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Presynaptic Inhibition of Corticothalamic Feedback by Metabotropic Glutamate Receptors

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Alexander, Georgia M. and Dwayne W. Godwin. Presynaptic inhibition of corticothalamic feedback by metabotropic glutamate receptors. *J Neurophysiol* 94: 163–175, 2005. First published March 16, 2005; doi:10.1152/jn.01198.2004. The thalamus relays sensory information to cortex, but this information may be influenced by excitatory feedback from cortical layer VI. The full importance of this feedback has only recently been explored, but among its possible functions are influences on the processing of sensory features, synchronization of thalamic firing, and transitions in response mode of thalamic relay cells. Uncontrolled, corticothalamic feedback has also been implicated in pathological thalamic rhythms associated with certain neurological disorders. We have found a form of presynaptic inhibition of corticothalamic synaptic transmission that is mediated by a Group II metabotropic glutamate receptor (mGluR) and activated by high-frequency corticothalamic activity. We tested putative retinogeniculate and corticogeniculate synapses for Group II mGluR modulation within the dorsal lateral geniculate nucleus of the ferret thalamus. Stimulation of optic-tract fibers elicited paired-pulse depression of excitatory postsynaptic currents (EPSCs), whereas stimulation of the optic radiations elicited paired-pulse facilitation. Paired-pulse responses were subsequently used to characterize the pathway of origin of stimulated synapses. Group II mGluR agonists (LY379268 and DCG-IV) applied to thalamic neurons under voltage-clamp conditions reduced the amplitude of corticogeniculate EPSCs. Stimulation with high-frequency trains produced a facilitating response that was reduced by Group II mGluR agonists, but was enhanced by the selective antagonist LY341495, revealing a presynaptic, mGluR-mediated reduction of high-frequency corticogeniculate feedback. Agonist treatment did not affect EPSCs from stimulation of the optic tract. NAAG (reported to be selective for mGluR3) was ineffective at the corticogeniculate synapse, implicating mGluR2 in the observed effects. Our data are the first to show a synaptically elicited form of presynaptic inhibition of corticothalamic synaptic transmission that is mediated by presynaptic action of mGluR2. This presynaptic inhibition may partially mute sensory feedback and prevent reentrant excitation from initiating abnormal thalamic rhythms.

INTRODUCTION

The dorsal lateral geniculate nucleus (LGN) of the thalamus receives massive excitatory feedback from visual cortex. Among the possible functions of this feedback are influences on the processing of sensory features, synchronization of thalamic firing, and transition in the response mode of thalamic relay cells (Emri et al. 2003; Godwin et al. 1996b; Jones and Sillito 1991; Murphy et al. 1999; Rivadulla et al. 2002; Sillito and Murphy 1988). In a different context, corticothalamic

feedback is implicated in abnormal thalamic rhythms associated with certain neurological disorders (Avoli and Kostopoulos 1982; Bal et al. 2000; Blumenfeld and McCormick 2000). In normal circumstances this glutamatergic feedback appears to be controlled, in part, through the distributed nature of the feedback projection and the low probability of glutamate release at these synapses compared with the retinal glutamatergic inputs (Granseth and Lindstrom 2003).

Corticothalamic feedback is also controlled by G-protein-coupled metabotropic glutamate receptors (mGluRs) that may dynamically amplify or mute the influence of cortex on the complement of ionotropic glutamate receptors at the corticothalamic synapse. Eight distinct mGluRs, along with a number of splice variants, have been reported in the CNS (Sladeczek et al. 1985; Sugiyama et al. 1987; Tanabe et al. 1992). These have been organized into 3 groups based on sequence homology, second-messenger system involvement, and pharmacology (Conn and Pin 1997). Of the 3 groups, only Group I and Group III mGluRs have been characterized at the corticothalamic synapse.

Group I mGluRs (mGluR1 and 5) stimulate phosphoinositide hydrolysis and phospholipase C, and are generally located postsynaptically (see Conn and Pin 1997). Within the LGN, mGluR1 is found on relay cell distal dendrites that are postsynaptic to axon terminals from cortical layer VI (Godwin et al. 1996a; Vidnyanszky et al. 1996). High-frequency synaptic activation stimulates mGluR1 and produces a slow excitatory postsynaptic potential (EPSP) that shifts LGN cells from burst to tonic mode of firing (Godwin et al. 1996b; Golshani et al. 1998; Lee and McCormick 1997; McCormick and von Krosigk 1992; Turner and Salt 1998; von Krosigk et al. 1999). mGluR5 is found on GABAergic terminals of local interneurons that receive retinal input (Godwin et al. 1996a), and activation of these receptors has been shown to produce local inhibitory postsynaptic potentials in relay cells (Cox et al. 1998; Govindaiah and Cox 2004).

Group III mGluRs (mGluR4, 6, 7, and 8), in addition to Group II mGluRs (mGluR2 and 3), inhibit cAMP signaling and are generally found at presynaptic sites in the CNS (see Conn and Pin 1997). Group III mGluR activity has been reported at the corticogeniculate synapse on the basis of pharmacological reduction of the corticogeniculate EPSP, possibly through a presynaptic mechanism. However, endogenous activation of Group III mGluRs through high-frequency stimulation of the corticogeniculate pathway could not be demonstrated (Turner

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and Salt 1999). Group III mGluRs have also been shown to inhibit thalamic reticular nucleus (TRN) input to thalamic relays (Salt and Eaton 1995a). The role of Group II mGluRs are less understood in the LGN. Group II mGluRs have been reported to be present in rat thalamic relay nuclei, with staining localized to profiles resembling glial processes, GABAergic terminals, and cortical terminals (Liu et al. 1998; Mineff and Valtchanoff 1999; Tamaru et al. 1992). Activation of Group II mGluRs has been shown to reduce inhibition in the ventrobasal thalamus arising from the adjacent TRN (Salt and Eaton 1995a,b), consistent with the staining of GABAergic terminals. However, physiological involvement of Group II mGluRs has not been assessed at corticothalamic terminals. Given the absence of immunocytochemical evidence for Group II receptors at retinal terminals, there is an intriguing possibility of a selective, presynaptic modulation of corticothalamic neurotransmission by this group of receptors. Their presynaptic location, sensitivity to glutamate, and physiological characterization in other systems (Kew et al. 2001; Kilbride et al. 1998) suggest that the influence of Group II mGluRs may be inhibitory.

Using whole cell patch-clamp recordings we tested the hypothesis that Group II mGluRs selectively reduce corticogeniculate glutamatergic neurotransmission. Stimulating retinal or cortical pathways to LGN relay cells while pharmacologically activating Group II mGluRs revealed a reduction of corticogeniculate neurotransmission, with no effect on retinogeniculate transmission. Train stimulation of the corticogeniculate pathway engaged Group II mGluRs, as evidenced by Group II mGluR pharmacological blockade, which elicited larger train responses. Group II mGluR reduction of corticogeniculate excitatory postsynaptic currents (EPSCs) could not be produced by pharmacologically active concentrations of *N*-acetyl-L-aspartyl-L-glutamic acid (NAAG), a selective mGluR3 agonist, implicating mGluR2 in the reduction of the synaptically elicited Group II mGluR effects that we observed. The presence of a frequency sensitive Group II mGluR at corticogeniculate presynaptic terminals is a novel, pathway-selective mechanism that reduces cortical feedback to the thalamus.

METHODS

Slice preparation

Male ferrets older than postnatal day 42 were anesthetized with halothane and decapitated in accordance with the Wake Forest University Animal Care and Use Committee and in agreement with National Institutes of Health and U.S. Department of Agriculture guidelines. The brain was rapidly removed and immersed in oxygenated (95% O₂-5% CO₂), ice cold sucrose substituted artificial cerebrospinal fluid (ACSF) containing (in mM): 220 sucrose, 12 MgSO₄, 10 glucose, 2 KCl, 1.5 NaH₂PO₄, 26 NaHCO₃, 0.2 CaCl₂ (pH 7.4, osmolarity 290–300 mOsm). A block of tissue containing the LGN was sectioned sagittally on a vibratome (model OTS 4000, Electron Microscopy Sciences, Fort Washington, PA) at 400 μm, and slices were maintained in oxygenated, warm (34°C) ACSF containing (in mM): 124 NaCl, 5 KCl, 2 MgSO₄, 2 CaCl₂, 23 NaHCO₃, 3 NaH₂PO₄, 10 glucose (pH 7.4, osmolarity 290–300 mOsm), for ≥1.5 h before being transferred to a submerged recording chamber for recordings (Harvard Apparatus, Holliston, MA).

