Activity-Dependent Activation of Presynaptic Metabotropic Glutamate Receptors in Locus Coeruleus

G. R. DUBÉ AND K. C. MARSHALL
Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada

Dubé, G. R. and K. C. Marshall. Activity-dependent activation of presynaptic metabotropic glutamate receptors in locus coeruleus. J. Neurophysiol. 83: 1141-1149, 2000. Synaptic activation of metabotropic glutamate receptors (mGluRs) in the locus coeruleus (LC) was investigated in adult rat brain slice preparations. Evoked excitatory postsynaptic potentials (EPSPs) resulting from stimulation of LC afferents were measured with current clamp from intracellularly recorded LC neurons. In this preparation, mGluR agonists (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (t-ACPD) and L(+)-2-amino-4-phosphonobutyric acid (L-AP4) activate distinct presynaptic mGluRs, resulting in an inhibition of EPSPs. When two stimuli were applied to afferents at intervals >200 ms, the amplitude of the second [test (T)] EPSP was identical in amplitude to the first [control(C)]. However, when a stimulation volley was delivered before T, the amplitude of the latter EPSP was consistently smaller than C. The activity-dependent depression (ADD) was dependent on the frequency and duration of the train and the interval between the train and T. ADD was potentiated in the presence of an excitatory amino acid (EAA) uptake inhibitor L-trans-pyrrolidine-2,4-dicarboxylic acid (t-PDC, 100 μM), changing the T/C ratio from 0.84 ± 0.05 (mean ± SE) in control to 0.69 ± 0.04 in t-PDC (n = 9). In the presence of t-PDC, the depolarizing response of LC neurons to focally applied glutamate was also increased. Together, these results suggest that accumulation of EAA after synaptic stimulation may be responsible for ADD. To test if ADD is a result of the activation of presynaptic mGluRs, the effect of selective mGluR antagonists on ADD was assessed. In the presence of t-PDC, bath applied (S)-amino-2-methyl-4-phosphonobutyric acid (MAP4, 500 μM), a mGluR group III antagonist, significantly reversed the decrease in T/C ratio after a train stimulation [from 0.66 ± 0.04 to 0.81 ± 0.02 (mean ± SE), n = 5]. The T/C ratio in the presence of MAP4 was not different from that measured in the absence of a stimulation volley. Conversely, ethyl glutamic acid (EGLU, 500 μM), a mGluR group II antagonist, failed to alter the T/C ratio. Together, these results suggest that, in LC, group III presynaptic mGluR activation provides a feedback mechanism by which excitatory synaptic transmission can be negatively modulated during high-frequency synaptic activity. Furthermore, this study provides functional differentiation between presynaptic groups II and III mGluR in LC and suggests that the group II mGluR may be involved in functions distinct from those of group III mGluRs.

INTRODUCTION

Neurotransmission by excitatory amino acids (EAA) is mediated via multiple receptors that are divided into two classes, ionotropic and metabotropic. Ionotropic receptors convey EAA signals through cation permeable channels, mediating postsynaptic depolarization within milliseconds after the release of neurotransmitters (see Clements et al. 1992). Metabotropic glutamate receptors (mGluRs) mediate EAA signals through a G-protein–dependent signal transduction pathway. Because of this, their effects are relatively slower, with an onset >50 ms after EAA binds to the receptor (Wickman and Clapham 1995). To date, eight mGluRs have been cloned and termed mGluR 1–8 (Anwyl 1999; Conn and Pin 1997). These can be classified into three groups based on their homology, pharmacology, and signaling pathways. Functional expression and microscopic localization demonstrated that mGluRs can be found pre- and postsynaptically and that they can be localized at the synapse and outside the synaptic zone (see Li et al. 1997; Martin et al. 1992; Petralia et al. 1996; Shigemoto et al. 1996, 1997). Thus these receptors likely contribute in different ways to synaptic transmission and modulation.

