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Metabotropic Glutamate Receptor Modulation of Voltage-Gated Ca^{2+} Channels Involves Multiple Receptor Subtypes in Cortical Neurons

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Metabotropic glutamate receptor (mGluR) modulation of voltage-gated Ca^{2+} channels was examined in isolated deep layer frontoparietal cortical neurons under conditions designed to isolate calcium-independent modulatory pathways. *Trans*-1-aminocyclopentane-1,3-dicarboxylate (*t*-ACPD), a nonspecific mGluR agonist, produced rapid and reversible inhibition of Ca^{2+} channels. This effect was mimicked by agonists for group I and group II, but not group III, mGluRs. Effects of group I and II agonists often were observed in the same neurons, but separate subgroups of neurons were unresponsive to the group I agonist quisqualate or the group II agonist 2-(2,3-dicarboxycyclopropyl) glycine (DCG-IV). Inhibition by quisqualate and DCG-IV was nonocclusive in neurons responding to both agonists. These agonists thus appear to act on different mGluRs. The mGluR antagonist α-methyl-4-carboxylphenylglycine attenuated inhibition by *t*-ACPD, quisqualate, and DCG-IV. Inhibition by quisqualate and DCG-IV was voltage-dependent. Although the effects of both agonists were greatly reduced by *N*-ethylmaleimide (NEM), inhibition by DCG-IV was more sensitive to NEM than inhibition by quisqualate. *t*-ACPD-induced inhibition was reduced by ω-conotoxin GVIA (ω-CgTx) and ω-agatoxin IVA (ω-AgTx) but was affected little by nifedipine. Inhibition by DCG-IV and quisqualate also was reduced by ω-CgTx. We conclude that multiple mGluR subtypes inhibit Ca^{2+} channels in cortical neurons and that N- and possibly P-type channels are inhibited. Modulation is via a rapid-onset, voltage-dependent mechanism that likely involves a pertussis toxin (PTX)-sensitive G-protein. Type I mGluRs may work via additional PTX-insensitive pathways.

Key words: calcium channels; metabotropic glutamate receptors; G-proteins; cortex; ω-conotoxin; ω-agatoxin; nifedipine; quisqualate; DCG-IV; ACPD; N-ethylmaleimide

Glutamate, the major excitatory neurotransmitter in the mammalian central nervous system, produces its actions via both ionotropic and metabotropic receptors (Nakanishi, 1992). Molecular cloning studies have revealed the existence of at least eight different metabotropic glutamate receptor (mGluR) subtypes labeled mGluR1–8 (Houamed et al., 1991; Masu et al., 1991; Abe et al., 1992; Nakanishi, 1992; Tanabe et al., 1992; Nakajima et al., 1993; Okamoto et al., 1994; Duvoisin et al., 1995). The mGluRs can be subdivided into three groups based on primary amino acid sequence, signal transduction, and pharmacology (Houamed et al., 1991; Masu et al., 1991; Manzoni et al., 1991; Prezeau et al., 1992; Nakanishi, 1992; Thomsen et al., 1992; Abe et al., 1992; Tanabe et al., 1992; Nakajima et al., 1993; Tanabe et al., 1993; Hayashi et al., 1993; Okamoto et al., 1994; Gereau and Conn, 1995a).

Activation of mGluRs alters the function of a variety of subtypes of high-voltage-activated Ca^{2+} channels in neurons from the brain and nodose ganglion (Lester and Jahr, 1990; Sahara and Westbrook, 1993; Swartz and Bean, 1992; Trombley and Westbrook, 1992; Sayer et al., 1992; Hay and Kunze, 1994; Stefani et al., 1994; Chavis et al., 1994). mGluR inhibition of N-type, L-type, and other Ca^{2+} channels has been observed in hippocampal and cortical neurons (Sayer et al., 1992; Sahara and Westbrook, 1993; Swartz and Bean, 1992; Swartz et al., 1993). Quisqualate, a group I mGluR agonist, inhibits Ca^{2+} channels in hippocampal, cortical, and nodose ganglion neurons (Lester and Jahr, 1990; Sayer et al., 1992; Swartz and Bean, 1992; Hay and Kunze, 1994) and facilitates L-type channel function in cerebellar granule cells (Chavis et al., 1995). Ca^{2+} channels are inhibited by L-AP4, a group III mGluR agonist, in cultured olfactory bulb neurons and in a subset of cultured hippocampal neurons (Trombley and Westbrook, 1992; Sahara and Westbrook, 1993). (2*S*,1'*S*,2'*S*)-2-(carboxycyclopropyl) glycine (L-CCG-I), an agonist with some selectivity for group II mGluRs, inhibits the function of L-type Ca^{2+} channels (Chavis et al., 1994). However, the actions of agonists selective for each mGluR group on particular Ca^{2+} channels have not been examined in a single population of neurons. In addition, few studies have demonstrated antagonist blockade of mGluR agonist effects on Ca^{2+} channels (Sahara and Westbrook, 1993).

Neurotransmitters can modulate Ca^{2+} channel function through a variety of mechanisms (reviewed by Anwyl, 1991; Hille, 1992). One well characterized mechanism involves a fast, calcium-independent, G-protein-mediated pathway that appears to be membrane-delimited (Anwyl, 1991; Hille, 1992). This mechanism has been subcategorized using the criteria of PTX–NEM sensitivity and voltage dependence (Shapiro et al., 1994). mGluR modulation of N- and non-N-type Ca^{2+} channels in cortical, hippocampal, and nodose ganglion neurons involves a fast, voltage- and calcium-independent pathway (Swartz and Bean, 1992; Swartz et al., 1993; Sahara and Westbrook, 1993; Hay and Kunze, 1994). A quisqualate-sensitive mGluR appears

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to be involved in this action (Swartz and Bean, 1992; Hay and Kunze, 1994). PTX-sensitive G-proteins are involved in modulation by mGluRs in some neurons (Sahara and Westbrook, 1993; Hay and Kunze, 1994; Chavis et al., 1994). mGluRs also act through other mechanisms, including slowly developing, possibly Ca^{2+}-dependent mechanisms in hippocampal and cortical neurons (Sayer et al., 1992; Sahara and Westbrook, 1993), and a slowly developing, possibly calcium-independent mechanism in cerebellar granule cells (Chavis et al., 1994).

We wished to characterize further mGluR modulation of Ca^{2+} channels involving calcium-independent pathways in deep-layer neocortical neurons by determining the following: (1) whether different mGluR subtypes inhibit Ca^{2+} channels in the same cortical neurons; (2) whether the modulation of Ca^{2+} channels by different mGluR subtypes involves similar mechanisms; and (3) what subtypes of Ca^{2+} channels are modulated.

