Group I Metabotropic Glutamate Receptors Mediate Slow Inhibition of Calcium Current in Neocortical Neurons

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Sayer, Rod J. Group I metabotropic glutamate receptors mediate slow inhibition of calcium current in neocortical neurons J. Neurophysiol. 80: 1981–1988, 1998. Metabotropic glutamate receptor (mGluR)-mediated inhibition of high-voltage-activated Ca\(^{2+}\) currents was investigated in pyramidal neurons acutely isolated from rat dorsal frontoparietal neocortex. Whole cell recordings were made at 30–32°C, with Ca\(^{2+}\) as the charge carrier. Selective agonists were used to classify the subgroup of mGluRs mediating the response. Ca\(^{2+}\) currents were inhibited by (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) and by the group I agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) but not by the group II agonist (2S,2’R,3’R)-2-(2,3’-dicarboxycyclopropyl)-glycine (DCG-IV) or the group III agonist l(-)-2-amino-4-phosphonobutyric acid (l-AP4). (2S,1’S,2’S)-2-(carboxycyclopropyl)-glycine (l-CCG-I) was effective at 10 and 100 μM but not at 1 μM, consistent with involvement of group I mGluRs. Variable results were obtained with the putative mGluR5-selective agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) and the putative mGluR1-selective antagonist (S)-4-carboxycyclohexylglycine [(S)-4CPG], indicating that the group I mGluR subtypes may vary between cells or that these compounds were activating other receptors. The actions of (+)-α-methyl-4-carboxycyclohexylglycine [(+)-MCPG] were consistent with it being a low-potency antagonist. Several features of the Ca\(^{2+}\) current inhibition evoked by DHPG distinguished it from the rapid modulation typical of a direct action of G proteins on Ca\(^{2+}\) channels; the inhibition was slow to reach maximum (tens of seconds), current activation was not slowed or shifted in the positive voltage direction, and the inhibition was not relieved by positive prepulses. Nm赞赏pine and ω-conotoxin GVIA blocked fractions of the current and also reduced the magnitude of the responses to DHPG, indicating that both L- and N-type Ca\(^{2+}\) channels were regulated. These results further differentiate the slow modulatory pathway observed in neocortical neurons when Ca\(^{2+}\) is used as the charge carrier from the rapid voltage-dependent mechanism reported to inhibit Ba\(^{2+}\) currents under Ca\(^{2+}\)-free conditions.

INTRODUCTION

Metabotropic glutamate receptors (mGluRs) mediate inhibition of high-voltage-activated Ca\(^{2+}\) current in a number of neuronal types, including neocortical and hippocampal pyramidal neurons (Choi and Lovinger 1996; Lester and Jahr 1990; Sahara and Westbrook 1993; Sayer et al. 1992; Swartz and Bean 1992). Regulation of Ca\(^{2+}\) channels at somata and dendrites may influence neuronal excitability or synaptic plasticity, and at presynaptic terminals it may play a role in autoinhibition of transmitter release (reviewed by Stefani et al. 1996b). mGluR subtypes are encoded by eight known genes and are classified into three groups based on amino acid sequence similarities and pharmacology: group I, mGluR1 and mGluR5; group II, mGluR2 and mGluR3; and group III, mGluR4, mGluR6, mGluR7, and mGluR8. Splice variants have been found for several mGluR subtypes (for a review of mGluR classification and pharmacology see Conn and Pin 1997). Recent studies used selective agonists to identify which groups of mGluRs are involved in Ca\(^{2+}\) channel regulation. In neocortical pyramidal neurons, receptors from all three groups have been implicated; Ca\(^{2+}\) channel inhibition was observed in response to the group I agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) and the group II agonist (2S,2’R,3’R)-2-(2,3’-dicarboxycyclopropyl)-glycine (DCG-IV) (Choi and Lovinger 1996) and also to l(-)-2-amino-4-phosphonobutyric acid (l-AP4), a group III agonist (Stefani et al. 1996a 1998). The Ca\(^{2+}\) channel subtypes targeted for modulation by mGluRs in neocortical neurons were N-type and possibly P-type (Choi and Lovinger 1996) or predominantly L-type (Sayer et al. 1992), depending on experimental conditions.