Recordings

During recording, thalamic slices were continuously perfused with oxygenated ACSF at a flow rate of 1.5–2 ml/min. Drugs were infused into the ACSF by a calibrated syringe pump. Recordings were made from either lamina A or A1 of the LGN. Patch pipettes (5–10 MΩ) were pulled from borosilicate glass (Sutter Instruments, Novato CA) with a PC-10 vertical puller (Narishige International USA, East Meadow, NY), and were filled with an internal solution containing (in mM): 100 gluconic acid, 100 CsOH, 10 NaCl, 10 HEPES, 20 TEA-Cl, 1 EGTA, 4 ATP (pH 7.3 with 2N CsOH, osmolarity 270–290 mOsm). A liquid junction potential of +14 mV was corrected for post hoc. For experiments using the G-protein blocker GDPβS, 1 mM GDPβS was added to internal solution. Train stimulation experiments were run at physiological temperature (34°C), and all other recordings were made at room temperature. To minimize the *N*-methyl-D-aspartate (NMDA) component of synaptic responses, cells were held at a hyperpolarized membrane potential, between –75 and –85 mV, and the NMDA antagonist [2-amino-5-phosphonovaleric acid (APV)] was included in the ACSF. A bipolar stimulating electrode, powered by a stimulus isolator (World Precision Instruments, Sarasota, FL), was positioned in the optic radiations for corticogeniculate stimulation, and in the optic tract for retinogeniculate stimulation. Cellular activity was acquired with an AxoClamp 2B amplifier (Axon Instruments, Union City, CA), digitized with a Digidata 1322 (Axon Instruments), and analyzed using pCLAMP 9.0 software (Axon Instruments). To acquire cells, patch pipettes were advanced “blind” through tissue in bridge mode until encountering a cell, a >1 GΩ seal was formed, the membrane ruptured to allow whole cell access, and the amplifier was then switched to cSEVC mode.

Single pulses were delivered to the fiber pathways for 100 μs to record single EPSCs. For paired-pulse experiments, dual pulses at 10 Hz frequency were delivered. This frequency was chosen for several reasons: 10 Hz stimulation is within the range of α-rhythms (8–13 Hz) observed in the electroencephalogram during relaxed wakefulness (Castro-Alamancos and Calcagnotto 1999; da Silva et al. 1973); this frequency is near the peak of facilitation effects at corticothalamic synapses yet still allows isolation of individual EPSCs; and this frequency allows comparisons with a prior study of Group III mGluRs at the corticogeniculate synapse (Turner and Salt 1999). Before running a pharmacological experiment, we tested for paired-pulse facilitation (PPF; corticogeniculate stimulation) or paired-pulse depression (PPD; retinogeniculate stimulation) for further confirmation of the pathway being stimulated. To ensure that the PPF results observed in the presence of Group II mGluR agonists were not a result of the length of the interstimulus interval, several cells were tested at 20 and 40 Hz frequency paired stimulation, and the results did not differ from those found with the 10 Hz stimuli (data not shown). Before all experiments, synaptic responses were observed for a range of stimulus intensities, and the intensity that produced approximately half-maximal response was used to allow the EPSC to either increase or decrease in amplitude. During the course of experiments, single stimuli were delivered every 20 s, whereas paired stimuli were delivered every 30 s. Baseline responses were measured for 7–10 min, followed by drug delivery for 7–10 min, and subsequent observation for drug effects.

During train experiments, trains of stimulation 10 s in duration at 10 Hz were delivered to the corticogeniculate pathway. After 5–10 min, a treatment of either the Group II mGluR antagonist (2*S*)-2-amino-2-[(1*S*,2*S*)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl)propanoic acid (LY341495; 200 nM), the Group II mGluR agonist (–)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268; 0.1 μM), or ACSF (control) was administered for 7–10 min. After treatment, train stimulation was delivered again. For these experiments, stimulation intensity was kept <80 μA. Cells had a resting membrane potential of –62.6 ± 0.8 mV and access resistance was <30 MΩ. Access resistance was monitored for all experiments,

and if it changed more than 10% over the course of the experiment, the cell was eliminated from analysis.

Analyses

A pharmacological effect on single EPSCs was defined as a significant change in the amplitude of the EPSC from baseline. For PPF experiments, the ratio of the amplitude of peak 2 to the amplitude of peak 1 was measured for all sweeps. The average of 7 values was taken during control conditions, 12 min after beginning the drug delivery, and after washout. One-way ANOVA was used to analyze these data, followed by a Dunnett's post hoc analysis when necessary or a Neuman-Keuls post hoc analysis for concentration response experiments. The decay constant was calculated for EPSCs during Group II mGluR agonist administration. To determine the decay constant, an exponential function with one term was fitted to the EPSC decay from peak amplitude, and a τ value was calculated. For train experiments, we performed a log transformation of the stimulus number within the train and plotted the EPSC amplitude versus the transformed axis for each response in the train to linearize the control and treatment data. Significance of drug treatment on the trains was then assessed as a significant difference in the slope of the best fitting lines through these data (mean error per data point = 0.85% for control; 0.23% for treatment). In all bar graphs and throughout text, values are reported as means \pm SE.

Pharmacology

Picrotoxin (100 μ M; Sigma-RBI, St. Louis, MO) and APV (50–100 μ M; Sigma-RBI) were always included in ACSF to block γ -aminobutyric acid-A (GABA_A) inhibition and NMDA excitation, except during analysis of NAAG effects on GABAergic inhibitory postsynaptic currents (IPSCs). The adenosine A₁ receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; Tocris, Ellisville, MO), the mGluR3 agonist NAAG (spaglumic acid; Tocris), 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX; Sigma-RBI), picrotoxin, and APV were made fresh daily. The Group II mGluR agonist, LY379268 (a gift from Eli Lilly), the Group II mGluR agonist (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV; Tocris) and the Group II mGluR antagonist LY341495 (Tocris) were made daily from stock solutions. Because NAAG is a neuropeptide, we were careful to make the drug solution immediately before administration to avoid breakdown and we also used higher drug concentrations to compensate for such breakdown. LY379268 and DCG-IV were used together to elucidate specificity of drug effects to Group II mGluRs. LY379268 has EC₅₀ values of >100 μ M at mGluR1, 5 and 7, 2 μ M at mGluR8, 21 μ M at mGluR4, 0.4 μ M at mGluR6, and <0.005 μ M at Group II mGluRs, as shown in expression systems (Cartmell and Schoepp 2000). To minimize the possibility that the observed effects of LY379268 were a result of activation of Group III mGluRs, DCG-IV was used to complement the LY379268 studies. DCG-IV has antagonist activity at mGluR4, 5, 6, 7, and 8. At mGluR1, DCG-IV has an EC₅₀ value of >1,000 μ M, and at mGluR2 and mGluR3 DCG-IV has an EC₅₀ value of <0.3 μ M (Cartmell and Schoepp 2000).

Although local drug administration using dropwise application or local perfusion may provide greater temporal resolution of onset and offset of drug effects (especially with fast-acting ionotropic receptors), in our study we were interested in determining a more precise concentration than is possible with local application. We therefore used full-bath substitution to administer drugs through perfusion of drug into the ACSF flow line by a syringe pump, which was adjusted based on the exact flow rate. The ACSF containing drug was delivered for 7–10 min. At a flow rate of 2 ml/min, drug was delivered for 10 min, and at a flow rate of 1.5 ml/min, drug was delivered for 7 min. Allowing time for nondrug ACSF to be fully replaced by ACSF containing drug (3 min), the time at which the known bath-substituted concentration plateau was first achieved was 7–11 min at a flow rate

of 1.5 ml/min and 5–13 min at a flow rate of 2 ml/min. Once the peak concentration has reached the recording chamber, there is an additional time delay before sampling in which the drug infuses into the slice. For a thick (400 μ M) slice, nearly 5 min is required for infusion (Nicholson and Hounsgaard 1983), thus shifting the peak concentration in the slice 5 min. To adopt a consistent sampling time for all experiments we sampled effects at 12 min after initiation of drug-containing ACSF. In figures, we show the drug treatment as the time during which the syringe pump was activated, even though the plateau concentration of drug in the slice occurred after the indicated delay.

RESULTS

We collected data from 139 LGN relay cells. Before studying a cell, it was verified as a relay neuron by testing for the presence of burst firing, which is rare or absent in interneurons because of masking by a transient K⁺ current (*I_A*) (Pape and McCormick 1995; Steriade and Llinas 1988).

