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Inhibition of Calcium Channels in Rat CA3 Pyramidal Neurons by a Metabotropic Glutamate Receptor

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L-Glutamate rapidly and reversibly suppressed Ca channel current in freshly dissociated pyramidal neurons from the CA3 region of the rat hippocampus. L-Glutamate inhibition of Ca channel current could be distinguished from activation of background conductance by appropriate ionic conditions and by distinct pharmacological profiles. Ca channel inhibition by glutamate was mimicked by quisqualate, ibotenate, racemict-ACPD and 1*S*,3*R*-ACPD but not by kainate, AMPA, L-aspartate, NMDA, L-2-amino-4-phosphonobutyric acid, or 1*R*,3*S*-ACPD; 6-cyano-7-nitroquinoxaline-2,3-dione did not inhibit the response. All agonists inhibited a similar fraction of high-voltage-activated Ca channel current, typically ~30%. Concentration–response relations for the agonists were consistent with mediation by a metabotropic glutamate receptor. The stereospecific agonist 1*S*,3*R*-ACPD was especially useful since it did not activate background conductances. The fraction of Ca channel current sensitive to 1*S*,3*R*-ACPD was partially blocked by ω-conotoxin GVIA but was not sensitive to dihydropyridine antagonists or agonists. The suppression of Ca channels by 1*S*,3*R*-ACPD became irreversible when cells were dialyzed with GTP-γ-S. 1*S*,3*R*-ACPD suppressed Ca channel currents in outside-out membrane patches but not in cell-attached patches when applied outside the patch. These results suggest that metabotropic glutamate receptors suppress the activity of N-type Ca channels in CA3 neurons by a mechanism involving G-proteins but not readily diffusible second messengers.

A number of examples are known in which neurotransmitters can inhibit their own release by acting on presynaptic autoreceptors (Koelle, 1961; Illes, 1986; North, 1986; Starke, 1987; Lipscombe et al., 1989; Kalsner and Westfall, 1990). One mechanism mediating such presynaptic inhibition is likely to be neurotransmitter inhibition of Ca channels in the presynaptic terminal. Virtually all neurotransmitter receptors known to be coupled to G_i or G_o-like G-proteins have been shown to inhibit Ca channels in one cell type or another (Carbone and Swandulla, 1991; Anwyl, 1991). In vertebrate neurons, suppression of Ca currents has been studied primarily in cell bodies, but the mechanism has also been demonstrated in presynaptic terminals (Maguire et al., 1989). Other mechanisms might also be involved in presynaptic suppression of transmitter release since many of the same transmitters also activate potassium channels (Illes, 1986; North, 1986).

L-Glutamate is probably the predominant fast excitatory neurotransmitter in the mammalian brain (Mayer and Westbrook, 1987; Dingledine et al., 1988). Recently, two groups reported that stimulation of glutamate receptors can inhibit Ca channels in hippocampal neurons (Lester and Jahr, 1990; Chernevskaya et al., 1991). In one study on cultured hippocampal neurons, the suppression of Ca current could be produced by L-glutamate and quisqualate, but not *RS*-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), or L-2-amino-4-phosphonobutyric acid (L-AP4) (Lester and Jahr, 1990). Suppression was readily detected only if GTP-γ-S was present in the patch pipette, and was slow, taking about 40 sec to reach completion. In this study, suppression of Ca channel current had a requirement for external or internal Ca (Lester and Jahr, 1990). In contrast, another study on freshly dissociated hippocampal neurons (Chernevskaya et al., 1991) found that the suppression of Ca current by glutamate was mimicked by NMDA receptor agonists and could be blocked by NMDA receptor antagonists. The onset of suppression by NMDA receptor agonists required minutes to develop fully and was irreversible.

We have further studied glutamate inhibition of Ca channels in hippocampal neurons, using freshly dissociated pyramidal neurons from the CA3 region of the rat hippocampus. We found rapid (t_{on} ~ 2 sec) and reversible inhibition of Ca channel current with no apparent requirement for Ca or involvement of internal Ca stores. In agreement with Lester and Jahr (1990), the effect of glutamate was mimicked by quisqualate but not by NMDA, AMPA, or kainate. In addition to glutamate and quisqualate, ibotenate, ±-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (racemic *t*-ACPD), and 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD) inhibited Ca channel current; agonist affinities were consistent with mediation by a metabotropic glutamate receptor. Inhibition was selective for N-type over L-type Ca channels. Metabotropic glutamate receptors were found to be coupled to Ca channels by a pathway involving G-proteins but not a readily diffusible second messenger.