One of the commonly described effects of specific mGluR agonists is the reduction of synaptic transmission presynaptically at glutamatergic and GABAergic synapses (Conn and Pin 1997). All three groups of mGluRs have been implicated in presynaptic inhibition and, in many instances, more than one group and subtype can be present at the same synapse (Pin and Duvoisin 1995). Recently, activity-dependent endogenous activation of presynaptic group II mGluRs was demonstrated in hippocampal slices (Min et al. 1998; Scanziani et al. 1997). Furthermore, activation of presynaptic mGluRs resulting from blockage of EAA uptake was reported in hippocampal cultures, an effect mediated by a group II mGluR (Fitzsimonds and Dichter 1996; Maki et al. 1995).

In the locus coeruleus (LC), excitatory synaptic transmission can be inhibited after the activation of either a group II or a group III mGluR (Dubé and Marshall 1997a). Concomitant activation of both receptors with low doses of agonists results in an additive response whereas activating them with permaximal concentrations of agonists gives a response that is significantly less than additive, suggesting that both receptors share a common step in their signal transduction pathway. This is in agreement with observations in various expression systems that indicate that both group II and III receptors are coupled to a similar transduction pathway, i.e., negatively coupled to adenylate cyclase through a pertussis toxin–sensitive (G_ia G-proteins) pathway (Conn and Pin 1997). Although neither group of receptors inhibited excitatory postsynaptic potentials (EPSPs) via a cyclic adenosine monophosphate (cAMP)-dependent pathway in LC, both receptors appeared to be coupled to a G_ia G-protein (Dubé and Marshall 1997b). The LC is
the principal central noradrenergic nucleus, providing innervation throughout the neuraxis, including exclusive noradrenergic innervation of the neocortex and the hippocampus. Interestingly, it was recently demonstrated that administering a mGluR2/3 agonist reduced the behavioral effects associated with opioid withdrawal (Vandergriff and Rasmussen 1999). This was associated with a specific and significant decrease in the glutamate-mediated overactivity of the LC nucleus. Given the apparent involvement of LC neurons in opioid addiction (Aghajanian 1978; Rasmussen et al. 1990), the modulatory actions of mGluRs on this nucleus could become therapeutic targets.

In this study, we investigated the role of group II and III mGluRs in relation to the activity of excitatory synaptic transmission to LC. We hypothesized that if one or both mGluRs are located presynaptically and function as feedback receptors, these should inhibit EPSPs in a synaptic activity--dependent fashion. A portion of this work was previously presented in abstract form (Dubé and Marshall 1996).

**Methods**

All experiments were carried out using a brain stem slice preparation from young adult male Sprague-Dawley rats (4–8 wk, 50–150 g; Charles River, St. Constant, Quebec) under conditions designed to minimize animal suffering. A detailed account of these procedures can be found in Dubé and Marshall (1997a). In brief, animals were fully anesthetized with oxygen-rich halothane (2%) and surgically decapitated. A portion of this work was previously presented in abstract form (Dubé and Marshall 1996).