Dynamics of synaptic activation from cortical and retinal pathways

In the rat thalamus, corticogeniculate synaptic responses characteristically display PPF, whereas retinogeniculate synaptic responses show PPD (Granseth et al. 2002; Turner and Salt 1998). The magnitude of the paired-pulse facilitation ratio (PPFR) at the corticogeniculate synapse and paired-pulse depression ratio (PPDR) at the retinogeniculate synapse depends on the interstimulus interval; shorter intervals increase the magnitude of the effect. This phenomenon has been shown *in vitro* in the rat LGN (Granseth et al. 2002; Turner and Salt 1998), but has not been examined in the ferret LGN. We measured paired pulse ratios at 10, 25, 100, 300, 600, 1,000, and 1,500 ms (Fig. 1; *n* = 8 cells for each pathway). In response to stimulation of the optic radiations (containing presumptive corticogeniculate fibers), we consistently observed PPF, with the largest mean facilitation at 25 ms (PPFR: 2.5 \pm 0.1). A shorter interval of 10 ms elicited a smaller facilitation ratio (PPFR: 1.9 \pm 0.2). Facilitation was observed at all interstimulus intervals tested. The data points collected were best fit with a single exponential decay function with a time constant of 181.8 ms. In response to stimulation in the optic tract (containing presumptive retinogeniculate fibers), we consistently observed PPD at all stimulus intervals tested. The greatest mean depression occurred at 10 ms (PPDR: 0.2 \pm 0.05), whereas a longer interval of 25 ms elicited less depression (PPDR: 0.4 \pm 0.05). The data points collected were best fit with a double-exponential decay function with time constants of 13.57 and 388.7 ms. These data are consistent with that observed in the rat dorsal LGN (Granseth et al. 2002; Turner and Salt 1998), including the observation that the maximal depression ratio at the retinal synapse is observed at the shortest interval tested, whereas the maximal facilitation ratio at the cortical synapse is observed at a slightly longer interval.

Group II mGluRs selectively reduce cortical input to the LGN

We assessed paired-pulse ratios to characterize facilitation or depression of the pathway being stimulated before each experiment as additional confirmation of the type of synapse being studied. EPSCs that were elicited from stimulation of the

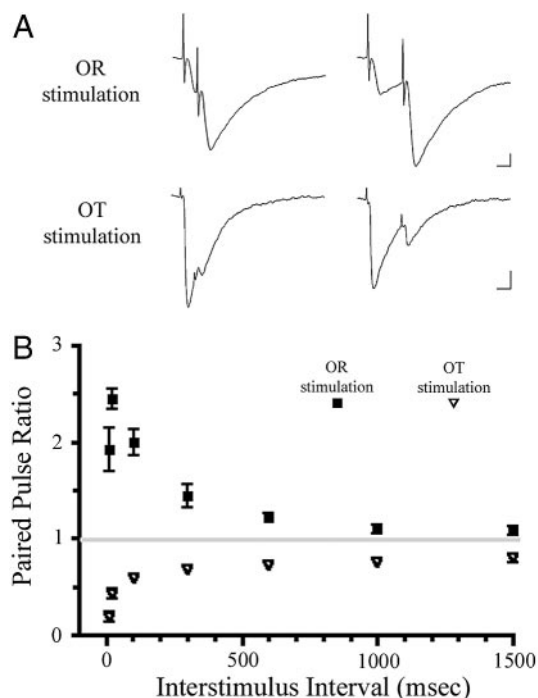


FIG. 1. Stimulation of ferret corticogeniculate and retinogeniculate pathways produced characteristic facilitation and depression. Paired pulses were delivered to either the optic radiations (OR; eliciting presumptive corticogeniculate EPSCs) or the optic tract (OT; eliciting presumptive retinogeniculate EPSCs) at various interstimulus intervals and the resulting facilitation or depression ratios were calculated. *A*: representative examples of paired-pulse facilitation (PPF) on OR stimulation and paired-pulse depression (PPD) on OT stimulation at 10 ms (*left*) and 25 ms (*right*) interstimulus intervals. *B*: facilitation and depression ratios are shown, and were calculated at increasing interstimulus intervals. At the corticogeniculate synapse (black boxes) the greatest facilitation was found at the 25 ms interval, whereas the greatest depression for the retinogeniculate synapse (white triangles) was found at the 10-ms interval. Scale bars in *A* = 10 pA, 10 ms.

optic tract and which showed PPD were presumed to be generated at the retinogeniculate synapse, whereas EPSCs elicited on stimulation of the optic radiations and which showed PPF were presumed to be generated at the corticogeniculate synapse. With the inclusion of both picrotoxin (100 μ M) and APV (50 μ M) in the recording buffer to isolate non-NMDA-mediated EPSCs, bath application of LY379268 (1 μ M) reduced the amplitude of the corticogeniculate EPSC (see Fig. 2*A*). LY379268 reduced EPSC amplitudes in all cells tested. The mean control EPSC amplitude was reduced to $50.0 \pm 3.0\%$ ($P < 0.01$, $n = 18$) in the presence of 1 μ M LY379268, and recovered after drug washout ($P > 0.05$). In an attempt to assign the specificity of the LY379268's effect to Group II mGluRs we studied the effect of the drug on the corticogeniculate EPSC at a range of concentrations.

LY379268 reduced the amplitude of the corticogeniculate EPSC in a dose-dependent manner with a peak effect found at both 0.1 and 1 μ M LY379268 (reduced to $50.0 \pm 3.6\%$ of control for 0.1 μ M; Fig. 2*B*). At all concentrations tested, LY379268 significantly reduced the corticogeniculate EPSC amplitude from control ($P < 0.01$). Each increase in concentration elicited a significant reduction in the EPSC amplitude ($P < 0.01$), but the effect of 0.1 μ M LY379268 did not differ significantly from that of 1 μ M ($P > 0.05$), suggesting that the maximal reduction of the corticogeniculate EPSC elicited by

Group II mGluRs with LY379268 is about 50%. At all concentrations, the EPSC recovered to an amplitude not significantly different from control ($P > 0.05$). LY379268 also decreased the EPSC decay constant, an effect that was observed at all concentrations tested, but was significant only at 1 μ M. At 1 μ M, LY379268 reduced the decay constant to $81.4 \pm 4.1\%$ of control ($P < 0.05$). LY379268 did not produce a significant change in holding potential or access resistance ($P > 0.05$) at any of the tested concentrations.

In contrast to the marked reduction in the corticogeniculate EPSC, LY379268 did not reduce the amplitude of the retinogeniculate EPSC at a concentration of 1 μ M ($P > 0.05$, $n = 9$; Fig. 2*C*). The mean control amplitude of the EPSC was 86.5 ± 22.5 pA, which did not differ during exposure to LY379268 (87.6 ± 24.7 pA). There was also no significant change in the EPSC decay constant ($P > 0.05$).

To assign the specificity of the LY379268 effect to Group II mGluRs, we preincubated sections in the Group II mGluR antagonist LY341495 and then challenged the cells displaying PPF with the agonist LY379268. LY341495 was infused in the ACSF by syringe pump for 10 min before beginning an experiment, and continuously delivered throughout the experiment. LY341495 alone had no effect on the amplitude of the single EPSC or the PPF. These experiments were performed with 2 separate concentrations of agonist and antagonist because of the observation that LY379268 produced 2 different effects on the PPF when used at 1 μ M as compared with lower concentrations (see following text). Preincubation of slices in 1 μ M LY341495 blocked the 1 μ M LY379268 reduction of the amplitude of the corticogeniculate EPSC ($P > 0.05$, $n = 6$; Fig. 3*A*). We then repeated this experiment at a lower concentration for both agonist and antagonist. Preincubation of slices in 0.1 μ M LY341495 blocked the reduction of the corticogeniculate EPSC by 0.1 μ M LY379268 ($P > 0.05$, $n = 8$; Fig. 3*B*). Because of the observation that the saturating concentration of LY379268 (0.1 μ M) was fully blocked by a concentration of LY341495 specific for Group II mGluRs (0.1 μ M), the observed agonist effect is likely specific to action at Group II mGluRs.

LY379268 has a concentration-dependent effect on corticogeniculate paired-pulse facilitation

To determine whether the action of LY379268 involved a presynaptic mechanism, we studied the effect of the drug on the PPF in which dual pulses were delivered to the optic radiations at 10 Hz. LY379268 altered the PPF at corticogeniculate synapses in a concentration-dependent manner. LY379268 reduced the facilitation ratio to $84.1 \pm 6.3\%$ of control at the highest concentration tested (1 μ M). Figure 4*A* shows an example of a 1 μ M concentration effect in which the facilitation ratio was decreased concomitant with the single EPSC response inhibition. However, when the concentration was reduced the PPF showed enhancement during the reduced-amplitude effect on the single EPSC (Fig. 4*B*). Although this effect is different from that observed with the higher concentration, the effect is specific to mGluRs, as shown by the antagonist experiments (see Fig. 3, *A* and *B*). At a concentration of 0.1 μ M, LY379268 increased the PPF to $116.9 \pm 11.99\%$ of control (Fig. 4*C*). The large SE may be attributed to the finding that at 0.1 μ M, 4 of the 10 cells tested showed a