Materials and Methods

Cell preparation. Pyramidal neurons were dissociated from the CA3 region of hippocampal slices (400 μm) obtained from 7–21-d-old Long–Evans rats using solutions modified from those of Furshpan and Potter (1989). Slices were incubated at 37°C for 8–10 min, in an O_2 atmosphere, in a solution containing 3 mg/ml protease XXIII (Sigma, St. Louis, MO) (see Kiskin et al., 1990), 82 mM Na_2SO_4, 30 mM K_2SO_4, 5 mM $MgCl_2$, 2 mM *N*-[2-hydroxyethyl]piperazine-*N*'-[2-ethanesulfonic acid] (HEPES), 10 mM glucose, and 0.001% phenyl red indicator, pH 7.4. Slices were transferred to a solution containing 1 mg/ml bovine serum albumin (Sigma), 1 mg/ml trypsin inhibitor (Sigma), 82 mM Na_2SO_4, 30 mM

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K_2SO_4, 5 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, and 0.001% phenyl red indicator, pH 7.4, and allowed to cool to room temperature in an O_2 atmosphere. As cells were needed, slices were withdrawn and the CA3 region dissected and triturated to release individual cells. Cells were placed in the recording chamber in a Tyrode's solution containing (in mM) 150 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose, and 10 HEPES, pH 7.4. Cells were typically used within 4 hr of the preparation of slices.

Whole-cell recordings. Whole-cell voltage-clamp recordings (Hamill et al., 1981) were obtained using pipettes pulled from 100 μl Boralex micropipettes (Dynalab, Rochester, NY) coated with Sylgard and fire polished. Pipette resistances ranged from 2 to 6 MΩ when filled with the internal solution consisting of (in mM) 117 tetraethylammonium (TEA) chloride, 4.5 $MgCl_2$, 9 HEPES, 9 ethyleneglycol-bis-(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA), 14 creatine phosphate-Tris (Sigma), 4 adenosine 5'-triphosphate magnesium salt (Mg-ATP) (Sigma), and 0.3 guanosine 5'-triphosphate Tris salt (GTP-Tris) (Sigma), pH adjusted to 7.4 with TEAOH. Creatine phosphate, ATP, and GTP were stored as aliquots at −70°C, and the solution was kept on ice after they were added. The external recording solution contained (in mM) 25 $BaCl_2$, 145 TEACl, 10 HEPES, and 0.1 EGTA, pH 7.4 with TEAOH. Drug solutions were applied by gravity-driven perfusion from a linear array of 12 microcapillary tubes (Drummond Microcaps; 2 μl, 64 mm length). Solution was exchanged in <250 msec (cf. Friel and Bean, 1988) as confirmed by block of Ba currents by $CdCl_2$ or the change in holding potential produced by 100 μM kainate. Seals were formed and the whole-cell configuration obtained in Tyrode's solution before perfusing the cell with the external solution containing TEA and Ba. Whole-cell currents recorded with a List EPC-7 patch amplifier were filtered at either 2 KHz (8-pole Bessel low-pass) or 3 KHz (4-pole Bessel low-pass), digitized, and stored using a BASIC-FASTLAB analog/digital interface and software (Indec Systems, Sunnyvale, CA).

Outside-out patch recordings. Pipettes had resistances ranging from 4 to 10 MΩ when filled with the same internal solution that was used for whole-cell recording. The external recording solution contained (in mM) 110 $BaCl_2$, 10 HEPES, and 0.1 EGTA, pH 7.4 with $BaOH_2$. Drugs were applied as described for whole-cell recording. Cells were fixed to the bottom of the recording chamber by first coating it with polylysine and laminin.

Cell-attached patch recordings. Pipettes had resistances ranging from 10 to 20 MΩ when filled with a solution containing (in mM) 110 $BaCl_2$, 20 TEACl, 10 HEPES, and 0.1 EGTA, pH 7.4 with $BaOH_2$. Seals were formed in the bath Tyrode's solution before perfusing the cell with a high-K solution to zero the cell membrane potential. The zeroing solution contained (in mM) 145 K-gluconate, 10 HEPES, 5 $MgCl_2$, and 1 EGTA, pH 7.4 with KOH.

Leak subtraction. In early experiments with Cs as the main internal cation, we discovered that agonist-gated currents through glutamate receptor channels could interfere with measurements of Ca channel currents. For example, kainate acting on non-NMDA receptor channels can produce an apparent reduction of inward Ca channel current near 0 mV by activating outward current carried by Cs. In order to suppress currents gated by excitatory amino acids, TEA was therefore used as the main cation in both internal and external solutions. Under these conditions, the leak conductance (determined after block of Ca channels by Cd) was small and reversed near 0 mV (Fig. 1*A*). Often the leak conductance was non-ohmic, with a concave downward shape negative to 0 mV. In such cells, it would be inappropriate to use standard leak correction by linear scaling of currents elicited near the holding potential since linear extrapolation would incorrectly predict large outward leak currents near 0 mV (Fig. 1*A*). Since leak currents were always small even at −80 mV and were much smaller near 0 mV, where we measured Ca channel currents, it was more accurate to make measurements of test current on raw currents. Figures 1–6 and 10–12 show raw, uncorrected currents; capacity transients were reduced by the electronic compensation circuit in the patch-clamp amplifier. When tail currents were measured, leak and capacitative currents were subtracted using linearly scaled currents elicited by a step from −80 to −90 mV; these experiments were confined to cells (e.g., Figs. 8, 9) in which the leak current was nearly linear and current at −10 mV was little affected by subtraction. Leak current in outside-out and cell-attached patches was generally linear and was subtracted, along with capacity transients, by extrapolation from negative potentials (see Figs. 13, 14).