MATERIALS AND METHODS

Cortical neuron preparation. Brain slices were prepared from 9- to 20-d-old Sprague–Dawley rats using previously described techniques (Lovinger, 1991; Lovinger et al., 1993). Rats were killed by decapitation, and the brains were cooled in ice-cold artificial CSF (aCSF) containing 124 mM NaCl, 4.5 mM KCl, 2.0 mM $CaCl_2$, 1.5 mM $MgCl_2$, 26 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 10 mM D-glucose adjusted to pH 7.4 by bubbling with 95% O_2/5% CO_2. Coronal sections (400 μm thick) were cut in ice-cold medium using a manual vibroslice (World Precision Instruments, New Haven, CT) vibrating slicer. Slices were then transferred onto a nylon net submerged in aCSF at room temperature (21–24°C), and the aCSF was constantly bubbled with 95% O_2/5% CO_2. After 1 hr, a chosen slice was hemisected. Hemislices containing the cortex and striatum just anterior to the globus pallidus were incubated at 37°C for 20–25 min in aCSF containing 0.05 mg/ml pronase (Calbiochem, La Jolla, CA) bubbled with 95% O_2/5% CO_2. Slices were transferred to a solution containing (in mM): 130 *N*-methylglucamine, 20 NaCl, 1 $MgCl_2$, 10 HEPES, 10 D-glucose, adjusted to pH 7.4 with HCl, osmolarity adjusted to 328 mmol/kg with sucrose. The deep layer of dorsal cortex overlying the striatum was removed and transferred to another culture dish containing the above solution and triturated through a p200 Eppendorf micropipette tip and then through flame-polished Pasteur pipettes of decreasing tip apertures. The cell suspension in the culture dish was placed onto the stage of an inverted microscope, and cells were allowed to settle for 10–15 min before normal external solution containing 150 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 10 mM HEPES, and 10 mM D-glucose (pH = 7.4 adjusted with NaOH, osmolarity adjusted to 340 mmol/kg with sucrose) was reintroduced to the culture dish.

Whole-cell recording. Whole-cell voltage clamp recordings were obtained using pipettes made from borosilicate glass capillaries pulled on a Flaming–Brown micropipette puller. Pipette resistances ranged from 0.5 to 2 MΩ when filled with internal solution. Two internal solutions were used. The first consisted of 117 mM tetraethylammonium (TEA) chloride, 4.5 mM $MgCl_2$, 9 mM HEPES, 9 mM EGTA, 14 mM creatine phosphate (Tris salt), 4 mM ATP (magnesium salt), and 0.3 mM GTP (Tris salt), pH adjusted to 7.4 with TEA-OH, osmolarity adjusted to 315 mmol/kg with sucrose. The second contained 125 mM *N*-methyl-D-glucamine, 20 mM TEA-OH, 10 mM HEPES, 11 mM EGTA, 1 mM $CaCl_2$, 14 mM creatine phosphate (Tris salt), 4 mM ATP (magnesium salt), and 0.3 mM GTP (Tris salt), pH adjusted to 7.4 with methanesulfonic acid, osmolarity adjusted to 315 mmol/kg with sucrose. Creatine phosphate, ATP, and GTP were stored as aliquots at −20°C and added just before experiments, and solution was then kept ice-cold. There was no difference between cells filled with the two internal solutions in terms of I_{Ba} amplitude or modulation by mGluR agonists. The external recording solution contained 25 mM $BaCl_2$, 145 mM TEACl, 10 mM HEPES, and 1 μM tetrodotoxin (TTX), pH adjusted to 7.4 with TEA-OH, osmolarity adjusted to 335 mmol/kg with sucrose.

Drug solutions were applied by gravity-driven perfusion from a linear array of 12 microcapillary tubes (inner diameter > 150 μm). Solution flow was controlled with a three-way stopcock. To assess the kinetics of DCG-IV- and quisqualate-induced inhibition, solution exchange was performed by simultaneously opening one valve and closing the adjacent valve. This allowed solution exchange within ~200 msec as judged by the time course of Ca^{2+} channel inhibition by cadmium. Seals were formed and the whole-cell configuration was obtained in normal external solution before perfusing the cell with the external solution containing TEA and Ba^{2+}.

The electrode series resistance was compensated 70–80%, and the leak current was determined by 10 mV hyperpolarizing steps from the holding potential. All data except the current traces shown in the figures are derived from leak-subtracted current values. Capacitance artifacts were not subtracted from the current traces shown in the figures. For display purposes, the full amplitude of capacitance artifacts was not shown in Figures 1*A*, 3*A*, and 5*D*. Whole-cell currents recorded with Axopatch 200 or 1D amplifiers were filtered at 5 kHz and digitized at up to 50 kHz and stored on a microcomputer using pClamp software. The amplitude of Ba^{2+} currents was measured isochronally at the time point at which peak current was seen in the absence of drug. The data for the concentration–response curves were fit with KaleidaGraph (Synergy Software, Reading, PA) using the logistic equation to obtain IC_{50} values. Percent inhibition by mGluR agonists before and after depolarizing prepulses was calculated as the current amplitude in the presence of agonist divided by the amplitude of the response in the absence of drug before the prepulse. The percentage of total current inhibited by agonist before and after application of channel blockers was calculated by determining the percentage of the original total baseline current amplitude that was inhibited in the presence of agonist before and after channel blocker treatment. All averaged values are given as mean ± SEM. The statistical criterion for significance was $p < 0.05$.

Drugs. *Trans*-1-aminocyclopentane-1,3-dicarboxylate (*t*-ACPD) and quisqualate were purchased from Research Biochemicals (Natick, MA). ω-Conotoxin GVIA (ω-CgTx), ω-agatoxin IVA (ω-AgTx), creatine phosphate, ATP, GTP, guanosine 5′-*o*-(3-thiotriphosphate) (GTPγS; tetralithium salt), ibotenate, D,L-AP4, L-SOP, NEM, TTX, and nifedipine were from Sigma (St. Louis, MO). (*R,S*)-α-Methyl-4-carboxylphenylglycine [(*R,S*)-MCPG], (+)-α-methyl-4-carboxylphenyl glycine [(+)-MCPG], and 4C3HPG were purchased from Tocris Neuramin (Bristol, UK). (*R,S*)-DHPG was from Tocris Cookson (Bristol, UK). DCG-IV was a gift from Drs. H. Shinozaki and Y. Ohfune.

RESULTS

Pharmacological properties of mGluRs that modulate Ca^{2+} channels

Barium current was recorded from 183 acutely isolated cortical neurons, which were identified as pyramidal neurons under phase-contrast microscopy. To investigate the involvement of mGluRs in the modulation of Ca^{2+} channels, *t*-ACPD, quisqualate, and DCG-IV were applied to acutely isolated cortical neurons. Inhibition of I_{Ba} developed within 2 sec after the onset of application of each agonist. Inhibition was largely reversible 8–16 sec after removal of agonist. Figure 1*A* shows current traces recorded immediately before, during, and after application of 200 μM *t*-ACPD, 200 nM quisqualate, and 5 μM DCG-IV to separate neurons. The rate of current onset was slowed considerably during agonist application. Maximal inhibition of I_{Ba} by 200 μM *t*-ACPD, 1 μM quisqualate, and 5 μM DCG-IV was 22.4 ± 2.0% (n = 15), 19.1 ± 1.9% (n = 18), and 14.9 ± 1.5% (n = 18), respectively (Fig. 1*B*). The concentration–response curves for *t*-ACPD-, quisqualate-, and DCG-IV-induced effects on I_{Ba} are shown in Figure 1*C*. The estimated IC_{50} values for *t*-ACPD, quisqualate, and DCG-IV were 11.5 μM, 0.12 μM, and 0.51 μM, respectively.