**Protocol for activity-dependent depression**

To assess synaptic depression after high-frequency synaptic transmission, stimulation volleys preceded and followed by single stimuli were delivered to the stimulating electrode. The first single stimulation resulted in a control (C)-evoked EPSP and the last resulted in the test (T) EPSP. Volleys were applied 100 ms after the first stimulation. For each condition, tests were carried out as follows (e.g., Fig. 1): 1) one test evoking the C and T EPSPs without a stimulation volley (Fig. 1A), 2) one test where only the stimulation volley was applied (Fig. 1B), and 3) three consecutive tests where both the single stimuli and the volley were applied (Fig. 1C). The individual tests consisted of four sweeps recorded at 0.2 Hz and were performed at 30 s intervals. All acquisition and measurements were made using pClamp 6.0 software (Axon Instruments Inc.). Data from voltage-current protocols were averaged online using four individual sweeps per averaged waveform. Data for EPSPs were averaged offline (with the clampfit module) using four individual sweeps per averaged waveform. For tests where only single EPSPs were evoked, the amplitudes of T and C were measured offline with respect to the baseline a few ms after the stimulus artifact. For the two other tests, the averaged volley waveform was subtracted from the averaged volley + EPSP waveform (Fig. 1D). The resulting waveform, comprising roughly only the C and T EPSPs, was analyzed in the same way. The ratio of T to C amplitudes measured from the resulting waveform (Fig. 1D) was used as an index of the effects of activity on LC excitatory synaptic transmission. Ratios >1 indicated a potentiation and ratios <1 a depression. Averaged data were presented as mean ± SE. For statistical analysis purposes, we tested whether or not the difference between two conditions (obtained by subtracting the respective T/C) was significant (as assessed by the Pearson correlation coefficient). For other statistical analysis, where indicated, analysis of variance (ANOVA) was performed followed by a Tukey post hoc test. Differences were considered significant at P < 0.05.

**Results**

**Basic characterization of activity-dependent depression of EPSPs**

To determine the conditions leading to activity-dependent depression (ADD), preliminary experiments were designed to delineate the range of durations and frequencies of the stimulation volley and the interval between the end of the volley and T, which resulted in maximal ADD of T with respect to C. Four parameters were examined: 1) the interval between C and T required to avoid paired-pulse facilitation in the absence of a pressure ejection using single pipettes with tip diameters of 8–12 μm. Pressure electrode tips were placed 50–100 μm away from the slice. The pressure (3–20 psi, 5–20 ms) applied to the electrode was adjusted through the pneumatic valve of a Picospirtizer II (General Valve Corp.). All other drugs were dissolved in ACSF and introduced through the perfusion line by gravity-induced flow. The pH was adjusted to 7.4 when necessary. Drugs used were (S)-amino-2-methyl-4-phosphonobutanoic acid (MAP4), ethyl glutamic acid (EGLU), L-trans-pyrrolidine-2, 4-dicarboxylic acid (t-PDC; Toceis Cookson), [-](-)-2-carboxypropyl-4-yl-propyl-1-phosphonate ([R]-CPP), (+)-α-methyl-4-carboxyphénylglycine (MCPG), baclofen, phacolol, scaphol, 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX), mianserin, 8-phenyl-theophylline and aminophylline (Research Biochemical International, Natick, MA), (-)-bicuculline methiodide, picrotoxin, and yohimbine HCl (Sigma).
stimulation volley, 2) the interval between the volley and T, 3) the duration of the volley, and 4) the frequency of the volley for optimal ADD. Figure 2 summarizes results pertaining to the first two parameters. Facilitation between a pair of EPSPs was observed when evoked 20–100 ms apart but was not observed at greater intervals (Fig. 2A). When a stimulation volley was applied (300 ms, 70 Hz) and the amplitude of T subsequently compared with C, T was consistently smaller when evoked 200–300 ms after the end of the volley (Fig. 2C). Because noradrenergic-presumptive slow IPSPs inherent to the LC nucleus were often observed during the tests, we assessed the effects of yohimbine on these. As shown in Fig. 2, B and D, 1 μM yohimbine reduced the slow IPSPs observed in both sets of tests but did not significantly affect the amplitudes and ratios of the EPSPs. We included yohimbine in subsequent experiments.