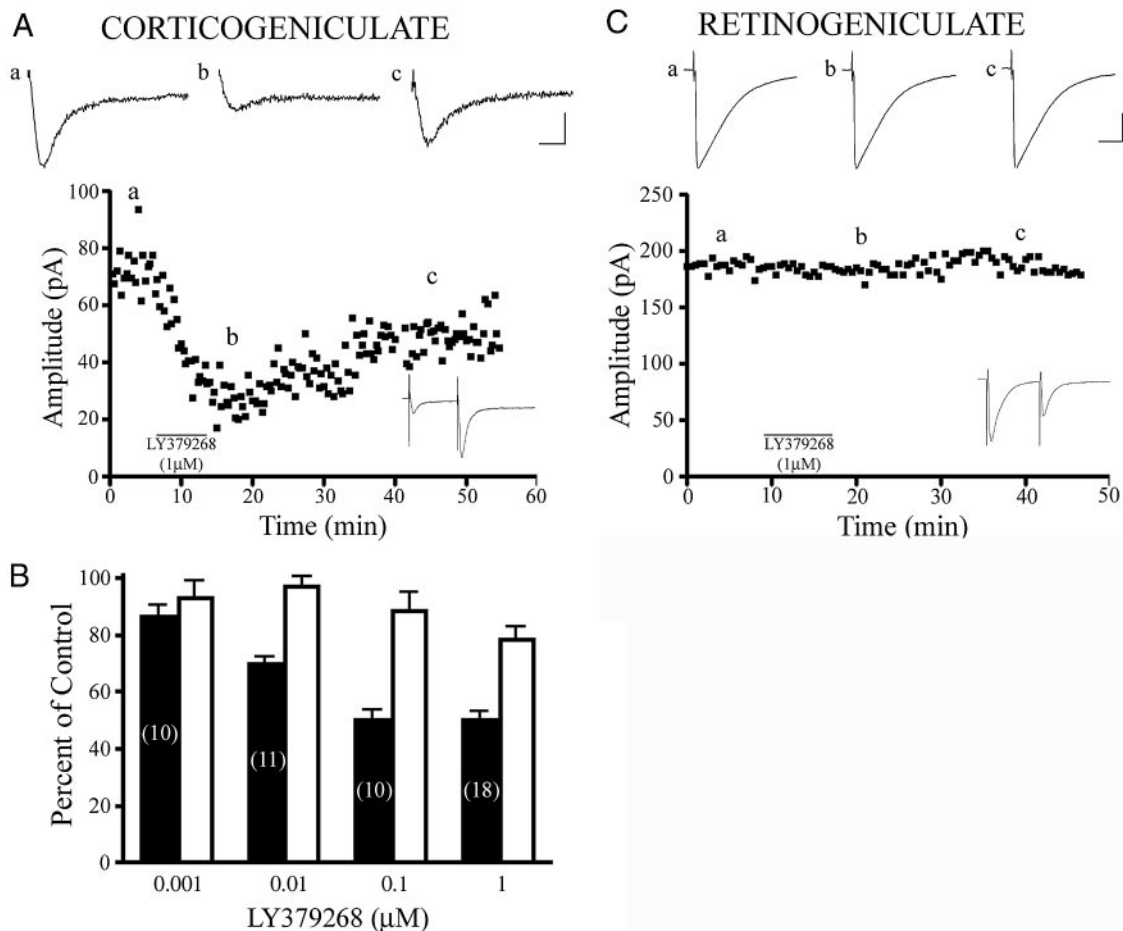


FIG. 2. LY379268 reduced the amplitude of EPSCs elicited from the corticogeniculate pathway, whereas retinogeniculate EPSCs were unaffected. *A*: corticogeniculate EPSC was reduced in amplitude by LY379268 ($1 \mu\text{M}$). *Inset trace* in the graph illustrates the LYPF at this synapse, suggesting that the recorded EPSC is of corticogeniculate origin. Graph shows the time course of the effect in which the letters refer to the corresponding *traces* taken during control (*a*), peak drug effect (12 min after beginning drug delivery) (*b*), and after recovery (*c*). *B*: LY379268 dose-dependently reduced the EPSC amplitude from control. Black bars represent maximal drug effect and white bars represent recovery. Number of observations for each concentration is shown on the respective bar. *C*: LY379268 had no effect on the amplitude of the retinogeniculate EPSC under the same recording conditions as in *A* and *B*. *Inset trace* on the graph displays PPF at the recorded synapse indicating the retinogeniculate origin of the EPSC. Graph shows the time course of the effect in which the letters refer to *traces* above representing control (*a*), 12 min after initiation of drug delivery (*b*), and the time point corresponding to observed recovery from drug effects on the corticogeniculate EPSC (*c*). Scale bar in *A* = 25 pA, 20 ms; *C* = 50 pA, 20 ms.

reduction in the PPF, whereas in the remaining 6 the PPF increased; therefore this concentration may be near threshold for the direction change of the PPF during the agonist effect. Reducing the concentration further to $0.01 \mu\text{M}$ increased the facilitation ratio to $119.2 \pm 7.6\%$ of control. Using a repeated-measures ANOVA, the effect of $1 \mu\text{M}$ LY379268 on the PPF was found to differ significantly from the effect of $0.1 \mu\text{M}$ ($P < 0.05$). No significant difference was found between any other groups ($P > 0.05$).

To determine whether the Group II mGluR effect involved a postsynaptic mechanism, we tested a group of cells at all concentrations with the inclusion of the intracellular G-protein blocker GDP β S (1 mM) in the internal pipette solution (Wang and Daw 1996). Because mGluRs work through G-proteins, intracellular administration of GDP β S would prevent LY379268 from exerting effects if the EPSC attenuation was attributable to postsynaptic mechanisms. At all concentrations, there was no significant difference in the direction or magnitude of the Group II mGluR agonist effect on the single corticogeniculate EPSC or PPF when GDP β S was included

in the intracellular solution ($P > 0.05$, $n = 4$ for $1 \mu\text{M}$ and $0.1 \mu\text{M}$, $n = 5$ for $0.01 \mu\text{M}$ and $0.001 \mu\text{M}$ groups). These data were therefore pooled with the data acquired without GDP β S. These experiments along with the PPF experiments suggest that the Group II mGluR effect is mediated presynaptically.

DCG-IV replicates PPF effects of LY379268

In examining the bidirectional effect of LY379268 on the PPF, we considered the possibility that the concentration of LY379268 was high enough that the drug also activated Group III mGluRs to influence PPFs at higher concentrations. Although the high concentration of LY341495 ($1 \mu\text{M}$) did fully block the effect of $1 \mu\text{M}$ LY379268, this concentration of LY341495 may also have antagonist action at Group III mGluRs (Kingston et al. 1998). We therefore used another Group II mGluR agonist, DCG-IV, which has no reported agonist activity at Group III mGluRs at concentrations $\leq 1 \text{ mM}$ (Cartmell and Schoepp 2000), and may have antagonist action at Group III mGluRs at high concentrations ($>20 \mu\text{M}$) (Brabet

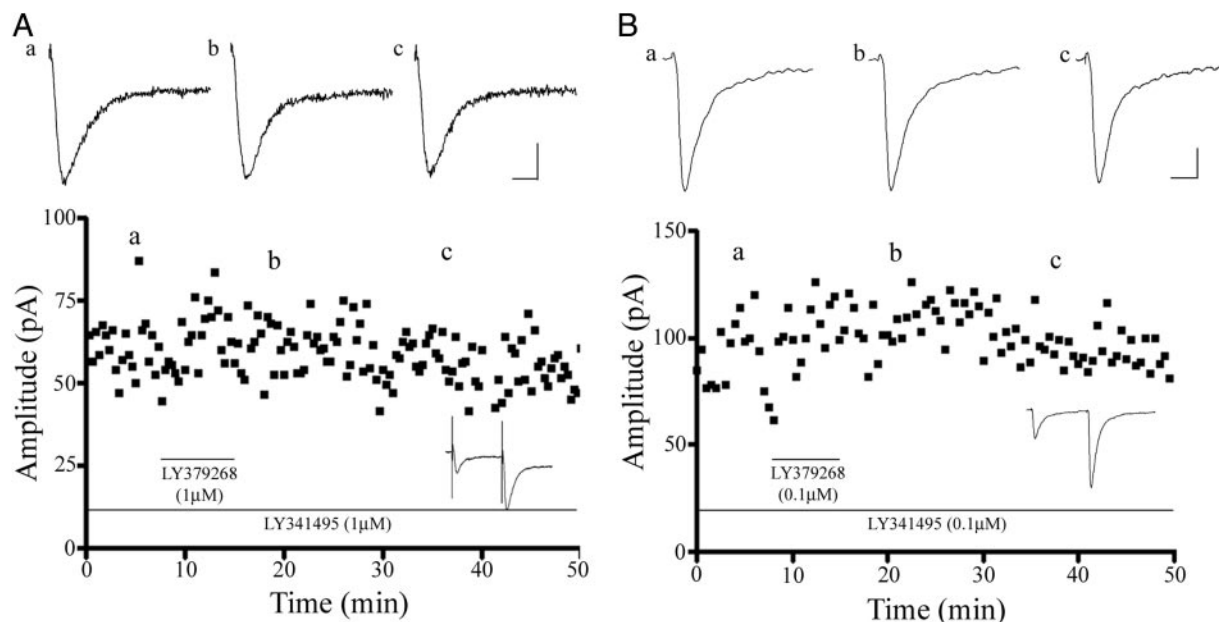


FIG. 3. Preincubation with the Group II mGluR antagonist, LY341495, blocked the reduction of the corticogeniculate EPSC by LY379268. *A*: 1 μM LY341495 blocked the reduction of the EPSC by 1 μM LY379268. Time course of the experiment is shown in which the letters refer to the corresponding traces above taken during control (*a*), 12 min after initiation of LY379268 delivery (*b*), and recovery (*c*). Inset trace shows PPF consistent with other experiments showing agonist effects. LY379268 (1 μM) did not reduce the amplitude of the corticogeniculate EPSC in the presence of LY341495 (1 μM) for a population of lateral geniculate nucleus (LGN) relay cells. *B*: 0.1 μM LY341495 blocked the reduction in EPSC by 0.1 μM LY379268. Description of *B* is the same as that for *A*. Scale bar in *A* and *B* = 25 pA, 10 ms.