The effects of other mGluR agonists were subsequently examined. Ibotenate is a relatively nonselective agonist (see Pin and Duvoisin, 1995). DHPG is a selective agonist for group I mGluRs (Gereau and Conn, 1995a). 4C3HPG is known to be an agonist for mGluR2 and an antagonist for mGluR1 (Thomsen et al., 1994). All three agonists were effective at concentrations that have been shown to activate mGluRs. 4C3HPG (200 μM), DHPG (50 μM), and ibotenate (30 μM) inhibited I_{Ba} by 16.7 ± 3.3% (n = 6), 17.7 ± 4.4% (n = 6), and 21.5 ± 2.7% (n = 7), respectively (Fig. 1*B*). These three agonists also produced slowing of current acti-

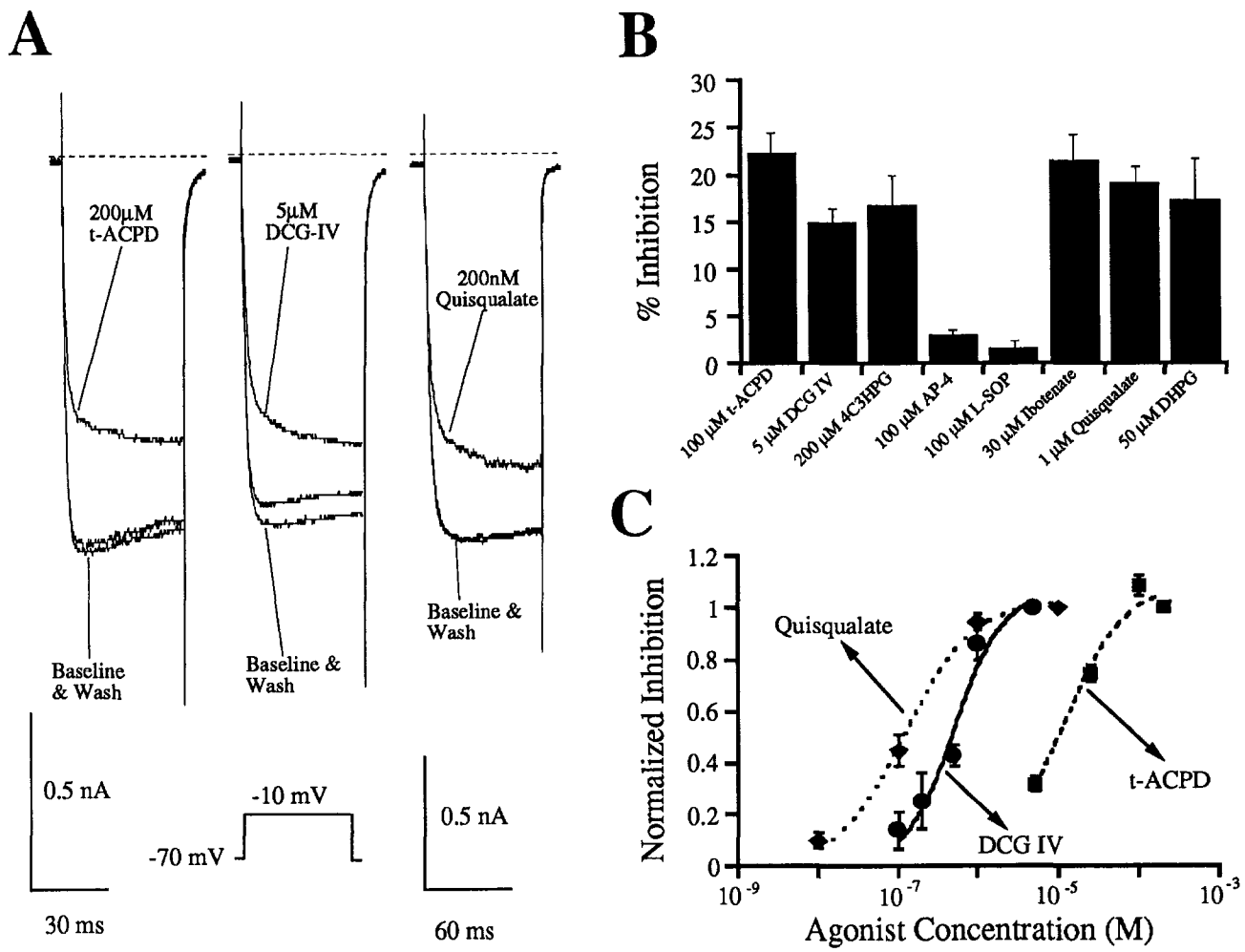

Figure 1. mGluR agonists inhibit I_{Ba} in a dose-dependent manner in acutely isolated cortical neurons. *A*, Currents activated by indicated voltage step before, during, and 8 sec after application of 200 μM *t*-ACPD, 200 nM quisqualate, and 5 μM DCG-IV. Data are from separate neurons. Left calibration bar is for *t*-ACPD and DCG-IV, and right calibration bar is for quisqualate. *B*, Percent inhibition of I_{Ba} by different mGluR agonists. The concentrations tested were chosen to be at the high end of concentration–response curves from studies of mGluR clones. *C*, Concentration–response curves for *t*-ACPD, DCG-IV, and quisqualate inhibition of I_{Ba}. The data set includes only neurons tested with at least four agonist concentrations, and each data point is the average of data from five to seven neurons. Inhibition by each agonist is normalized to inhibition by the maximally effective agonist concentration (100 μM *t*-ACPD, 1 μM quisqualate, and 5 μM DCG-IV). I_{Ba} was evoked by 50 msec voltage steps from −70 to −10 mV in *A*, *B*, and *C*. Data points and error bars represent ±SEM.

vation. We also examined the actions of the selective group III mGluR agonists [D,L-AP4 (100 μM) and L-SOP (100 μM)]. These agonists produced little inhibition of I_{Ba} (percent inhibition by D,L-AP4 = 3.1 ± 0.5, n = 8; L-SOP = 1.7 ± 0.7, n = 6) in neurons in which *t*-ACPD produced >15% inhibition (Fig. 1*B*). There was no change in holding current during the application of any mGluR agonist at concentrations that produced maximal responses in these experiments in which NMG or TEA and Ba^{2+} were the major cations in the external and internal recording solutions.

The observation that both quisqualate and DCG-IV modulated Ca^{2+} channel function was interesting given that these agonists have selective effects on different mGluR subtypes. It thus appeared possible that more than one mGluR subtype was involved in Ca^{2+} channel modulation. The first piece of information that was consistent with this hypothesis was the observation that DCG-IV was effective in fewer neurons than either *t*-ACPD or quisqualate. Inhibition of current amplitude of >5% was observed in 95% of neurons in which 100 μM *t*-ACPD was tested (n = 63), 93% of neurons in which 1 μM quisqualate was tested (n = 42), but only 75% of neurons in which 5 μM DCG-IV was tested (n = 48). In 11 of these neurons, DCG-IV failed to produce significant inhibition, whereas *t*-ACPD or quisqualate produced >15% inhibition (Fig. 2*A*). In addition, three neurons were sensitive to *t*-ACPD but did not respond to quisqualate (Fig. 2*B*). Thus, a proportion of the neurons tested did not respond to particular mGluR agonists even though effects of other agonists could be demonstrated in the same neuron.

We next correlated the inhibition produced by a maximally effective concentration of a given mGluR agonist with that produced by other agonists in the same neurons. The correlation between the percent inhibition produced by quisqualate and DCG-IV was quite low (r = 0.11, n = 10), as was the correlation between inhibition by *t*-ACPD and quisqualate (r = 0.01, n = 12) or *t*-ACPD and DCG-IV (r = 0.28, n = 10) (*t* test, $p > 0.1$).