To test the last two parameters, i.e., the optimal duration and frequency of the volley, we used a stimulation train of 300 ms and 70 Hz. Figure 2 summarizes the results. A: The effect of varying the interval between C and T in the absence of stimulation volleys (9 independent experiments). Paired-pulse facilitation [(EPSP2/EPSP1)1/2] was observed at intervals <200 ms, but there was no significant change in T at intervals >200 ms. Bath-applied yohimbine (1 μM Yoh) did not affect the amplitude of the individual EPSP (dotted line). C: Superimposed waveforms showing the effect of yohimbine on the baseline potential after the first EPSP and the lack of effect on the amplitude of the second EPSP. B: The effect of varying the interval between T and the end of a 300 ms train (70 Hz) on ADD. Under these conditions, the ratio of T/C was significantly reduced when T was delivered 200–300 ms after the train (*, significance from 0 ms, p < 0.01, n = 7, as tested by analysis of variance (ANOVA) and Tukey post hoc test). Bath-applied yohimbine did not affect the amplitude of the individual EPSP (dotted line; †, significance from 0 ms, p < 0.05, n = 6, as tested by ANOVA and Tukey post hoc test), but decreased the hyperpolarization after the EPSP, as shown in D.
frequency of the stimulation volley, the C and T EPSPs were separated by an 800 ms interval (corresponding to 0 Hz stimulation). Keeping the interval between C and T constant, 100, 200, or 300 ms-long stimulation volleys were delivered to the LC afferents. Frequencies of stimulation between 10 and 90 Hz (increment of 10 Hz) were tested for each time duration, and the amplitude of T was compared with C (Fig. 3A). Results from these experiments showed significant ADD when 300 ms stimulation volleys were delivered to the LC afferents with frequencies ranging from 50 to 70 Hz. Figure 3B shows a representative example displaying how C and T EPSPs varied when 300-ms stimulation volleys of different frequencies (0–90 Hz) were applied. Note that a prolongation of the repolarization phase of the T EPSP, as compared with C, was sometimes observed, although not consistently, even within the same series of tests. This was not NMDA dependent (n = 3, not shown) or mGluR dependent and was not affected by any of the pharmacological treatments performed in this study (listed in Table 1).

Roles of mGluRs in ADD

To test the hypothesis that presynaptic mGluRs can be activated after stimulation volleys through the release of high amounts of EAA in the synaptic cleft, specific mGluR antagonists were tested for their effects on ADD. First, three mGluR antagonists were tested because of their actions on mGluR in LC (Dubé and Marshall 1997a). Of these, both (R)-MCPG and EGLU were shown to inhibit the effects of exogenously applied t-ACPD on excitatory synaptic transmission whereas MAP4 was found to antagonize selectively the L-AP4 effects. However, results from these experiments showed that after 15 min perfusion with each antagonist (500 μM), none caused a significant change in T/C although a tendency toward higher values was observed with each drug [control T/C vs. drug (mean ± SE) for MCPG: 0.74 ± 0.06 vs. 0.76 ± 0.06, n = 8; EGLU: 0.83 ± 0.04 vs. 0.94 ± 0.04, n = 7; MAP4: 0.67 ± 0.06 vs. 0.70 ± 0.06, n = 9].

We subsequently tested whether or not specifically blocking the uptake of EAA could potentiate ADD. As a control, the ability of t-PDC, an EAA uptake inhibitor, was assessed on focally applied glutamate-evoked depolarizations of LC neurons. As exemplified in Fig. 4, glutamate-evoked depolarizations were markedly potentiated in the presence of t-PDC (100 μM) in all of the five cells tested, suggesting that the clearance of the focally applied glutamate depends, in part, on the activity of the glutamate transporters. Occasionally, the firing frequency and the membrane potential of the impaled neuron were reversibly increased in the presence of t-PDC (Fig. 4). This was presumably caused by the accumulation of glutamate resulting from the clearance block. The changes in membrane potentials were compensated with injection of hyperpolarizing current, but the enhanced glutamate response remained despite the current injection. No overall significant differences in cell input resistance were observed with t-PDC (n = 9).

