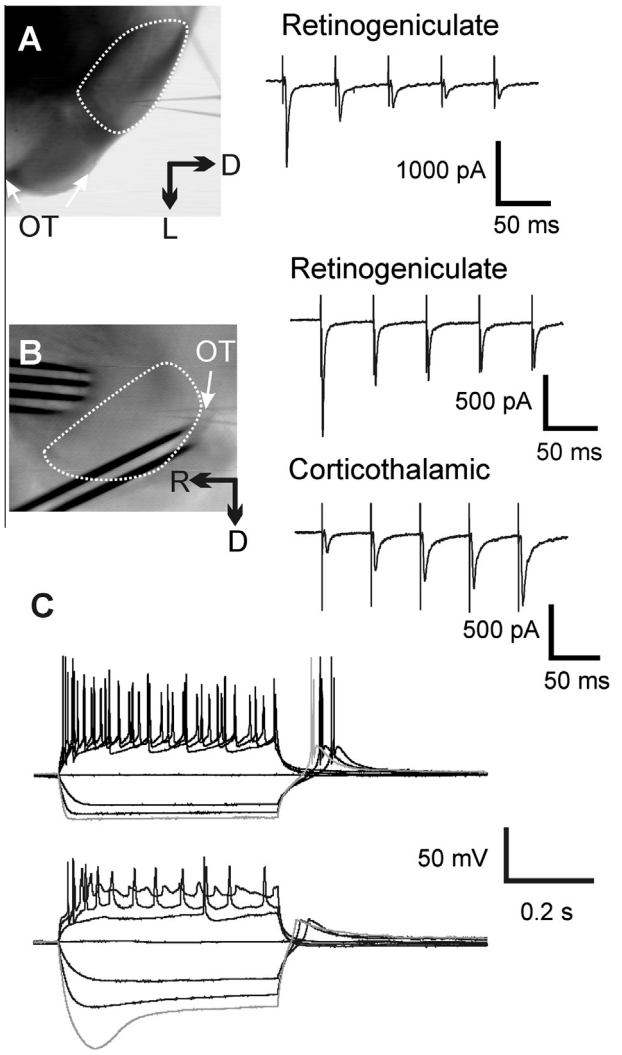

Fig. 1. Experimental setup and methods. (A) Left, photomicrograph taken during a recording from a coronal slice. Right, an example of the EPSC response to optic tract stimulation. (B) Left, photomicrograph taken during a recording from a parasagittal slice. Right, response to optic tract (upper) and corticothalamic axon (lower) stimulation. The dotted line in A and B encircles the LGN and white arrows indicate the location of optic tract (OT). (C) Response of a relay cell (upper) and an interneuron (lower) to current step injection. Interneurons can be distinguished by a distinctive ''sag'' in their response to hyperpolarizing current injection (gray traces).

Retinogeniculate synapses were stimulated using a bipolar electrode straddling the optic tract. For coronal slices, the electrode was placed at a location further ventral to the region shown in Fig. 1A, and so it is not visible in the photomicrograph. The corticothalamic pathway was stimulated by placing a 4×1 electrode array across the incoming corticothalamic axons, near the LGN (Fig. 1B) and the two electrodes with the lowest stimulation response threshold were used for bipolar stimulation. Electric current was generated using a stimulus isolator (A365, World Precision Instrument, Sarasota, FL, USA). Response threshold to optic tract thresholds were determined before each experiment, and the stimulation intensity used was 150–250% above threshold, which turned out to be between 40 and 200 μA.

Whole cell recordings were performed at room temperature (22 °C) using a visualized slice setup (Cox and Sherman, 2000; Lam and Sherman, 2005). Recording pipettes were pulled from borosilicate glass capillaries and had a tip resistance of 3–6 MΩ when filled with a pipette solution containing the following (in mM): 127 K-gluconate, 3 KCl, 1 $MgCl_2$, 0.07 $CaCl_2$, 10 HEPES, 2 Na_2-ATP, 0.3 Na-guanosine triphosphate (Na-GTP), 0.1 EGTA. The pH of the pipette solution was adjusted to 7.3 with KOH or gluconic acid, and the osmolality was 280–290 mOsm.

The experiments were performed in voltage-clamp mode at a holding potential of −60 mV, using an Axopatch 200B (Molecular Devices, Sunnyvale, CA, USA). The access resistance of each cell was constantly monitored throughout the recordings, and experiments were discontinued if the access resistance exceeded 30 MΩ. Gabazine (20 μM, SR95531) was included in the ACSF to prevent any disynaptic IPSCs from contaminating the results.

The LGN of mice contains both relay cells and interneurons (Arcelli et al., 1997), and so we identified interneurons by the presence of a distinctive ''sag'' in their response to hyperpolarization current injection (Fig. 1C, Pape and McCormick, 1995; Zhu et al., 1999; Govindaiah and Cox, 2006).We did not study these cells further, and thus all data reported here are from relay cells.

Photostimulation

Methods for photostimulation have been described by us previously (Lam and Sherman, 2005, 2007, 2010, 2011a) and are briefly outlined here. Data acquisition and photostimulation were controlled by the program Tidalwave (Shepherd et al., 2003). Nitroindolinyl (NI)-caged glutamate (Canepari et al., 2001) was added to the recirculating ACSF to a concentration of 0.39 mM during recording. Focal photolysis of the caged glutamate was accomplished by a 2-ms pulsed UV laser (355-nm wavelength, frequency-tripled Nd:YVO4, 100-kHz pulse repetition rate, DPSS Laser, San Jose, CA, USA). The laser beam was directed into the side port of a double-port tube (U-DPTS) on top of an Olympus microscope (BX50WI) using UV-enhanced aluminum mirrors (Thorlabs, Newton, NJ, USA) and a pair of mirror-galvanometers (Cambridge Technology, Cambridge, MA, USA) and then focused onto the soma of the recording cells using a low-magnification objective (4×0.1 Plan, Olympus).

Chemicals

Various agents were bath applied, including: the Group I mGluR agonist, (R,S)-3,5-dihydroxyphenylglycine (DHPG); the Group II mGluR agonist (2S,2′R,3′R)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG IV); the $GABA_A$ antagonist gabazine (SR 95531 hydrobromide); and the $GABA_B$ antagonist (3-minopropyl)(cyclohexylmethyl) phosphinic acid (CGP 46381). The G-protein antagonist GDP-β-S (Guanosine 5′-[β-thio]diphosphate) was included in the pipette solution for some cells to block postsynaptic