We then examined the effect of combined application of maximally effective concentrations of the selective agonists DCG-IV

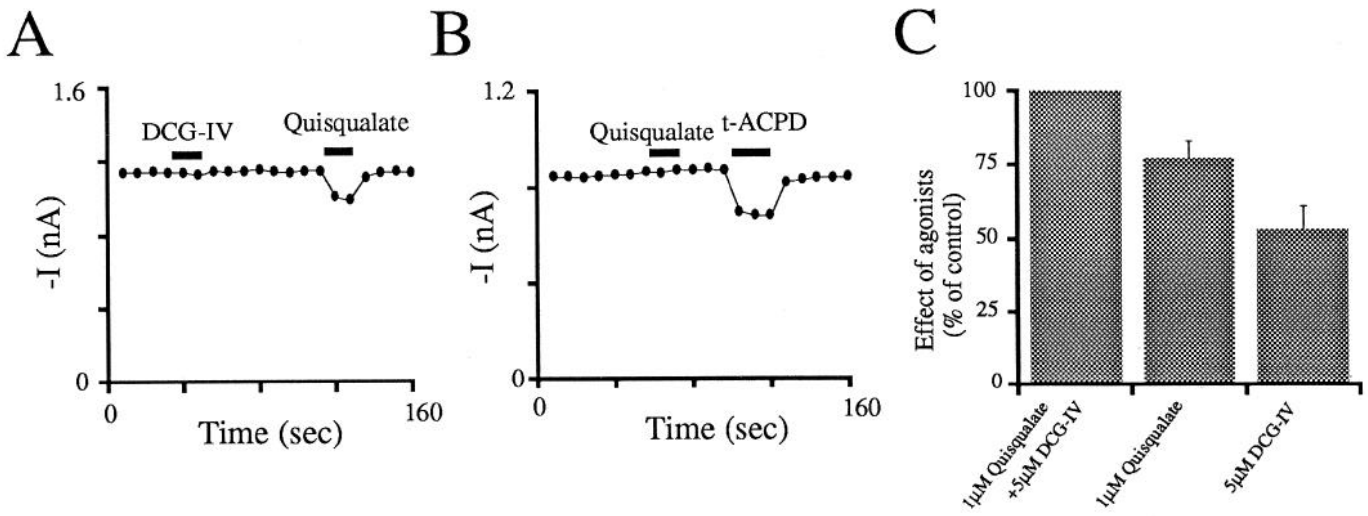

Figure 2. The effects of DCG-IV and quisqualate are not correlated and are nonocclusive. *A* and *B*, Plots of I_{Ba} amplitude over time for two neurons in which one mGluR agonist produced significant inhibition and another agonist did not. *C*, Bar graph showing the inhibition by quisqualate, DCG-IV, or the combination of quisqualate + DCG-IV normalized to the inhibition by combined agonist treatment ($n = 5$). All data are from the same set of neurons. The data set includes all cells showing >5% inhibition by both DCG-IV and quisqualate. Agonist concentrations were 100 μM *t*-ACPD, 1 μM quisqualate, and 5 μM DCG-IV in *A, B,* and *C*. I_{Ba} was evoked by 50 msec voltage steps from −70 to −10 mV. Error bars represent ±SEM.

and quisqualate. We hypothesized that if these agonists act on the same receptor, then inhibition by the combination of both agonists should be similar in magnitude to that produced by either agonist alone. If, however, the agonists act on separate receptors, one would expect that inhibition in the combined presence of both agonists would be greater than that observed with either agonist alone. Inhibition by the combination of 5 μM DCG-IV + 1 μM quisqualate was greater than that by either DCG-IV or quisqualate alone in every neuron in which both agonists produced >5% inhibition when applied separately ($n = 5$) (Fig. 2*C*). Furthermore, we observed that inhibition by 100 μM *t*-ACPD was well correlated with inhibition during the combined application of DCG-IV and quisqualate ($r = 0.96$, $n = 6$) (*t* test, $p < 0.001$).

Antagonism by MCPG

MCPG has been reported to be a competitive antagonist of mGluRs (Birse et al., 1993; Hayashi et al., 1994). The ability of MCPG to block mGluR agonist modulation of I_{Ba} was examined, as shown in Figure 3. (*R,S*)-MCPG (1 mM) or (+)-MCPG (500 μM) partially blocked I_{Ba} inhibition by *t*-ACPD, quisqualate, and DCG-IV at agonist concentrations that produced submaximal inhibition. Percent inhibition by 25 μM *t*-ACPD, 200 nM quisqualate, or 600 nM DCG-IV averaged 18.6 ± 2.9, 16.7 ± 5.1, and 16.7 ± 4.1%, respectively, in the absence of MCPG and 3.7 ± 0.2, 6.1 ± 1.9, and 2.9 ± 0.6, respectively, in the presence of MCPG. Taken together, these results suggest that *t*-ACPD, DCG-IV, and

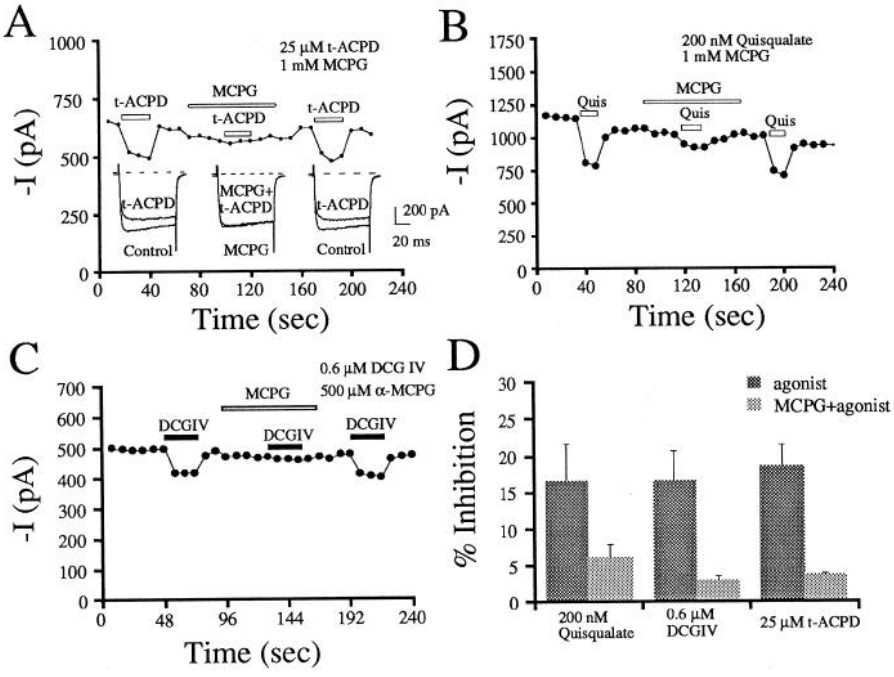

Figure 3. MCPG antagonizes the inhibition of I_{Ba} by mGluR agonists. *A–C*, Time course of experiments in which the effect of 25 μM *t*-ACPD, 200 nM quisqualate, or 600 nM DCG-IV on I_{Ba} was examined before, during, and after application of 1 mM (*R, S*)-MCPG or 500 μM (+)-MCPG. *Inset currents* were recorded at different times during the experiment plotted in *A*. *D*, Bar graph showing the percent inhibition by mGluR agonists in the absence or presence of MCPG ($n = 3$ for each agonist). One millimolar (*R, S*)-MCPG was used in the *t*-ACPD and quisqualate experiments, and 500 μM (+)-MCPG was used in the DCG-IV experiment. I_{Ba} was evoked by 50 or 80 msec voltage steps from −70 to −10 mV. Voltage pulses were given every 8 sec. Error bars represent ±SEM.