Figure 5 displays a representative example of the effect of 100 μM t-PDC on ADD. As depicted in this example, the amplitude of the C was not affected by the uptake inhibitor. Furthermore, when no stimulation volleys were delivered between C and T, the amplitude of T and T/C remained unchanged as compared with control (without t-PDC) (Fig. 5, C and E). Conversely, under the same conditions, when stimulation volleys were applied to the afferents, the amplitude of T was further reduced in the presence of t-PDC (Fig. 5, compare B and D). Thus in the presence of t-PDC, ADD was significantly larger than under control conditions (Fig. 5F) (mean T/C control vs. t-PDC: 0.84 ± 0.05 vs. 0.69 ± 0.04; P < 0.0001; n = 9). The effects of t-PDC were observed in every cell tested and thus were highly reproducible.

Using t-PDC, we tested the hypothesis that the potentiation of the depression observed with the uptake inhibitor was mediated by the activation of either or both groups of mGluRs
characterized in LC. Figure 6A displays representative examples of the effects of MAP4 (500 μM) on the relative amplitude of T in the presence of t-PDC. As expected, t-PDC significantly potentiated ADD. However, in the presence of MAP4, T/C was significantly increased not only as compared with conditions where t-PDC was present but also with respect to the control without stimulation volleys (Fig. 6C). Furthermore, this was not observed when EGLU was tested in the same manner (Fig. 6B and D). Thus application of MAP4, but not EGLU, under these conditions significantly reversed the t-PDC–dependent inhibition of T by stimulation volleys to levels that were not different from conditions where no stimulation volleys were applied. These results support the hypothesis that selective mGluRs can be activated to cause ADD of excitatory synaptic transmission.

Involvement of other factors in ADD

These tests present some caveats. One of these is that the focal stimulation applied to our preparations may result in the release of several neurotransmitters in addition to EAA. Two of these, GABA and noradrenaline (NA), have been addressed by inclusion of antagonists in the perfusion fluid. However, although the fast IPSPs mediated by GABA are blocked by inclusion of specific antagonists in the perfusate, other actions of GABA and other neurotransmitters could potentially interfere with the measurements. Therefore the involvement of three likely candidates, GABA (because of its action on the GABA B receptor), adenosine (a known neurotransmitter released during high synaptic activity), and serotonin [shown to depress excitatory synaptic transmission in LC (Bobker and Williams 1989; Charléty et al. 1993)] were investigated. Specific antagonists for each of the three neurotransmitters were tested as for mGluR antagonists. Table 1 summarizes the results obtained for all antagonists tested. Three specific antagonists of GABA B receptors were tested: phaclofen, saclofen, and CGP35348. We found that CGP35348 (100 μM) reversed the GABA B–induced (baclofen, 3 μM) presynaptic inhibition of excitatory synaptic transmission in LC (unpublished observations). However, at 100–500 μM concentrations, none of these antagonists significantly affected ADD. Three distinct antagonists of adenosine receptors were also tested. Aminophylline and 8-phenyl-theophylline are antagonists of the A1 and A2 receptor subtypes and DPCPX is a potent and selective A1 antagonist. Again, none of the antagonists affected ADD. The broad-range serotonin antagonist mianserin (500 μM) was also tested and was found to have no effect on ADD. Finally, we tested for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) desensitization using the desensitization blocker cyclothiazide (100 μM). Pooled results from eight experiments indicated that desensitization did not contribute significantly to the decrease in T/C. However, close inspection of the results revealed that when ADD produced a marked reduction in T/C (<65%), cyclothiazide caused a significant increase in T/C (P < 0.01). These results suggest that desensitization of AMPA does occur in our system under certain conditions. However, the reversal was only partial, contributing ~10% of the decrease in T/C (4 of 8 cells, Table 1).