Studies of Ca\(^{2+}\) channel regulation in sympathetic neurons have revealed a variety of G protein–mediated mechanisms (Hille 1994). A major pathway that inhibits N-type channels is fast, membrane delimited, does not require the presence of Ca\(^{2+}\), and involves a shift in the voltage dependence of channel gating. Another pathway is slower, probably involves a diffusible second messenger, requires Ca\(^{2+}\), is not associated with a gating shift, and targets L- and N-type channels. In neocortical pyramidal neurons, the mGluR-mediated inhibition of Ba\(^{2+}\) current through N-type (and possibly P-type) Ca\(^{2+}\) channels described by Choi and Lovinger (1996) showed the hallmarks of the fast pathway with a gating shift. These included a rapid agonist action (near-maximal responses in 2–4 s), a slowing of Ca\(^{2+}\) current activation, voltage-dependent inhibition (less inhibition at more positive potentials), and temporary relief of the inhibition by strongly positive voltage steps. This contrasted with an earlier study (Sayer et al. 1992) in which Ca\(^{2+}\) was used as the charge carrier, where a slow (1–2 min to maximum) inhibition of L-type Ca\(^{2+}\) channels was observed. The aim of this investigation was to characterize further the slow modulation of Ca\(^{2+}\) current by mGluRs in neocortical pyramidal neurons, by 1) using selective agonists to classify the group of mGluRs mediating the response and by 2) obtaining evidence as to whether the targeted Ca\(^{2+}\) channels undergo a gating shift.
METHODS

Cell preparation and recording

Rats aged 10–21 days were anesthetized with ketamine (150 mg/kg ip) and xylazine (10 mg/kg ip) and killed by carotid section. Transverse slices 500 μm thick were cut from dorsal frontoparietal (sensorimotor) cortex in bicarbonate-buffered saline (Sayer et al. 1992) saturated with 95% O₂-5% CO₂ at ~4°C. The slices were chopped into pieces 1–2 mm wide and incubated for 45–60 min with 19 U/ml papain in a saline containing (in mM) 5 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 5 Na-HEPES, 140 NaCl, 3 KCl, 1.25 NaH₂PO₄, 10 glucose, 2 MgCl₂, and 2 CaCl₂, pH 7.4, temperature 28°C, saturated with O₂. The tissue pieces were maintained in this solution (without papain) at room temperature until immediately before use. Cells were isolated by trituration through Pasteur pipettes of decreasing aperture in a similar saline but with 5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), no added CaCl₂, and 10 mM MgCl₂.

Recordings were made in whole cell voltage clamp (Axopatch 20A, pClamp: Axon Instruments, Foster City, CA) from neurons of pyramidal morphology with apical dendrites 200 μm in diameter. The recording micropipettes contained (in mM) 30 tris(hydroxymethyl)aminomethane (Tris)-base, 70 diTris-PO₄, 4 MgCl₂, 5 NaCl, 0.286 CaCl₂, 1.12-bis-(2-amino-phenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA), 0.1 leupeptin, 0.3 GTP (Tris salt), 4 ATP (Tris salt), 20 phosphocreatine, and 50 μM creatine phosphokinase, pH 7.2. Under these conditions a calcium association constant of 4.5–6.5 × 10⁻⁴ M⁻¹ would be expected for BAPTA (Harrison and Bers 1987), giving an estimated free intracellular Ca²⁺ concentration of 60–90 nM under resting conditions. The aim of using a relatively low concentration of BAPTA (1 mM rather than 10 mM BAPTA or EGTA) was to provide conditions more permissive to Ca²⁺-dependent mechanisms.