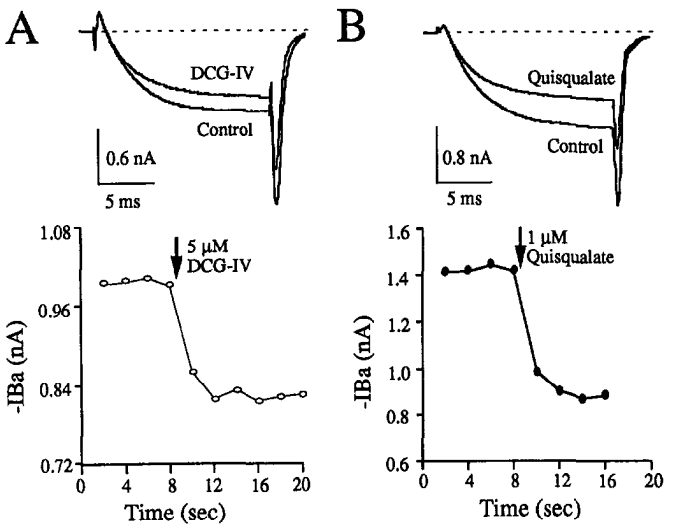

Figure 4. Time course of inhibition of I_{Ba} by DCG-IV and quisqualate. *A*, *B*, Current records are I_{Ba} evoked by 16 msec voltage steps from −70 to −10 mV before agonist application, and 12 sec (*A*) or 8 sec (*B*) after beginning application of the indicated agonist. Graphs illustrate I_{Ba} amplitude as a function of time during experiments determining the time course of Ca^{2+} channel inhibition by 5 μM DCG-IV and 1 μM quisqualate in isolated cortical neurons. Voltage pulses were given every 2 sec. DCG-IV and quisqualate were applied at the time indicated by the *arrow*.

quisqualate inhibit I_{Ba} through mGluR activation in acutely isolated cortical neurons.

Mechanism of inhibition of Ca^{2+} channels by different mGluR subtypes

Hille and co-workers have identified different pathways linking receptor activation and Ca^{2+} channel modulation. These pathways can be distinguished by the time course of onset of inhibition with "fast" modulation developing within a few seconds, and "slow" modulation developing over tens of seconds (Hille, 1992; Shapiro et al., 1994). To determine how fast the inhibition by different mGluR agonists was achieved, we compared the time course of inhibition by DCG-IV and quisqualate. I_{Ba} was evoked by 16 msec voltage steps from −70 to −10 mV every 2 sec (this protocol allowed for maximal current activation without measurable inactivation). Using this pulse protocol, no sign of run-down of I_{Ba} amplitude was seen. As shown in Figure 4, substantial inhibition by 5 μM DCG-IV and 1 μM quisqualate was achieved within 2 sec, and saturation of inhibition was observed in <4 sec (n = 5). This time course is comparable with that previously reported for "fast" inhibition and is considerably faster than "slow" modulation (Hille, 1992).

As mentioned earlier, mGluR agonists including *t*-ACPD, quisqualate, and DCG-IV slowed I_{Ba} activation kinetics. This slowing has been proposed to represent a voltage-dependent relief of inhibition during the depolarizing test pulse (Bean, 1989). To determine whether inhibition by mGluR agonists was voltage-dependent, we measured the current–voltage relationship of I_{Ba} before, during, and after application of 100 μM *t*-ACPD, 5 μM DCG-IV, and 1 μM quisqualate (Fig. 5*A–C*). Inhibition of I_{Ba} appeared to be voltage-dependent as previously reported for other G-protein-coupled neurotransmitter receptors (Bean, 1989; Elmslie et al., 1990). It has been reported that depolarizing prepulses transiently facilitate Ca^{2+} channel current by relieving G-protein-mediated inhibition induced by receptor activation (Jones, 1991; Ikeda, 1991, 1992; Boland and Bean, 1993). The relief of inhibition by depolarizing prepulses has been reported for both N- and non-N-type, presumably P-type, channels (Mintz and Bean, 1993; Swartz, 1993). To determine whether depolarizing prepulses relieved inhibition by mGluR agonists in isolated cortical neurons, we measured I_{Ba} amplitude during short, moderately depolarizing voltage steps separated by a large transient depolarization. We observed that depolarizing prepulses facilitated I_{Ba} to a greater extent in the presence than in the absence of mGluR agonists (Fig. 5*D*), suggesting that the inhibition by mGluR agonists was relieved by the prepulse. To compare the depolarization relief of Ca^{2+} channel inhibition by DCG-IV and quisqualate, we measured the percent inhibition by each agonist before and after a depolarizing prepulse. The percent inhibition by 5 μM DCG-IV (n = 7) and 1 μM quisqualate (n = 14) averaged 20.8 ± 3.9 and 19.7 ± 1.5 before and 8.9 ± 2.3 and 8.5 ± 1.4 after a depolarizing prepulse (Fig. 5*E,F*). Thus, no significant difference was found in the depolarization relief of the inhibition by DCG-IV and quisqualate.

Previous studies have suggested that calcium channel modulation by mGluRs is G-protein-mediated (Lester and Jahr, 1990; Swartz and Bean, 1992; Sayer et al., 1992; Sahara and Westbrook, 1993; Hay and Kunze, 1994; Stefani et al., 1994; Chavis et al., 1994). Thus, we predicted that introduction of the nonhydrolyzable GTP analog GTPγS into the cell would mimic the effect of agonists and/or render agonist-induced inhibition irreversible. Dialysis of the cell interior with 100–300 μM GTPγS produced gradual inhibition that resembled that produced by mGluR agonists and eliminated fast, reversible inhibition by agonists, including DCG-IV, quisqualate, and *t*-ACPD (n = 10) (data not shown). However, in some GTPγS-filled neurons (n = 8), mGluR agonists produced a slowly developing, irreversible inhibition (data not shown).

Because of the difficulty in using PTX in experiments on acutely isolated neurons, we used NEM, a sulfhydryl-alkylating agent (Jakobs et al., 1982), which has been shown to uncouple PTX-sensitive G-proteins from receptors (Nakajima et al., 1990; Shapiro et al., 1994). A recent study suggests that NEM blocks Ca^{2+} channel modulation by PTX-sensitive G-proteins in neurons (Shapiro et al., 1994). To determine the NEM sensitivity of Ca^{2+} channel inhibition by DCG-IV and quisqualate in isolated cortical neurons, we briefly applied mGluR agonists before and after a 120 sec application of 50 μM NEM (Fig. 6). To determine how much of the inhibition by mGluR agonists was sensitive to NEM, we measured the percent inhibition by DCG-IV and quisqualate before and after application of NEM. The percent inhibition by 1 μM quisqualate (n = 14) and 5 μM DCG-IV (n = 8) averaged 20.2 ± 1.6 and 20.7 ± 2.7, respectively, before and 8.6 ± 0.7 and 4.1 ± 1.0, respectively, after application of 50 μM NEM. Thus, inhibition by DCG-IV appeared to be affected by NEM to a greater extent than inhibition by quisqualate. These data suggest that a PTX-sensitive G-protein mediates the majority of DCG-IV and quisqualate-induced inhibition. However, part of the inhibition by quisqualate might be mediated by a PTX-insensitive G-protein(s).