**DISCUSSION**

We previously characterized two pharmacologically distinct presynaptic mGluRs that modulate excitatory synaptic transmission in LC (Dubé and Marshall 1997a). The two receptors belong to groups II and III, respectively, which have been shown in expression systems to be coupled to the same signal transduction pathway. We hypothesized that if both receptors

**TABLE 1. Effects of specific GABA B-, adenosine-, and serotonin-receptor antagonists on ADD**

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Antagonist</th>
<th>Control T/C</th>
<th>Test T/C</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>GABA B</td>
<td>Phaclofen (500 μM)</td>
<td>0.67 ± 0.08</td>
<td>0.70 ± 0.09</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Saclofen (500 μM)</td>
<td>0.68 ± 0.03</td>
<td>0.73 ± 0.06</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>CGP35348 (100 μM)</td>
<td>0.79 ± 0.04</td>
<td>0.75 ± 0.02</td>
<td>3</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Aminophylline (1 mM)</td>
<td>0.70 ± 0.02</td>
<td>0.67 ± 0.04</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>8-Phenyl-theophylline (500 μM)</td>
<td>0.74 ± 0.03</td>
<td>0.74 ± 0.02</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>DPCPX (0.1 μM)</td>
<td>0.79 ± 0.05</td>
<td>0.83 ± 0.09</td>
<td>4</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Mianserin (500 μM)</td>
<td>0.78 ± 0.03</td>
<td>0.72 ± 0.08</td>
<td>5</td>
</tr>
<tr>
<td>Desensitization</td>
<td>Cyclothiazide (100 μM)</td>
<td>0.50 ± 0.06</td>
<td>0.60 ± 0.06*</td>
<td>4</td>
</tr>
</tbody>
</table>

Values are means ± SE. *, significant difference at P < 0.01.

**FIG. 4.** Enhancing effect of glutamate uptake inhibition on exogenous glutamate-evoked depolarizations in LC neurons. Response of an LC neuron to focally applied glutamate (pressure application) under control conditions (top trace) and during bath application of L-trans-pyrididine-2,4-dicarboxylic acid (t-PDC; 100 μM). t-PDC consistently potentiated the effects of exogenous glutamate applications on LC neurons in all tests (n = 6). There were no significant changes in cell input resistance (right) under these conditions.
carried out identical functions, then both should be functionally activated in a similar fashion. However, if these receptors served different functions resulting in a similar effect, it might be possible to activate one independently of the other. Our results show that under conditions in which extracellular EAA levels should be strongly enhanced, the group III mGluR can be endogenously activated. In contrast, under the same conditions, activation of the group II mGluR was not observed.

Glutamate released into the synaptic cleft may be expected to activate the various EAA receptors differentially, depending on their sensitivity to the neurotransmitter and their location in relation to the synaptic cleft. The average effective concentration for 50% of maximal response of AMPA receptors for glutamate ranges from 250 to 1500 μM (Clements 1996; Liu et al. 1999) whereas that of mGluRs is between 3 and 20 μM with the exceptions of mGluR7, for which it is ~1000 μM, and mGluR8, for which it is ~0.02 μM (Conn and Pin 1997). Based on the time/concentration profile of glutamate released by a single quantum, as estimated by several laboratories (e.g., Clements et al. 1992; Holmes 1995; Otis et al. 1997), the concentration of glutamate stays at AMPA-activating concentrations for only ~1 ms but could be high enough to act on mGluRs for a relatively long time after release. Thus it is likely that mGluRs, if located in the synaptic cleft, would be activated. This is supported by the findings of Schrader and Tasker (1997) that show tonically active mGluRs.

FIG. 5. Potentiating effect of glutamate uptake inhibition on ADD of evoked EPSPs in LC neurons. Set of ADD experiments under control conditions (A and B) and after 15 min perfusion with 100 μM t-PDC (C and D) (average of 2–4 consecutive sets of measurements). A and C: conditions where no stimulation volley was applied. B and D: conditions where a 70-Hz stimulation volley (300 ms) was applied between C and T. Note that t-PDC caused an increase in ADD but failed to change the response of the cell when stimulation volleys were not applied. E and F: the average (*) and individual (○) values for each experiment testing the effects of t-PDC on ADD. Effect of t-PDC on ADD was significantly different from control (p < 0.0001, paired t-test).
et al. 1995). Group II mGluRs have been found to have a relatively broad distribution with rather diffuse presynaptic staining (Luján et al. 1997; Petralia et al. 1996; Shigemoto et al. 1997). Conversely, members of group III have been found to be more selectively associated with presynaptic sites of axon terminals in the hippocampus (Shigemoto et al. 1997), providing a possible morphological correlation for the differences we noted in these functional studies.