et al. 1998). We examined the effect of a range of DCG-IV concentrations (0.1–100 μM) on the single EPSC as well as the PPF. The maximal DCG-IV effect on the single stimulus was found at 100 μM (reduced to $19.4 \pm 2.9\%$ of control) and at lower concentrations, EPSC amplitude inhibition decreased in a dose-dependent manner (Fig. 5*A*). DCG-IV also decreased the EPSC decay constant at 100 μM ($63.6 \pm 9.4\%$ of control; $P < 0.05$). Examination of the DCG-IV dose-dependent effect on the PPF yielded the same pattern as was found with LY379268. At DCG-IV concentrations $\leq 1 \mu\text{M}$, the facilitation ratio increased (to $120.4 \pm 2.8\%$ of control for 0.1 μM , and $132.7 \pm 18.47\%$ of control for 1 μM) during the DCG-IV effect on the single stimulus. At a concentration of 10 μM DCG-IV, 4 cells were studied: 2 showed an enhancement of the PPF and 2 showed a reduction, demonstrating a pattern similar to that observed with LY379268, and resulting in a PPF of $102.8 \pm 33.3\%$ of control. Raising the DCG-IV concentration to 100 μM elicited a reduction in the PPF to $62.62 \pm 8.8\%$, as was observed with LY379268 (Fig. 5, *B* and *C*). Using a repeated-measures ANOVA, the effect of 100 μM DCG-IV on the PPF was found to differ significantly from the effect of 1 μM ($P < 0.05$). No significant difference was found between any other groups ($P > 0.05$).

mGluR3 agonist NAAG does not affect corticogeniculate EPSCs

NAAG is a neuropeptide found in the mammalian CNS and is a relatively selective agonist for mGluR3 (Wroblewska et al. 1997) over mGluR2. EC_{50} values for NAAG at mGluR3 have been found to be $< 100 \mu\text{M}$ in expression systems, whereas activation of mGluR2 requires concentrations $\leq 1,000 \mu\text{M}$

(Neale et al. 2000; Wroblewska et al. 1997). Because of this relative selectivity for mGluR3 over mGluR2, we used NAAG to probe whether the observed effects of LY379268 (which has very similar affinity for mGluR2 and mGluR3) stemmed from mGluR3 activation, combined mGluR2 and mGluR3 activation, or mGluR2 alone. Because NAAG is a neuropeptide found endogenously, we were careful to make the drug fresh immediately before administration, and we ran the experiment using a range of concentrations from 50 μM to 2 mM. As a positive control for biological activity of NAAG we first tested for NAAG modulation of the GABAergic TRN input to LGN relay cells, as shown by Turner and Salt (2003). In the presence of APV and DNQX to block ionotropic glutamate receptors, we stimulated the TRN and recorded the IPSC. NAAG (1 mM) significantly reduced the amplitude of the IPSC to $33.0 \pm 6.9\%$ of control ($P < 0.01$, $n = 3$; Fig. 6*A*), similar to the findings of Turner and Salt (2003). However, at a concentration close to the reported EC_{50} value for mGluR3 (50 μM), and well in excess of that we found no effect on the amplitude of the corticogeniculate EPSC ($P > 0.05$; 50 μM , $n = 2$; 200 μM , $n = 1$; 500 μM , $n = 1$; 1 mM, $n = 2$; 2 mM, $n = 1$; Fig. 6*B*). We conclude that the observed effect of LY379268 does not support the involvement of mGluR3, but is consistent with the involvement of mGluR2, or a yet undescribed mGluR with a similar pharmacological profile.

Group II mGluR effects persist in the presence of adenosine receptor blockade

In addition to its presence on cortical and GABAergic terminals, Group II mGluRs have been localized to astrocytes in the rat thalamus. Studies have suggested the involvement of astrocytes in mGluR-mediated inhibition of neurotransmitter

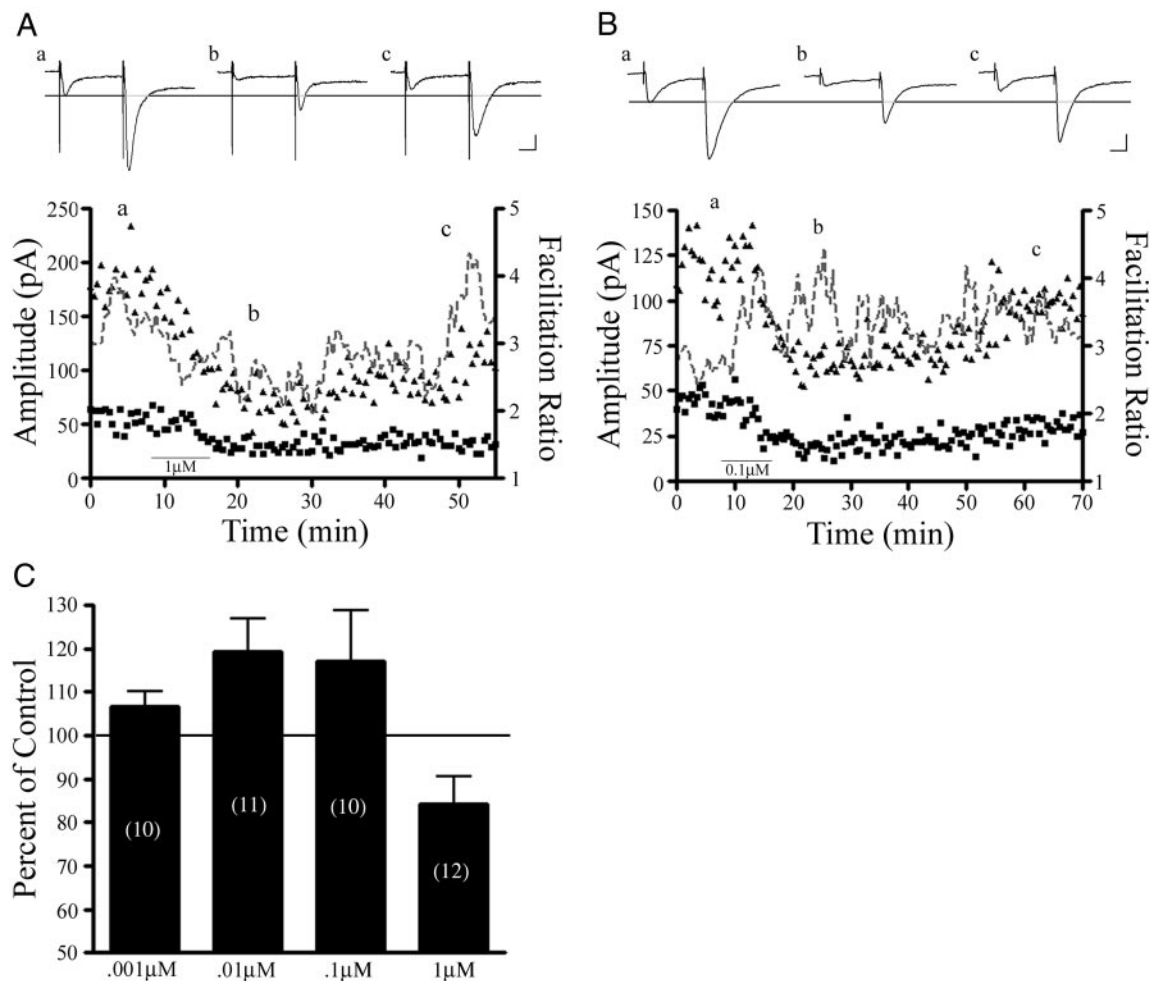


FIG. 4. Paired-pulse facilitation ratios (PPFRs) showed concentration dependency at the corticogeniculate synapse. *A*: at the highest concentration studied (1 μM), LY379268 reduced the amplitude of the single EPSC while also reducing the facilitation ratio between the first and the second EPSC. Graph shows the time course of the effect in which the letters refer to the corresponding traces above taken during control (*a*), peak drug effect (*b*), and after drug washout (*c*). Squares represent peak 1, triangles represent peak 2, and the dashed line represents a boxcar average of the facilitation ratio. *B*: at a lower concentration (0.1 μM), LY379268 still reduced the amplitude of the single EPSC while increasing the facilitation ratio between the first and second EPSC. Symbols and letters are the same as in *A*. *C*: effect of a range of LY379268 concentrations on the PPFR displayed as percentage of control PPFR value. At concentrations ≤ 0.1 μM the PPFR increased during the drug effect on the single stimulus. At 0.1 μM , most cells showed enhancement of the PPFR, although some showed a reduction in the PPFR, arising from the large error bar at that concentration. At 1 μM , PPFR decreased during the drug effect on the single stimulus. Number of observations for each concentration group is shown in the respective bar. Scale bar in *A* and *B* = 25 pA, 25 ms.