Ca^{2+} channel subtypes modulated by mGluRs

We examined the effects of Ca^{2+} channel blockers including nifedipine (an L-type channel blocker) (Fox et al., 1987a,b), ω-CgTx (an N-type channel blocker) (Aosaki and Kasai, 1989; Plummer et al., 1989), and ω-AgTx (a blocker of P- and other, channels) (Mintz et al., 1992). Application of each of these agonists inhibited some proportion of I_{Ba} activated by voltage steps to −10 mV from a holding potential of −70 mV. ω-CgTx (1 μM)

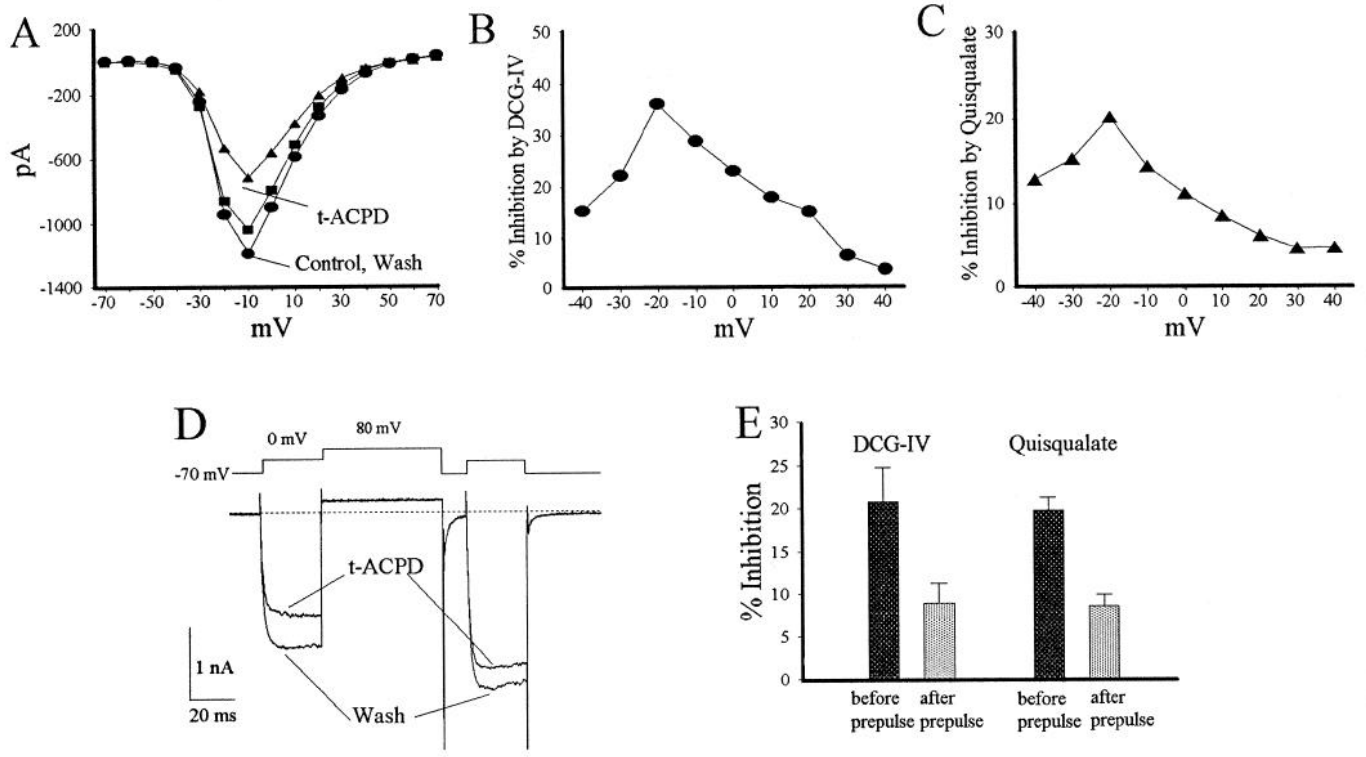

Figure 5. mGluR agonists inhibit I_{Ba} in a voltage-dependent manner. *A*, Current–voltage relationships determined before, during, and after application of 100 μM *t*-ACPD. Holding potential was −70 mV. Test potentials were given every 5 sec. *B* and *C*, Plot of I_{Ba} inhibition versus test pulse potential derived from current–voltage relationships taken before, during, and after 5 μM DCG-IV (*B*) or 1 μM quisqualate (*C*) treatment. Holding potential was −70 mV. Test potentials were given every 5 sec. *D*, Relief of *t*-ACPD-induced inhibition by depolarizing prepulses. Current activated by the indicated voltage steps before and during application of 100 μM *t*-ACPD. Note the increase in the ratio of the response to the second test pulse relative to the first test pulse in the presence of *t*-ACPD. *E*, Graph showing the percent inhibition by 5 μM DCG-IV and 1 μM quisqualate before and after a depolarizing prepulse. A single denominator (control response before the prepulse) was chosen to calculate percent inhibition before and after the prepulse. Prepulses increase the ratio of the response to the second test pulse relative to the first test pulse by 5.1 ± 2.3 and 22.3 ± 4.1% in the absence or presence of 5 μM DCG-IV ($n = 7$), respectively, and by 7.9 ± 1.9 and 23.3 ± 3.6% in the absence or the presence of 1 μM quisqualate ($n = 14$), respectively. Current was evoked by test pulses (−70 to −5 mV, 25 msec duration) given before and after a 50 msec prepulse to +80 mV, as in *D*.

blocked 31.5 ± 7.0% ($n = 5$) of I_{Ba}, nifedipine (5 μM) blocked 22.8 ± 1.1% ($n = 5$) of I_{Ba}, and ω-AgTx with 1 mg/ml cytochrome *C* (100 nM) blocked 33.5 ± 4.3% ($n = 5$) of I_{Ba} (Fig. 7*D*). We estimated that a substantial proportion of current (10–20% of total current amplitude) was not affected by any of these blockers. However, the proportion of the Ca^{2+} channel types varied among cells. In some cells, apparently unequal proportions of the different Ca^{2+} channels (e.g., <10% of L-type, 50% of N- or P-type) were present.

To determine the Ca^{2+} channel subtypes that were inhibited by *t*-ACPD, I_{Ba} was elicited before and during block of N-, P-, and L-type currents with 1 μM ω-CgTx, 100 nM ω-AgTx, and 5 μM nifedipine (Fig. 7). The percent of total current inhibited by *t*-ACPD was 18.3 ± 1.9 before and 6.4 ± 1.6 after application of 1 μM ω-CgTx ($n = 5$) (paired *t* test, $p < 0.001$). In another group of cells ($n = 5$), the percent of total current inhibited by *t*-ACPD was 28.5 ± 5.7 before and 16.7 ± 5.4 after application of 100 nM ω-AgTx (paired *t* test, $p < 0.001$), whereas *t*-ACPD inhibited I_{Ba} by 30.6 ± 5.9% before and by 24.5 ± 4.1% after application by 5 μM nifedipine in yet another group of cells ($n = 5$) (paired *t* test, $p < 0.05$) (Fig. 7*E*). We also examined the involvement of N-type channels in I_{Ba} modulation by DCG-IV and quisqualate. The percent of total current inhibited by 5 μM DCG-IV ($n = 4$) and 1 μM quisqualate ($n = 5$) was 26.6 ± 5.4 and 15.7 ± 2.6, respectively, before and 11.9 ± 4.2 and 7.36 ± 2.2, respectively, after application of 1 μM ω-CgTx (paired *t* test, $p < 0.01$ for both agonists). Thus, the effect of ω-CgTx on inhibition by DCG-IV and quisqualate appeared to be similar to that of ω-CgTx on inhibition by *t*-ACPD.