The lack of effect of any of the three mGluR antagonists in the absence of blockade of glutamate transport indicated that most of the ADD in this circumstance was caused by other mechanisms. Of the possible mechanisms, we were able to rule out the participation of other presynaptic receptors, i.e., GABA<sub>B</sub>, adenosine A<sub>1</sub>/A<sub>2</sub>, and serotonin. A relatively small but significant degree of AMPA receptor desensitization was observed when ADD was pronounced but was negligible otherwise. Thus AMPA desensitization could not account for most of the ADD. Alternatively, ADD of EPSPs could be attributed to the intrinsic properties of synapses. Of these, depletion of vesicles available for evoked release (Liu and Tsien 1995; Zucker 1989) or presynaptic calcium-dependent transient modifications of evoked release (Hsu et al. 1996; Mori et al. 1994) have been suggested. The lack of effect of mGluR antagonists on ADD suggests either that the t-ACPD and L-AP4 receptors in LC are not feedback receptors in normal function or that the conditions used in our tests were insufficient to produce a significant level of activation of those presynaptic mGluRs. This is consistent with recent findings in the calyx of Held, which demonstrated that mGluRs (group II and/or III) contributed only to a small portion (~6%) of the ADD observed (von Gersdorff et al. 1997).

We did observe that the glutamate uptake inhibitor t-PDC caused a significant increase in ADD and that this change was reduced by the group III mGluR antagonists, supporting the idea that increased ambient EAA can produce presynaptic inhibition by mGluR activation. However, t-PDC had no effect on single evoked EPSP amplitude, suggesting that although the transporters have little effect on clearance of EAA from the synaptic cleft during relatively slow synaptic transmission, they may play a significant role during robust presynaptic release of glutamate. Other reports also described activity-dependent endogenous activation of presynaptic mGluRs in the presence of EAA uptake blockade. In cultured hippocampal neurons, the frequency-dependent inhibition of EPSPs was not affected by the broad range antagonist MCPG (Maki et al. 1995) although depression of EPSPs by 250 μM t-PDC was

FIG. 6. Reversal of the effect of ADD on EPSP amplitude in the presence of t-PDC by group III but not group II mGluR antagonist. A1–B1: average of 4 consecutive sets of measurements displaying the ADD of EPSPs under control conditions in the presence of 100 μM t-PDC (A2–B2), t-PDC and a group III mGluR antagonist [500 μM (S)-amino-2-methyl-4-phosphonobutanoic acid (MAP4)] (A3), or 500 μM group II mGluR antagonist ethyl glutamic acid (EGLU) (B3), t-PDC, MAP4, and EGLU were applied by bath 10–15 min before testing. C: summary of the effects of MAP4 with t-PDC; average ratios in the absence and presence of trains under the conditions indicated. [n = 5 individual experiments; *, significantly different from no train (p < 0.005); **, significantly different from basal with trains (p < 0.02); ***, significantly different from t-PDC with trains (p < 0.006).] D: summary of the effects of EGLU with t-PDC; average T/C ratios in the absence and presence of trains under the conditions indicated. [n = 8 individual experiments; *, significantly different from no train (p < 0.02); **, significantly different from basal with trains p < 0.01.]
effects of EPSPs evoked at 1 Hz and, again, MCPG reversed the synaptic transmission in the hippocampal slice (Scanziani et al. 1997), an effect blocked by MCPG or (2S,3S,4S)-methyl-2-(carboxycyclopropyl)-glycine (MCCG), suggesting a group II mGluR. Similar to our results, (t)-ACPD did not affect basal synaptic transmission in the hippocampal slice (Scanziani et al. 1997). However, t-PDC significantly decreased the amplitude of EPSPs evoked at 1 Hz and, again, MCPG reversed the effects of t-PDC (Scanziani et al. 1997). Release of EAA from the corticostriatal system was enhanced by MCPG in the presence of t-PDC, but not in its absence (Lada et al. 1998). Finally, in the hippocampal slice, alteration of glutamate clearance by various means, such as changes in temperature or application of a glutamate-scavenging agent, decreased presynaptic mGluR activation by 1 Hz stimulation whereas decreasing clearance (by increasing extracellular viscosity) had the opposite effect (Min et al. 1998). Overall, these results indicate that presynaptic mGluRs can be endogenously activated under specific conditions but that these conditions may vary between different systems and protocols, likely because of the heterogeneity that exists between synapses in different parts of the CNS. These ideas are dealt with more extensively in a review by Anwyl (1999).