release (Moldrich and Beart 2003). Glutamate, acting on astrocytic mGluRs, could evoke adenosine release (or ATP release, which is broken down to adenosine in the extracellular space) which could then activate presynaptic adenosine receptors (Moldrich et al. 2002; Winder et al. 1996). Presynaptic adenosine A_1 receptor activation has been shown to reduce neurotransmitter release (Moldrich and Beart 2003). In an attempt to rule out astrocyte involvement in the observed Group II mGluR effects, we preincubated sections in DPCPX (1 μM), an A_1 receptor antagonist, for 10 min and challenged cells with LY379268 (1 μM). In this experiment, LY379268 replicated prior experiments, reducing the EPSC amplitude to $45.2 \pm 4.5\%$ of control and the PPFR to $76.5 \pm 3.2\%$ of control in the presence of DPCPX ($P > 0.05$ compared with LY379268 alone ($n = 7$); data not shown). These data support a direct presynaptic terminal effect by mGluRs, but we cannot rule out involvement of other unidentified astrocyte-mediated mechanisms.

Synaptic and pharmacological modulation of high-frequency corticogeniculate activity by Group II mGluRs

The frequency of synaptic activity has been shown to be an important factor in the engagement of metabotropic signaling. High-frequency stimulation has been specifically linked to activation of all mGluRs studied (Kew et al. 2001; Scanziani et al. 1997), including postsynaptic Group I mGluRs in the LGN (Govindaiah and Cox 2004; McCormick and von Krosigk 1992). We thus used stimulus trains in an attempt to elicit intrinsic Group II mGluR responses at the corticogeniculate synapse. As shown in Fig. 7A, during train stimulation responses within the train became successively larger, as previously shown at the corticogeniculate synapse (Granseth 2004; Kao and Coulter 1997; von Krosigk et al. 1999). Moreover, when Group II mGluRs were blocked with LY341495 (200 nM) train responses were significantly larger than those during control train stimulation ($P < 0.01$, $n = 9$), indicating that in the absence of Group II mGluR blockade, train stimulation

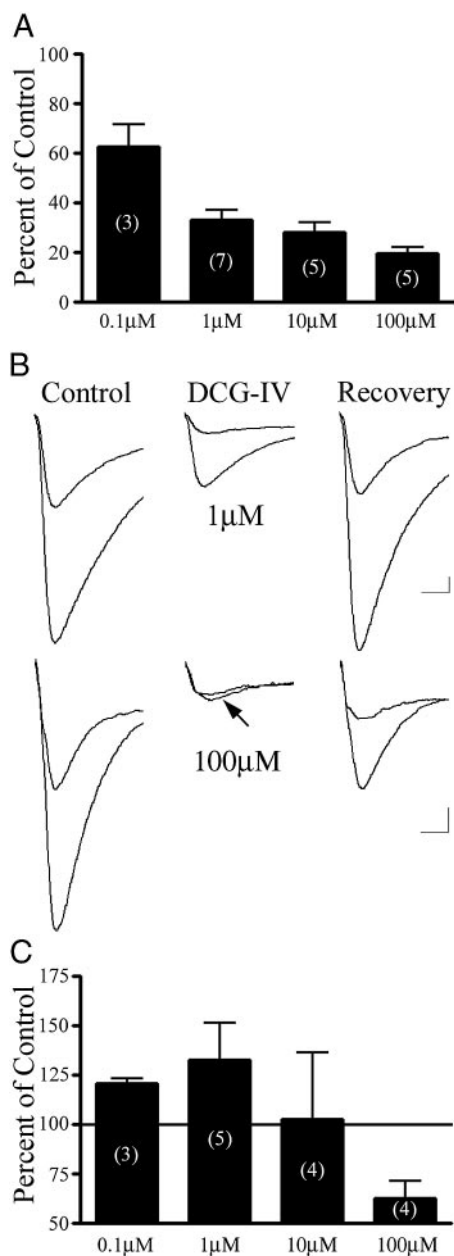


FIG. 5. DCG-IV reproduced the concentration dependent effect of LY379268 on the PPFR at the corticogeniculate synapse. *A*: DCG-IV dose-dependently reduced the amplitude of the corticogeniculate EPSC from control. Number of observations for each concentration group is shown on each respective bar. *B*: example traces showing the effect of 1 μM (*top row*) and 100 μM (*bottom row*) DCG-IV on the PPFR. Each pair of EPSCs displays peak 1 overlaid on peak 2. Larger response in each pair is the 2nd of the 2 stimuli (the arrow in the 100 μM DCG-IV effect pair points to peak 2). 1 μM DCG-IV produced an enhancement of the PPFR, whereas 100 μM produced a reduction in the PPFR. *C*: effect of a range of DCG-IV concentrations on the PPFR displayed as percentage of control PPFR. ≤ 1 μM, DCG-IV enhanced the PPFR. 10 μM DCG-IV enhanced the PPFR in 2 cells and reduced the PPFR in 2, thus accounting for the small percentage change from control PPFR and large error bar. 100 μM DCG-IV reduced the PPFR in 4 cells of 4 studied. Both scale bars in *B* = 20 pA, 10 ms.

activated Group II mGluRs and reduced subsequent glutamate release within the train. The EPSC areas were plotted for each response in the train during control and LY341495 conditions (Fig. 7*B*). The area of the first response in the train was

unchanged before and after LY341495 administration ($P > 0.05$). The second response was the first to show a significant increase in area over control ($P < 0.05$), suggesting that Group II mGluR modulation of train stimulation has a rapid onset. However, not all cells showed Group II mGluR modulation on the second response in the train (5 of 9 cells showed modulation). By the third response, all cells showed enhanced response area during LY341495 administration. After washout of LY341495, response areas returned to values near control ($P > 0.05$).

Corticothalamic activity patterns similar to the train stimulation used in this study have been shown to precede spike-and-wave discharge (SWD) activity (Timofeev et al. 1998). Therefore to study how Group II mGluR agonists may be beneficial in reducing the occurrence of such activity patterns, we examined the effect of LY379268 on train responses designed to mimic pre-SWD cortical activity patterns, termed "cortical fast runs" (Timofeev et al. 1998). Train stimulation was delivered as mentioned above, and in the presence of LY379268 (0.1 μM) responses within the train were significantly reduced from control in both amplitude and area ($P < 0.01$; $n = 6$) (Fig. 7, *C* and *D*), suggesting that Group II mGluR agonists may be effective in reducing thalamic EPSCs in response to cortical fast runs.

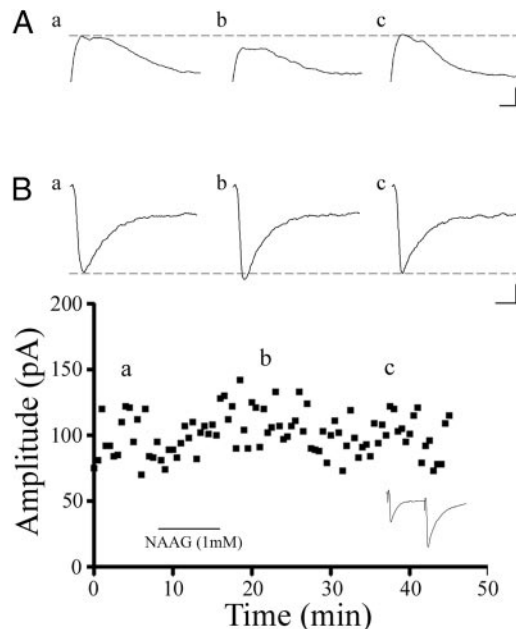


FIG. 6. NAAG, a selective mGluR3 agonist, had no effect on the amplitude of the corticogeniculate EPSC. *A*: positive control for the activity of NAAG. Stimulation of the TRN in the presence of APV and DNQX elicited a GABAergic outward current [response is GABA-mediated chloride current with Cs-gluconate internal solution, and reversed at -55 mV; see also Cox et al. (1997)] in an LGN relay cell with at a holding potential of -45 mV (*a*). NAAG (1 mM) reduced the amplitude of the current (*b*), which then recovered (*c*). *B*: stimulation of the optic radiations to elicit a corticogeniculate EPSC in the presence of APV and picrotoxin was not affected by NAAG. Time course and representative traces are shown during control (*a*), 12 min after application of NAAG (1 mM) (*b*), and during an interval corresponding to recovery (*c*). *Inset trace* in the graph shows PPF consistent with other cells showing Group II mGluR agonist effects. Scale bar in *A* = 25 pA, 10 ms; *B* = 25 pA, 20 ms.