After gaining whole cell access, the cells were superfused with a tris(hydroxymethyl)aminomethane (Tris)-base, 70 diTris-PO₄, 4 MgCl₂, 5 NaCl, 0.286 CaCl₂, 1.12-bis-(2-amino-phenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA), 0.1 leupeptin, 0.3 GTP (Tris salt), 4 ATP (Tris salt), 20 phosphocreatine, and 50 μM creatine phosphokinase, pH 7.2. Under these conditions a calcium association constant of 4.5–6.5 × 10⁻⁴ M⁻¹ would be expected for BAPTA (Harrison and Bers 1987), giving an estimated free intracellular Ca²⁺ concentration of 60–90 nM under resting conditions. The aim of using a relatively low concentration of BAPTA (1 mM rather than 10 mM BAPTA or EGTA) was to provide conditions more permissive to Ca²⁺-dependent mechanisms.

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Data were obtained from 75 neocortical pyramidal neurons. The standard protocol for the pharmacological experiments was to hold the cells at −60 mV and evoke Ca²⁺ currents by stepping to −10 mV for 50 ms at 20-s intervals. At a holding potential of −60 mV, the low-voltage-activated (T-type) Ca²⁺ current is inactivated in these neurons (Sayer et al. 1990), and the test potential of −10 mV was close to the negative peak of the current-voltage relationship, as determined for each cell at the beginning of recording (not shown).

Group-selective agonists

DHPG is a selective agonist at group I mGlurRs (Baker et al. 1995; Gereau and Conn 1995), whereas DCG-IV is selective for group II mGlurRs (Hayashii et al. 1993; Ishida et al. 1993). 1S,3R-ACPD is a more “broad-spectrum” mGlurR agonist, activating all receptors of groups I and II, although it is of low potency, where determined, at mGlurRs of group III (see Conn and Pin 1997). All three agonists were applied, in varying order, to 14 neurons. 1S,3R-ACPD (200 μM) and DHPG (200 μM) were similarly effective in inhibiting the Ca²⁺ current, producing reductions in amplitude of 31 ± 3.4 and 30 ± 3.6% respectively. DCG-IV (10 μM), in comparison, was ineffective (1 ± 0.4% inhibition; Fig. 1A and B). Individual current traces from one neuron are shown in Fig. 1C; note that when the current was reduced by 1S,3R-ACPD or DHPG there was no significant slowing of activation.

1S,3R-ACPD may be useful for distinguishing group II from group I mGlurRs, because the concentrations causing 50% ef-
High-voltage-activated Ca\(^{2+}\) currents were inhibited by (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD, 200 μM) and (RS)-3,5-dihydroxyphenylglycine (DHPG, 200 μM) but not by (2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV, 10 μM). Figure 1A: peak Ca\(^{2+}\) current amplitudes vs. time plotted for 1 neuron. Currents were evoked by a step depolarization from −60 to −10 mV (50-ms duration). Drugs were applied at the times indicated by the horizontal bars. B: mean Ca\(^{2+}\) current inhibition (n = 14) in response to the agonists (error bars represent SE). C: individual records from the neuron in A taken at times indicated by the lowercase letters. The scale bars represent 100 pA and 10 ms.

Compounds with differential action on group I mGluR subtypes

Two compounds putatively useful in distinguishing mGluR5a from mGluR1a were tested; CHPG, which was reported to activate mGluR5a but not mGluR1a (Doherty et al. 1997), and (S)-4CPG, which has competitive antagonist selectivity for mGluR1a over mGluR5a (Brabet et al. 1995).

Application of CHPG (1 mM) resulted in inhibition of the Ca\(^{2+}\) current in 7 of 10 neurons tested. The reduction

\[\text{Inhibition (\%)} = \frac{\text{Current before drug} - \text{Current after drug}}{\text{Current before drug}} \times 100\]

was 19 ± 0.6% for 1 mM CHPG, 21 ± 2.5% for 10 μM CHPG, 30 ± 4.9% for 100 μM CHPG, and 23 ± 3.4% for 200 μM 1S,3R-ACPD (Fig. 2B).