DISCUSSION

Previous studies have demonstrated that mGluR activation modulates Ca^{2+} channels in a variety of neurons (Lester and Jahr, 1990; Sayer et al., 1992; Swartz and Bean, 1992; Trombley and Westbrook, 1992; Sahara and Westbrook, 1993; Hay and Kunze, 1994; Stefani et al., 1994; Chavis et al., 1994; Chavis et al., 1995). The majority of studies have shown that *t*-ACPD and quisqualate activate Ca^{2+} channel modulation (Swartz and Bean, 1992; Sayer et al., 1992; Hay and Kunze, 1994; Chavis et al., 1995). In addition, some evidence for modulatory effects on Ca^{2+} channels of the group III mGluR agonist L-AP4 (Trombley and Westbrook, 1992; Sahara and Westbrook, 1993) and L-CCG-I (a moderately selective group II mGluR agonist) has been presented (Chavis et al., 1994). Interestingly, *t*-ACPD was able to inhibit Ca^{2+} channel function in hippocampal neurons from mutant mice lacking mGluR1, suggesting that this receptor cannot account for all of the Ca^{2+} channel-modulatory actions of mGluRs (Aiba et al., 1994). From these studies it has been hypothesized that several

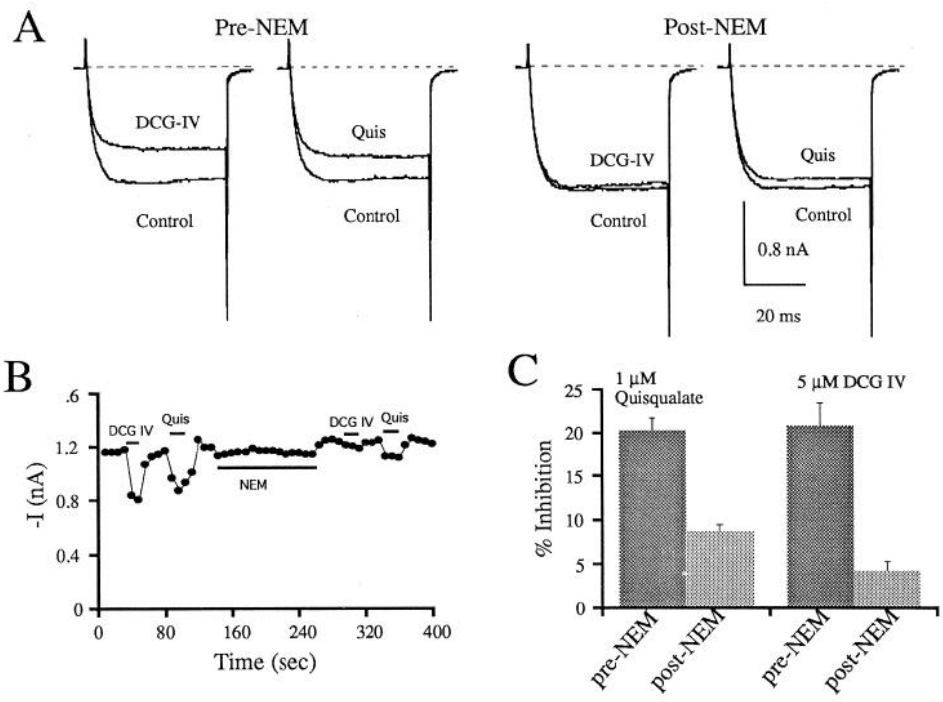

Figure 6. NEM reduces inhibition by DCG-IV more than inhibition by quisqualate. *A*, Current traces recorded during an experiment examining the effect of 5 μM DCG-IV and 1 μM quisqualate before and after application of 50 μM NEM. *B*, I_{Ba} amplitude plotted as a function of time for the experiment from which the records shown in *A* were taken. *C*, Graph illustrating the percent inhibition by DCG-IV and quisqualate before and after application of NEM. Although NEM produced a small change in I_{Ba} amplitude in some neurons, the percent of control I_{Ba} amplitude in the presence of NEM averaged 100.4 ± 3.0 (n = 12). I_{Ba} was evoked by 50 msec voltage steps from −70 to −10 mV. Voltage pulses were given every 8 sec.

mGluR subtypes can participate in Ca^{2+} channel modulation (Pin and Duvoisin, 1995). However, there is very little evidence that different mGluR subtypes participate in modulation in the same population of neurons. In the present study, we have obtained evidence that agonists selective for different mGluRs inhibit calcium current in some neurons, whereas in other neurons only one subtype-selective agonist was effective. In addition, inhibition during the combined application of DCG-IV and quisqualate was greater than that elicited by application of either agonist alone. Finally, inhibition by the nonspecific mGluR agonist *t*-ACPD was well correlated with the combined actions of DCG-IV and quisqualate in individual neurons but was not well correlated with the actions of either agonist alone. These findings suggest that more than one mGluR mediates Ca^{2+} channel inhibition in these neurons.

The mGluR agonists that elicited Ca^{2+} channel modulation in cortical neurons were nonspecific, selective for group I mGluRs (Manzoni et al., 1991; Nakanishi, 1992; Gereau and Conn, 1995a), or selective for group II mGluRs (Hayashi et al., 1993; Thomsen et al., 1994; Gereau and Conn, 1995a). The findings with MCPG suggest that each of these agonists works through mGluRs, and appear to exclude the involvement of mGluR4 because this subtype is not blocked by MCPG (Hayashi et al., 1994). We observed no evidence of inhibition by agonists selective for group III mGluRs. However, L-AP4 modulates Ca^{2+} channel function in olfactory bulb and hippocampal neurons (Trombley and Westbrook, 1992; Sahara and Westbrook, 1993). Thus, there is evidence for participation of all subgroups of mGluRs in Ca^{2+} channel modulation. However, members of subgroups I and II appear to be the best candidates for modulating Ca^{2+} channels in cortical neurons, at least under conditions used in the present study.

Activation of different mGluRs appears to modulate Ca^{2+} channels via a similar fast, reversible, G-protein-mediated, voltage-dependent mechanism in isolated cortical neurons. The only difference in inhibition mediated by group I and group II mGluRs was the sensitivity to NEM, a compound previously demonstrated to mimic the actions of PTX (Hille, 1992; Shapiro et al., 1994). Thus, inhibition by group I mGluRs may involve both PTX-sensitive and -insensitive G-proteins whereas group II mGluRs use mainly PTX-sensitive G-proteins. We have evaluated further the specificity of NEM for antagonizing PTX-sensitive G-protein-mediated modulatory pathways. NEM appears to be a fairly specific blocker of PTX-sensitive G-proteins under conditions similar to those used in the present study (Choi et al., 1995). Previous results suggest that group II mGluRs couple negatively to adenylate cyclase through PTX-sensitive G-proteins. Thus, involvement of PTX-sensitive G-proteins might be expected in the case of DCG-IV-induced Ca^{2+} channel inhibition. It has been reported that quisqualate-activated mGluRs and group I mGluRs can modulate Ca^{2+} channels, PI hydrolysis, and Ca^{2+} mobilization through PTX-sensitive or -insensitive G-proteins (Abe et al., 1992; Aramori and Nakanishi, 1992; Linden et al., 1994; Hay and Kunze, 1994) and, thus, the observed involvement of both PTX-sensitive and -insensitive G-proteins in the actions of quisqualate is also not surprising. Our findings suggest that mGluRs, particularly a group I mGluR, inhibit Ca^{2+} channels through multiple G-proteins in the same neuron, consistent with previous studies in which α2 adrenergic and pancreatic polypeptide receptors inhibit Ca^{2+} channels through multiple G-proteins in superior cervical ganglion neurons (see Shapiro et al., 1994).

It must be noted that there is strong evidence for mGluR inhibition of Ca^{2+} channels in cortical neurons via Ca^{2+}-dependent mechanisms excluded in the present study (Sayer et al., 1992). The mechanism described in this previous study appears to be involved in modulation of L-type currents. Thus, mGluRs likely inhibit different calcium channels using diverse mechanisms in cortical neurons.