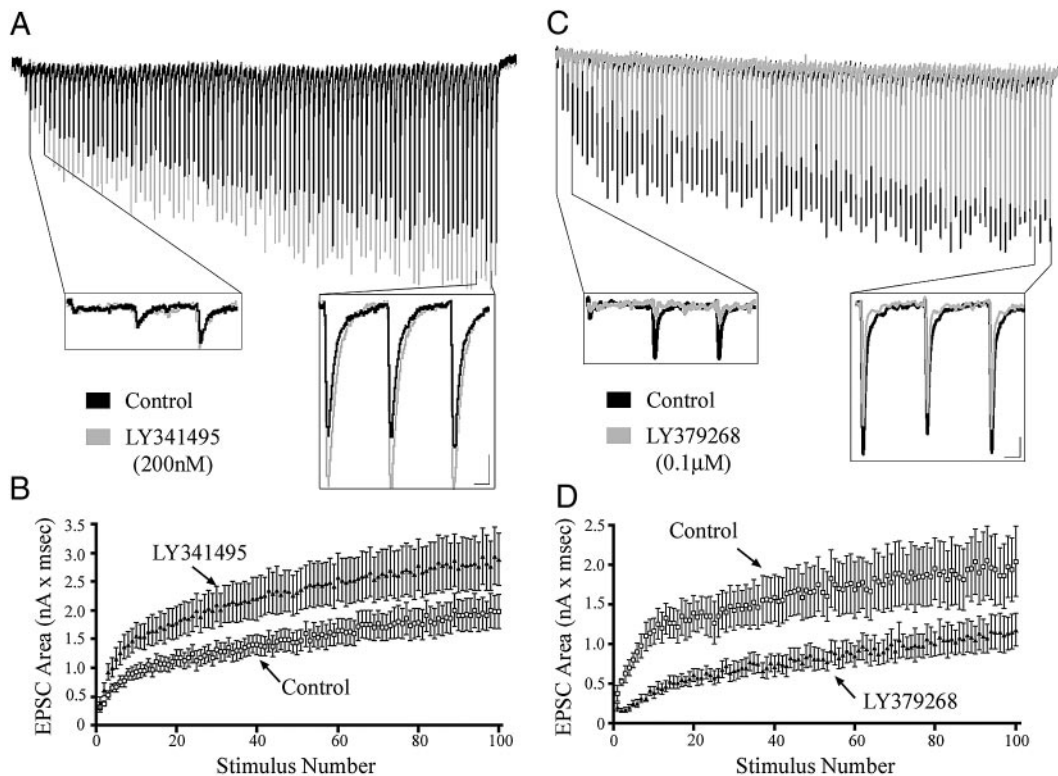


FIG. 7. Group II mGluR activation in the corticogeniculate pathway by 10 Hz frequency trains. *A*: in the presence of LY341495 (200 nM; *gray trace*), the response to train stimulation was enhanced in amplitude relative to control (*black trace*). First response of the train was unchanged in the presence of LY341495, as seen in the expanded *inset* showing the first 3 stimuli, although later EPSCs were enhanced, as seen in the *inset* showing the later EPSCs in the train. *B*: LY341495 (200 nM) increased the area of the individual EPSCs for the population of relay cells studied. *C*: in the presence of the Group II mGluR agonist, LY379268 (0.1 μ M; *gray trace*), the response to train stimulation was reduced relative to control (*black trace*). First few responses in the train were substantially reduced as shown in the *inset*, whereas later responses were less affected. *D*: LY379268 (0.1 μ M) reduced the area of individual EPSCs in the train for the population of relay cells studied. Scale bar in *A* = 35 pA, 300 ms for *full trace*; 30 pA, 25 ms for *insets*. Scale bar in *C* = 30 pA, 300 ms for *full trace*; 30 pA, 25 ms for *insets*.

DISCUSSION

Comparisons with other studies

The reduction in EPSCs we have observed in the corticogeniculate pathway in the presence of LY379268 and DCG-IV is similar to observations in several other areas of the CNS (Anwyl 1999). The reduction of excitatory input by Group II mGluRs has been well documented within the hippocampus in the lateral and medial perforant path of the dentate gyrus (Kilbride et al. 1998), the mossy fiber to CA3 path (Kamiya et al. 1996), and the perforant path to CA1 (Kew et al. 2001). The enrichment of mGluR2 in the perforant path, dentate gyrus, and CA1 is consistent with involvement of mGluR2 in the inhibition of synaptic transmission at these synapses (Kilbride et al. 1998; Ohishi et al. 1993a; Shigemoto et al. 1997). Also, compared with wild-type animals, mGluR2 deficient mice exhibit a large reduction in pharmacological response inhibition in the perforant path, implicating mGluR2 activation in response inhibition (Kew et al. 2002). In the present study, the mGluR3 selective agonist NAAG had no effect on the amplitude of the EPSC at concentrations ≤ 2 mM (the EC_{50} value for mGluR3 in expression systems is 50 μ M) (Wroblewska et al. 1997), implicating mGluR2 activation in the observed response inhibition. LY379268 did not alter retinogeniculate EPSCs (Fig. 2C), indicating that Group II mGluR reduction of EPSCs is specific to the corticogeniculate glutamatergic input. Immunocytochemical and in situ hybridization studies have previ-

ously localized Group II mGluRs within the thalamus. mGluR3 mRNA has been found in the TRN, with very little signal in the LGN, and double label for mGluR2/3 and a GABAergic marker in mGluR2-deficient mice indicate that mGluR3 TRN inhibitory terminals are found in relay nuclei (Lourenco et al. 2000; Ohishi et al. 1993b; Tamaru et al. 1992). Anatomical localization of mGluR3 to TRN terminals was supported by the sensitivity of IPSPs in thalamic relay cells to NAAG (Turner and Salt 2003; see also Fig. 6A). In contrast to the highly localized staining pattern within the TRN, mGluR2 protein has been shown to have a more diffuse staining pattern (Neki et al. 1996). mGluR2 mRNA has been shown in layer VI of neocortical regions (Ohishi et al. 1993a), and mGluR2/3 protein staining has been found in profiles resembling cortical axon terminals in thalamus (Liu et al. 1998). Our study is the first to identify a physiological role for mGluR2 at corticothalamic terminals.

Presynaptic nature of Group II mGluR-mediated inhibition

Our study supports the presynaptic origin of Group II mGluR-mediated response inhibition. Reflecting presynaptic release dynamics demonstrated in rat (Granseth et al. 2002; Turner and Salt 1998), we observed that optic tract stimulation produced PPD, whereas stimulation of the optic radiations yielded PPF. The alteration in PPF during Group II mGluR-mediated EPSC inhibition suggests a presynaptic influence on

corticogeniculate neurotransmission. The observed Group II mGluR agonist effects do not appear to be postsynaptic because inclusion of the intracellular G-protein blocker GDP β S had no effect on the magnitude or direction of the effects of LY379268. Additionally, during Group II mGluR agonist-induced reduction in EPSC amplitude, we noted a decrease in the EPSC decay constant. Changes in EPSC decay kinetics can be suggestive of a presynaptic mechanism. For example, spillover of glutamate out of the synaptic cleft and onto AMPA receptors in a neighboring synapse has been shown to contribute to an increased duration in synaptic response (DiGregorio et al. 2002; Otis and Trussell 1996). In our study, the decreased EPSC decay constant could reflect a decreased spillover of glutamate onto neighboring synapses during the EPSC response inhibition by Group II mGluR agonists.

Instead of expected increases in PPR during EPSC attenuation, LY379268 elicited a bidirectional effect depending on drug concentration. At lower concentrations, LY379268 increased the PPR, whereas at higher concentrations the PPR decreased. DCG-IV (which has only antagonist activity at Group III mGluRs) produced the same concentration-dependent bidirectional effect on the PPR as LY379268, indicating that nonspecific activity at Group III mGluRs is not responsible for the bidirectional effect. PPR values are dependent on external calcium concentration, which determines calcium availability within the synaptic terminal arising from calcium influx. Typically, lowering calcium concentration reduces neurotransmitter release and increases PPR (Katz and Miledi 1968; Rahamimoff 1968; Zucker and Regehr 2002). At the corticogeniculate synapse, lowering external calcium from 2 to 1 mM increases the PPR, and lowering it further to 0.5 mM decreases PPR (Granseth et al. 2002). Therefore the bidirectional effect of Group II mGluR agonists on PPR could reflect changes in calcium influx into corticogeniculate terminals. Consistent with this idea, one mechanism by which Group II mGluRs have been shown to have their effects on neurotransmitter release is through blockade of high-threshold calcium channels that mediate transmitter release (Anwyl 1999).