The group III-selective agonist L-AP4, at a concentration of 1 mM, did not inhibit the Ca\(^{2+}\) current consistently in these experiments. Instead, the most common finding for L-AP4 was a small increase in peak Ca\(^{2+}\) current (9/13 neurons, e.g., Fig. 3A). In the remainder there was no response, or in one case there was a 5% reduction. Overall L-AP4 enhanced the current by 6 ± 1.6%, and in the same cells 200 μM 1S,3R-ACPD induced a 26 ± 3.8% inhibition (Fig. 3B).
associated with CHPG was 17 ± 3.0%, whereas in the same cells the response to 200 μM DHPG was 35 ± 3.8% inhibition. In five of these seven neurons, the response to CHPG was biphasic; the inhibitions followed small (7 ± 0.9%) and transient (peak ≤40 s) increases in the current seen during wash-in of the drug (Fig. 4A). The responses to DHPG in the same neurons were purely inhibitory. In the remaining 3 of the 10 neurons tested, application of CHPG was associated with monophasic increases in the current (15 ± 4.3%), whereas in the same cells DHPG produced 23 ± 6.6% inhibition (Fig. 4B).

Antagonist activity of (S)-4CPG (500 μM) was assessed by application of 50 μM 1S,3R-ACPD in the absence and presence of the compound. In three of six neurons tested, (S)-4CPG had no apparent effect; the mean inhibition of the Ca²⁺ current in response to 1S,3R-ACPD alone was 16 ± 2.7% compared with 19 ± 3.3% in the presence of (S)-4CPG (Fig. 4C). However, for the remaining three cells in which 1S,3R-ACPD alone produced a mean inhibition of 15 ± 1.4%, the application of (S)-4CPG was associated with an increase in the Ca²⁺ current (mean 10 ± 1.9%). The subsequent application of 1S,3R-ACPD in the presence of (S)-4CPG produced no discernible effect in these neurons (Fig. 4D).

Antagonism by (+)-MCPG

(+)-MCPG is a competitive antagonist at some cloned mGluRs of groups I and II but not group III (Hayashi et al. 1994), and it has been shown to inhibit mGluR-mediated responses in a number of systems (reviewed by Roberts 1995). In six neurons, the response to 50 μM 1S,3R-ACPD was tested with and without 1 mM (+)-MCPG in the bath. In these cells, the Ca²⁺ current inhibition was 24 ± 6.4% for 1S,3R-ACPD alone and 9 ± 5.2% for 1S,3R-ACPD in the presence of 1 mM (+)-MCPG (Fig. 5). This difference was significant (P = 0.008, paired t-test). However, 1 mM (+)-MCPG had no effect on Ca²⁺ current inhibition evoked by 200 μM 1S,3R-ACPD in two other cells (not shown).

Time course of Ca²⁺ current inhibition

To determine the time course of the mGluR-mediated inhibition, Ca²⁺ currents were evoked every 2 s, and the turnover time of the recording chamber was minimized by reducing the fluid level and increasing the flow rate. The time course of drug delivery was estimated for each neuron by switching to a superfusion saline with 1.5 mM Mn²⁺ (instead of the usual 0.75 mM Mn²⁺), which produced further partial blockade of the Ca²⁺ current. DHPG (200 μM) applied to four of these neurons inhibited the Ca²⁺ current with a much slower time course than was caused by Mn²⁺ blockade (Fig. 6). When fitted with a single exponential, the time constants obtained for inhibition by DHPG in the four neurons were (in seconds) 21.9, 21.4, 27.3, and 9.7 (mean 20.1). In the same cells the time constants for Mn²⁺ blockade were almost an order of magnitude shorter, at 2.4, 3.4, 2.7, and 1.9 s.
happened to be the one with the shortest time constant for the response to DHPG, the delay was not resolvable at the 2-s interval between evoked currents.