Figure 7 is a schematic providing a scenario that could explain our results. The L-AP4–sensitive receptor was placed far enough away from the release site so that it would not be activated during high-frequency synaptic transmission and spillover of neurotransmitters. Conversely, the t-ACPD–sensitive receptor was placed far enough away from the release site so that it would not be activated during high-frequency synaptic transmission. In this schematic, application of exogenous agonists would result in the activation of either receptor. Based on the present study, we propose that only the group III mGluR is a feedback receptor in LC. The function of group II remains unknown. However, mGluRs have been found away from the synapse (e.g., on axon and dendrite shafts) and could be targets for other synapses (Martin et al. 1992; Petralia et al. 1996; Shigemoto et al. 1996). Note that the glutamate transporters were omitted from the schematic. These have been found on neurons and glia that envelop the terminals (Rothstein et al. 1994). High-density transporters have been shown to be associated with the postsynaptic terminal (Rothstein et al. 1994). In a recent study in Purkinje neurons, it was estimated that there is a 15:1 ratio between EAAT4 alone and AMPA receptors (Otis et al. 1997). Therefore, even though the transporter activity appears to be too slow to cause the termination of synaptic transmission, the high concentration of transporter on the postsynaptic membrane could substantially buffer EAA. Thus, in LC, MAP4-sensitive mGluRs appear to act as feedback receptors under conditions where the clearance of EAA is saturated or impaired. Physiological and pathophysiological implications for these findings can be better appreciated in light of recent findings implicating EAA transporters. For example, significant downregulation of the glial transporter GLAST (assessed by Western blot analysis) was reported in the piriform cortex/amygdala as early as 24 h after kindling-induced epilepsy (stage 3 seizure) and persisted through multiple stage 5 seizure (Miller et al. 1997). In contrast, the neuronal transporter EAAC-1 was found to be upregulated in the same areas, but only when the animal had reached stage 5. Therefore the ability for glutamate buffering between stages 3 and 5 was likely to be markedly reduced. Thus, although t-PDC application may not reflect physiological conditions, it clearly...
mimics conditions observed under specific pathologies such as epilepsy and perhaps other abnormal conditions.

In summary, two groups of presynaptic mGluRs are present on LC afferents, but only those belonging to the group III mGluR were found to be activated under conditions that promoted EAA accumulation and spillover. We conclude that these receptors function as negative feedback receptors in LC. Conversely, the presynaptic group II mGluRs were not activated under the same conditions, suggesting that these receptors may be involved in a negative modulation of presynaptic transmission distinct from group III mGluRs, and ruling out duplication in function at presynaptic terminals innervating LC neurons.

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Present address of G. R. Dubé: Center for Learning and Memory and Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, E25-435, Cambridge, MA 02139.

Address for reprint requests: K. C. Marshall, Dept. of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, 451 Smyth Rd., Ottawa, Ontario K1H 8M5, Canada.

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