Neurotransmitter release inhibition could also be an indirect effect of Group II mGluRs on astrocytes. Receptor activation may lead to release of adenosine from astrocytes that may then affect the presynaptic terminal (Moldrich and Beart 2003; Moldrich et al. 2002; Winder et al. 1996). However, Group II mGluR effects persisted in the presence of the A₁ receptor antagonist DPCPX, suggesting that adenosine is not responsible for the presynaptic effects we observed. Similar findings of presynaptic mGluR modulation of corticogeniculate transmission have been reported for Group III mGluRs in rat. The receptors believed responsible for this effect are mGluR7 and mGluR8 (Turner and Salt 1999).

Functional implications

THALAMIC PROCESSING. Group II mGluRs are generally found extrasynaptically (Shigemoto et al. 1997; Tamaru et al. 1992), so high-frequency stimulation sufficient to produce neurotransmitter spillover from the synaptic cleft is required to engage Group II mGluR-mediated physiological responses. In the present study, train stimulation at 10 Hz and several seconds in duration elicited successively larger EPSCs in the train. Blockade of Group II mGluRs enhanced the EPSCs in the train to an

even greater extent, indicating that Group II mGluR responses are elicited at similar frequencies. A significant increase in response area within the train was seen by the third response, with most cells showing an increased response on the second response (5 of 9 cells studied). This is in apparent contrast to prior observations of Group III mGluR activity. At the corticogeniculate synapse in rat, trains of 5 stimuli at 10 Hz were not significantly altered by Group III mGluR blockade with the Group III mGluR antagonist, (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid (MAP4). Interestingly, there was also no significant effect of either 3 μ M LY341495, a concentration with activity at both Group II mGluRs and Group III mGluRs, or the specific Group II mGluR antagonist, (*2S*)- α -ethyl-glutamic acid (EGLU), on EPSPs elicited by trains of 5 stimuli (Turner and Salt 1999). Differences between that study and the present one may be partly methodological. Whereas our study used patch electrodes to study voltage-clamped EPSCs, Turner and Salt (1999) studied relay cell responses using current-clamp recordings of EPSPs. Current-clamp recordings are a valuable approach that provides useful information on synaptic responses, but the membrane potential is not clamped and there is a possibility of triggering voltage-dependent currents, such as the T-type calcium current. Although measures were taken to reduce the contribution of the T-type calcium current in their study (Turner and Salt 1999) even a small contribution by T-type currents might influence the detection of Group II mGluR effects on EPSPs. Additionally, Turner and Salt (1999) used trains of 5 stimuli, which may have been too few stimuli to allow detection of Group II mGluR modulation of EPSPs with current-clamp techniques. An important difference between Group II and Group III mGluRs is their synaptic localization. Whereas Group II mGluRs are generally found extrasynaptically, Group III mGluRs are reported to be in the active zone (Shigemoto et al. 1997). This difference in localization may provide for frequency-selective mechanisms of activation that would depend on experimental conditions.

Stimuli of 10 Hz are of interest in thalamic processing because they are within the range of α -rhythms (8–13 Hz) observed in the electroencephalogram during relaxed wakefulness (Castro-Alamancos and Calcagnotto 1999; da Silva et al. 1973). A recent study found that in the LGN, activation of mGluR1, which is found in corticogeniculate recipient zones on distal dendrites of relay cells (Godwin et al. 1996a; Vidnyanszky et al. 1996), initiates postsynaptic α -rhythms (Hughes et al. 2004). Similar to our findings of presynaptic Group II mGluRs being engaged by trains of synaptic stimulation, postsynaptic Group I mGluRs are engaged by high-frequency trains of synaptic stimulation (Golschani et al. 1998; McCormick and von Krosigk 1992; Turner and Salt 1998; von Krosigk et al. 1999). Presynaptic Group II mGluRs may function to regulate the occurrence of α -oscillations in a complementary fashion with the postsynaptic mGluR1. mGluR1 may permit the expression of α -rhythms, whereas presynaptic Group II mGluRs may regulate the initiation and persistence of the rhythm.

The nature of the extensive corticogeniculate feedback projection may account for the need for a presynaptic Group II mGluR autoreceptor. Corticogeniculate terminal arbors diverge to contact multiple thalamic relay neurons (Murphy and Sillito 1996), and each relay neuron likely receives signals from multiple cortical inputs that under normal information processing demands may convey unique feature-related con-

tent (Murphy et al. 1999; Sillito and Murphy 1988). This feedback is communicated through synapses with a relatively low probability of release (Granseth et al. 2002). The complex divergence/convergence of this feedback signal may require that the Group II mGluR-mediated reduction of corticogeniculate feedback be implemented at the level of individual afferent fibers to preserve the integrity of spatial and temporal feedback that may result from multiple layer 6 neurons, while protecting the capacity of the pathway to respond dynamically to new information.

Because in the natural environment salient visual features are constantly shifting, in visual thalamic relays there may be a requirement that cortical inputs be capable of dynamic regulation to preserve the information content of corticogeniculate feedback and allow the system to update. Group II mGluRs may function in this context by allowing early EPSCs to occur, but may attenuate later EPSCs that have less information content.

NEUROPROTECTION. Similar to our findings, Group II mGluR-mediated response attenuation during train stimulation has been observed in several hippocampal pathways (Kew et al. 2001; Scanziani et al. 1997). Presynaptic Group II mGluRs are thought to protect brain circuits from hyperexcitation stemming from excess glutamate release (Chapman et al. 1996; Schoepp 2001). Whereas Group I mGluR activation is epileptogenic in the hippocampus (Moldrich et al. 2001; Thuault et al. 2002), Group II mGluR activation blocks seizure-related activity (Moldrich et al. 2001). Group II mGluR agonists reduce development of kindling in the hippocampus (Attwell et al. 1998a) and reduce seizures in fully kindled animals by inhibiting neurotransmitter release (Attwell et al. 1998b).

High-intensity corticothalamic activity has been implicated in the etiology of absence epilepsy. Animal models have shown that spike-and-wave activity that is characteristic of absence seizures may originate in the neocortex (Meeren et al. 2002; Steriade and Amzica 2003), and normally occurring spindle oscillations can transition to hypersynchronous rhythms through high-frequency corticothalamic activation (Bal et al. 2000; Blumenfeld and McCormick 2000; Destexhe et al. 1998; Steriade et al. 1993). Fast runs of tonic cortical activity occurring at 10–15 Hz have been shown to precede the characteristic 2- to 4-Hz rhythms (Timofeev et al. 1998), and we have shown that thalamic responses to stimulus trains similar to such cortical fast runs are attenuated in the presence of an Group II mGluR agonist (Fig. 7, C and D).

Group II mGluRs may be useful therapeutic targets for the treatment of thalamic dysrhythmias that develop as a consequence of aberrant corticothalamic input. Presynaptic Group II mGluRs, perhaps in conjunction with Group III mGluRs could interrupt the transition from normal to abnormal thalamic synchrony by muting cortical fast runs at the level of the corticothalamic synapse (Fig. 7). One study has shown that activation of Group II mGluRs reduces absence rhythms in *lh/lh* mice (Moldrich et al. 2001). In addition to our observed Group II mGluR-mediated corticogeniculate response inhibition, Group II mGluR activation has also been shown to reduce GABAergic inhibition arising from the TRN (Salt and Eaton 1995b), which could further reduce the tendency of thalamo-cortical circuitry to engage in abnormal oscillations.

In conclusion, we have found a new intrinsic mechanism for the control of corticogeniculate feedback involving mGluR2

acting as a presynaptic autoreceptor on corticogeniculate terminals that have been physiologically characterized using paired-pulse protocols. Group II mGluR agonists reduced corticogeniculate input while having no effect on retinogeniculate input. NAAG application at physiologically active concentrations did not replicate the effects on EPSCs, suggesting a specific contribution of mGluR2 to our results. Stimulus trains of 10 Hz frequency applied to the corticogeniculate pathway elicited intrinsic mGluR2 responses that reduced train EPSCs and the overall amplitude of the late phase of the stimulus train. Group II mGluRs represent an important modulatory influence on excitatory transmission that allows fast information transmission through ionotropic glutamate receptors to occur, but squelches activity that may reduce the fidelity of information transmission in thalamocortical loops. Further studies at the in vivo level will elucidate the full impact of these receptors in normal sensory processing, and how such regulation can be targeted in the treatment of abnormal thalamic oscillations.

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