Voltage dependence of \( \text{Ca}^{2+} \) channel inhibition

A shift in the voltage dependence of channel gating is a common mechanism of \( \text{Ca}^{2+} \) channel inhibition, and it was reported to occur for mGluR responses in neocortical neurons (Choi and Lovinger 1996). To investigate whether this was happening under the conditions of the current experiments, activation curves were constructed from tail currents evoked before and after application of 100 \( \mu \text{M} \) DHPG. DHPG did not alter the voltage dependence of \( \text{Ca}^{2+} \) channel activation (Fig. 7A). In the same cells, the inhibition of peak \( \text{Ca}^{2+} \) current induced by DHPG was not relieved by a 50-ms prepulse to 80 mV (Fig. 7, B and C). Facilitation (an increase in \( \text{Ca}^{2+} \) current after a depolarizing prepulse) was not seen in either the presence or absence of DHPG (Fig. 7C).

Inhibition of \( \text{Ca}^{2+} \) channel subtypes

To determine which \( \text{Ca}^{2+} \) channel subtypes were inhibited under the conditions of the current experiments, DHPG (200 \( \mu \text{M} \)) was applied before and after successive fractions of the \( \text{Ca}^{2+} \) current were blocked by \( \nu \)-CgTX (an N-type channel blocker) and nimodipine (an L-type channel blocker). An example of the effects of the drugs on peak \( \text{Ca}^{2+} \) current amplitude is shown in Fig. 8A. In these cells (\( n = 7 \)),
ω-CgTX (≈3 μM) blocked 23 ± 2.3%, and nimodipine (5 μM) blocked 33 ± 2.7% of the total (baseline) current (Fig. 8B), leaving 43 ± 1.8% resistant to both. Note that the fractions of current blocked are similar to previous findings (Brown et al. 1994), suggesting that the Mn²⁺ present did not selectively block of any one type of Ca²⁺ channel. Neither ω-CgTX nor nimodipine abolished the response to DHPG, but each reduced the magnitude of the inhibition (i.e., the absolute amount of Ca²⁺ current inhibited), suggesting that each blocked some of the channels modulated by DHPG. Nimodipine reduced the DHPG-sensitive current by 36 ± 7.9%, whereas ω-CgTX reduced it by 30 ± 7.2% (Fig. 8C). After both ω-CgTX and nimodipine were applied, the remaining current sensitive to DHPG represented 34 ± 8.2% of the original (baseline) current inhibited by DHPG.

**DISCUSSION**

The effectiveness of DHPG implicates group I mGluRs in a slow pathway for Ca²⁺ current inhibition in neocortical pyramidal neurons. The finding that L-CCG-I was active at ≈10 μM is consistent with involvement of group I receptors because, although this agonist would be expected to selectively activate group II receptors at 1 μM, it also activates group I mGluRs at higher concentrations (Hayashi et al. 1992; Pin et al. 1994). In addition, the results fit with the rank order of potency previously found for Ca²⁺ current inhibition in these neurons (quisqualate > glutamate > trans-ACPD) (Sayer et al. 1992), a profile that is now considered characteristic of group I mGluRs.

The use of CHPG and (S)-4CPG to distinguish subtypes of group I mGluRs produced variable results. In the majority of neurons tested, the Ca²⁺ currents were inhibited by 1 mM CHPG, implicating mGluR5. The smaller amplitude of the inhibition, compared with 200 μM DHPG, is consistent with CHPG being a low-potency (and/or partial) agonist at this receptor (Doherty et al. 1997). However the results were complicated by the biphasic nature of the responses and, in a minority of cases, by pure increases of the current. Without further information on the specificity of CHPG, an action on non-group I receptors cannot be ruled out; such activity could converge on the same pathways or Ca²⁺ channels to oppose the group I mGluR-mediated inhibition. Alternatively, variable expression of group I subtypes (or their splice variants) between cells might account for the findings. Both mGluR1 and mGluR5 have been localized to neocortex by immunohistochemistry, although mGluR1 expression is low relative to mGluR5 (Baude et al. 1993; Martin et al. 1992; Romano et al. 1995; Shigemoto et al. 1993).