The different mGluRs appear to modulate similar Ca^{2+} channel subtypes. Inhibition of N-type channels accounted for the bulk of the effects of *t*-ACPD, DCG-IV, and quisqualate. The effects of *t*-ACPD and quisqualate are similar to those observed in previous studies (Swartz and Bean, 1992; Sahara and Westbrook, 1993; Swartz et al., 1993; Hay and Kunze, 1994; Stefani et al., 1994). However, inhibition of non-N-type channels accounted for a sizable proportion of receptor-mediated modulation by all three agonists. The observation that

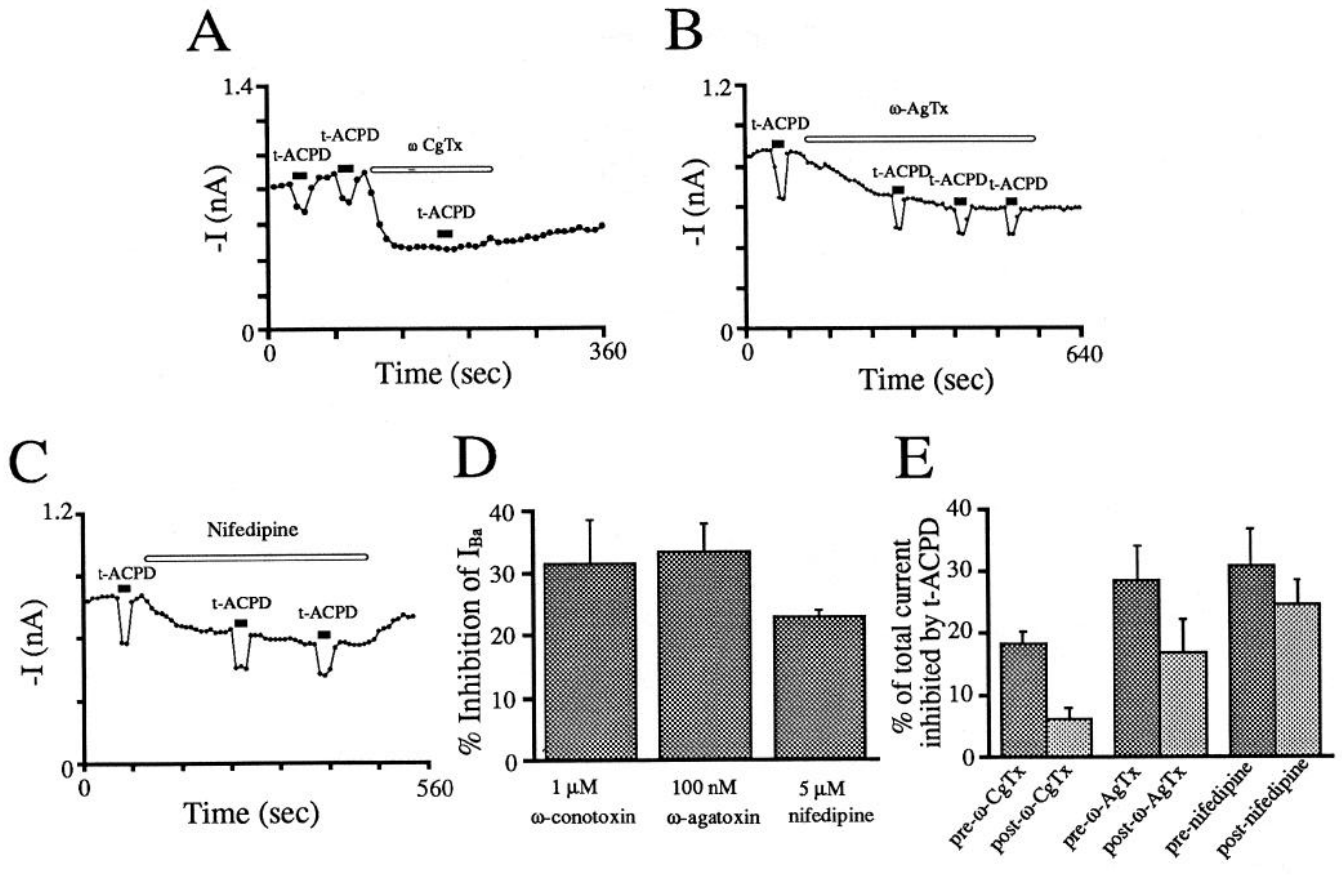

Figure 7. *t*-ACPD inhibits predominantly ω-CgTx-sensitive and ω-AgTx-sensitive Ca^{2+} channels. *A–C*, I_{Ba} amplitude plotted as a function of time during experiments examining the effect of 100 μM *t*-ACPD before and during application of 1 μM ω-CgTx (*A*), 100 nM ω-AgTx (*B*), or 5 μM nifedipine (*C*). *D*, Graph illustrating the percent inhibition of I_{Ba} by each Ca^{2+} channel blocker. *E*, Graph illustrating the percent inhibition by 100 μM *t*-ACPD before and after application of each channel blocker. A single denominator (control response before the application of channel blockers) was chosen to calculate the percent of the total current inhibited in the presence of mGluR agonists before and during the application of channel blockers. I_{Ba} was evoked by 50 msec voltage steps from −70 to −10 mV. Voltage pulses were given every 8 sec.

some of the current inhibited by *t*-ACPD was ω-AgTx-sensitive suggests that one or more of the mGluR subtypes inhibits non-N-type channels affected by this blocker. The concentration of ω-AgTx used was in the range that is fairly selective for P-type channels, suggesting the involvement of this channel type. We were not able to distinguish further the Ca^{2+} channel subtypes modulated by DCG-IV because of depletion of our limited supply of this agonist. $GABA_B$ receptors modulate P-type Ca^{2+} channels in cerebellar Purkinje and spinal cord neurons through a G-protein-mediated mechanism similar to the mechanism described at present (Mintz and Bean, 1993). Thus, it is becoming evident that non-N-type, non-L-type channels, including the P-type, can be modulated by G-protein-coupled receptors via a fast modulatory pathway.

Inhibition of Ca^{2+} channels has been suggested as a mechanism for regulation of transmitter release by presynaptic autoreceptors (Lipscombe et al., 1989; Bley and Tsien, 1990). Also, N- and non-N-type Ca^{2+} channels are responsible for synaptic transmission at corticostriatal and hippocampal synapses (Luebke et al., 1993; Lovinger et al., 1994; Wheeler et al., 1994; Wu and Saggau, 1994), and P-type channels appear to be involved in synaptic transmission at hippocampal synapses (Luebke et al., 1993; Wu and Saggau, 1994; Castillo et al., 1994). N- and P-type channels involved in excitation–secretion coupling may be modulated by mGluRs in presynaptic terminals, leading to synaptic depression. Our results indicate that a group II mGluR inhibits glutamatergic synaptic transmission presynaptically at corticostriatal synapses (Lovinger and McCool, 1995). Thus, it is conceivable that modulation of Ca^{2+} channels by group II mGluR agonists represents the mechanism by which synaptic transmission is presynaptically inhibited. However, low concentrations of quisqualate that do not activate group II mGluRs have been shown to inhibit synaptic transmission in hippocampus and striatum (Baskys and Malenka, 1991; Calabresi et al., 1992). It is thus possible that a group I mGluR subtype also functions as a presynaptic inhibitory receptor and that inhibition of Ca^{2+} channels is a viable mechanism for the action of this receptor on transmission. It should be noted, however, that some neurotransmitter receptors can produce presynaptic inhibition independent of the involvement of Ca^{2+} channels (Scholz and Miller, 1992; Scanziani et al., 1992, 1993). Furthermore, recent evidence indicates that mechanisms downstream from calcium entry contribute to modulation of synaptic transmission by mGluR autoreceptors in corticostriatal cocultures and in hippocampus (Tyler and Lovinger, 1995; Gereau and Conn, 1995b). Clearly, further work is needed to assess the importance of Ca^{2+} channel inhibition in the action of inhibitory presynaptic glutamate receptors.

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