The same possibilities could underlie the findings for (S)-4CPG. The lack of antagonist action for some neurons would be consistent with mediation by mGluR5 (Brabet et al. 1995), whereas the slight enhancement of the current and block of the inhibition in other cells might reflect antagonism of an mGluR1 with a small degree of tonic activity. Alternatively, there may have been interference from agonist actions at non-group I mGluRs (Bedingfield et al. 1995).

The lack of response to 10 μM DCG-IV or 1 μM L-CCG-I suggests that group II mGluRs are rarely or never involved in the slow inhibition of Ca²⁺ current in these neurons. It is unlikely that the ineffectiveness of DCG-IV was due to the concentration being too low, given that the EC₅₀ for the action of DCG-IV on group II mGluRs expressed in oocytes were submicromolar (Hayashi et al. 1993), and 5 μM DCG-IV evoked a fast inhibition of Ca²⁺ channels in 75% of neocortical neurons tested when Ba²⁺ was used as the charge carrier (Choi and Lovinger 1996). The results of Choi and Lovinger (1996) indicate that group II mGluRs are present in these cells and that they have modulatory influence on Ca²⁺ channels, which can be revealed under certain conditions. That at least a subset of neocortical projection neurons produce group II mGluRs is also suggested by the presynaptic depression induced by DCG-IV at corticostriatal synapses (Lovinger and McCool 1995), although this is not necessarily mediated by regulation of Ca²⁺ channels.

The present findings for the group III agonist L-AP4 do not support a role for group III mGluRs in the slow inhibitory pathway for Ca²⁺ current inhibition, at least not under the conditions of these experiments. Instead, L-AP4 induced a small enhancement of Ca²⁺ current in the majority of neurons. Choi and Lovinger (1996) likewise reported only small effects of group III agonists on Ba²⁺ current in neocortical neurons (mean 3% inhibition with 100 μM L-AP4). More significant inhibition of Ba²⁺ current (mean 22% with 100 μM L-AP4) was observed by Stefani et al. (1996a, 1998) in the same type of neuron, but in older rats [aged 1–2 mo instead of 9–21 days for Choi and Lovinger (1996) and this study]. There is evidence for group III mGluRs being involved in suppression of excitatory synaptic transmission in the neocortex (Burke and Hablitz 1994), although again the inhibition of Ca²⁺ channels in presynaptic terminals is just one of a number of possible mechanisms by which this could occur.

Similar to the findings for the fast inhibition of Ba²⁺ current in neocortical neurons (Choi and Lovinger 1996), (+)-MCPG produced a partial and variable block of Ca²⁺ current inhibition at submaximally effective concentrations of 1S,3R-ACPD. The findings of this study are consistent with (+)-MCPG acting as an antagonist, albeit of low potency, at group I mGluRs. Note that (+)-MCPG has previously been shown to antagonize responses to 1S,3R-ACPD in cells expressing mGluR1a and in cells expressing mGluR5a (Brabet et al. 1995).

A number of features distinguished this form of modulation from the typical fast inhibition of Ca²⁺ channels that is associated with a shift in the voltage dependence of channel gating. The responses to 1S,3R-ACPD and DHPG were slow, taking tens of seconds to reach a maximum. The time courses were an order of magnitude slower than those observed for mGluR agonists in other studies in neocortical neurons where Ca²⁺ was absent and Ba²⁺ was used as the charge carrier (Choi and Lovinger 1996; Stefani et al. 1996a, 1998). No evidence was found for the Ca²⁺ current inhibition evoked by DHPG being voltage dependent. There was no obvious slowing of current onset, there were no shifts in the activation curves, and the inhibition was not relieved by strongly positive prepulses. This is in marked contrast to the voltage-dependent inhibition seen in the same cells in the absence of Ca²⁺ (Choi and Lovinger 1996) and that ob-
erved in many examples of Ca\(^{2+}\) channel inhibition in other types of neuron (reviewed by Dolphin 1995). However, the voltage-independent pathway for inhibition of N- and L-type channels in sympathetic neurons, activated by muscarinic and angiotensin II receptors, is slow in onset, sensitive to intracellular Ca\(^{2+}\) chelation, and involves a diffusible messenger (Hille 1994). This appears similar to the mechanism of inhibition described here, although the experiments required to establish the involvement of a diffusible messenger have not yet been performed in neocortical neurons.

The experiments with Ca\(^{2+}\) channel antagonists showed that the mGluRs activated by DHPG regulate multiple subtypes of Ca\(^{2+}\) channel. \(\omega\)-CgTX and nimodipine each caused a reduction in the amount of Ca\(^{2+}\) current inhibited by DHPG, suggesting that N- and L-type Ca\(^{2+}\) channels were inhibited in the baseline (initial) response to DHPG. The Ca\(^{2+}\) current that remained after block by \(\omega\)-CgTX and nimodipine was probably mostly conducted by P-type channels, along with some through channels resistant to organic blockers (\(\approx 30\) and 10\% of the original current, respectively) (Brown et al. 1994; Choi and Lovinger 1996). Therefore the residual response to DHPG after block by \(\omega\)-CgTX and nimodipine probably involved inhibition of P-type Ca\(^{2+}\) channels, although modulation of the resistant ("R-type") channels cannot be ruled out. In the previous study (Sayer et al. 1992), all of the Ca\(^{2+}\) current inhibition evoked by trans-ACPD appeared to be accounted for by modulation of L-type channels. Two factors that might have allowed additional channel modulation to be revealed in this study were the higher temperature and the reduced Ca\(^{2+}\) buffering. Using different conditions again (21–24°C, Ba\(^{2+}\) substituted for Ca\(^{2+}\)), Choi and Lovinger (1996) suggested that the fast inhibitory pathway also regulated more than one type of Ca\(^{2+}\) channel, with evidence that N-type and possibly P-type Ca\(^{2+}\) channels were modulated.

It remains to be explained why, if fast and slow mechanisms for mGluR-mediated Ca\(^{2+}\) channel inhibition exist in neocortical neurons, both are not observed under the same experimental conditions. Even when currents were recorded every 2 s, there was no evidence for a fast component to the Ca\(^{2+}\) current inhibition. This argues against the possibility that a fast pathway was activated, but then desensitized within the first 20-s interval of drug application, between the evoked currents of the standard experimental protocol. The important variable may be Ca\(^{2+}\); when it is used as the charge carrier, the only slow inhibition is observed (Sayer et al. 1992 and this study), but when Ba\(^{2+}\) is substituted for Ca\(^{2+}\), only the fast inhibition is apparent (Choi and Lovinger 1996). The effect of Ca\(^{2+}\) may be intracellular, as a similar slowing of Ca\(^{2+}\) channel inhibition by 1S,3R-ACPD was found in cultured hippocampal neurons when intracellular Ca\(^{2+}\) concentration was buffered at 100 nM instead of \(<1\) nM (Sahara and Westbrook 1993). However, strong intracellular Ca\(^{2+}\) buffering does not necessarily produce Ca\(^{2+}\) channel modulation identical to that seen when Ba\(^{2+}\) is used as the charge carrier (Jones et al. 1992; Sayer et al. 1992). Of possible relevance to this issue is the recent finding that group I mGluRs are activated by extracellular Ca\(^{2+}\) and that other divalent ions are less effective (Kubo et al. 1998, Kubokawa et al. 1996). However, the implications of mGluR sensitivity to Ca\(^{2+}\) for the mechanisms modulating Ca\(^{2+}\) channels are not yet clear.

In neocortical neurons it seems that two separate (fast and slow) mechanisms for Ca\(^{2+}\) channel inhibition exist rather than one that is accelerated or slowed under different conditions. They differ in the subtypes of receptors involved: both group I and group II mGluRs for the fast pathway but only group I mGluRs for the slow pathway. Although both pathways target multiple types of Ca\(^{2+}\) channel, the mechanisms of inhibition are different, involving a shift in the voltage dependence of gating for the fast pathway but not for the slow one, and it appears that each pathway is inhibited or occluded under conditions that favor the other.

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