All statistical results are given as mean ± SEM.

Agonist-gated conductances. Using the internal and external solutions with TEA as the main cation, glutamate-activated current was greatly reduced (compared with Na outside and K or Cs inside), but not completely eliminated. Figure 1*B* shows an experiment in which we determined the current–voltage relationship for glutamate-activated current under the standard ionic conditions we used in these studies. The glutamate-activated current reversed near 0 mV (+4 mV in this cell). Presumably, the inward current is carried mainly by Ba ions and outward current mainly by Mg ions (with a small permeability of TEA also possible). In the absence of glycine, cation channels gated by L-glutamate are predominantly of the non-NMDA (AMPA/kainate) receptor type (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) is a relatively selective antagonist of non-NMDA receptor channels in vertebrate neurons (Honore et al., 1988; Verdoorn et al., 1989). We tested what concentration of CNQX was needed to inhibit the glutamate-activated current with these ionic conditions, since this is a crucial tool for distinguishing glutamate-activated current from glutamate modulation of Ca channels. Concentration–response relations were first obtained for CNQX against current activated by 25 μM L-glutamate or 100 μM kainate in a series of eight cells dialyzed with an internal solution in which 108 mM CsCl replaced 108 mM TEACl; internal Cs resulted in large outward currents activated by glutamate or kainate at potentials ranging from −30 to +30 mV, facilitating accurate measurements of CNQX block of L-glutamate- or kainate-gated conductances. Half-maximal inhibition of either L-glutamate- or kainate-gated conductances were obtained with ~1 μM CNQX; 5 μM CNQX produced ~90% block, and 25 μM CNQX provided complete block. In 10 other pyramidal neurons dialyzed with the

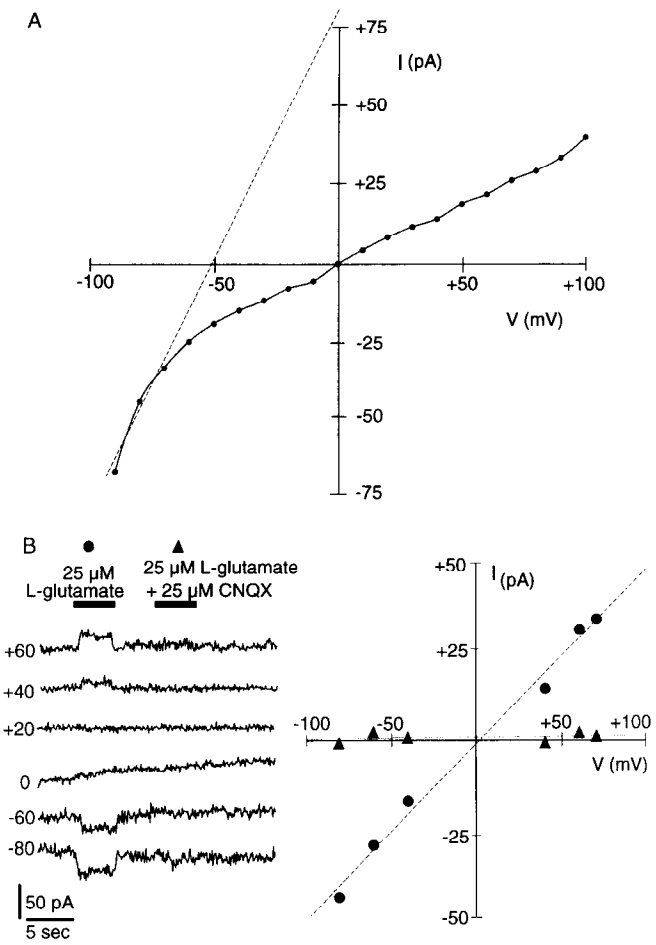

Figure 1. Current–voltage relationships for leak and L-glutamate–activated conductances. *A*, Current–voltage relations for leak current after block of Ca channels by 2 mM $CdCl_2$. *B*, Current–voltage relation for currents activated by 25 μM L-glutamate alone (*circles*) and with 25 μM CNQX (*triangles*). Glutamate was applied for 5 sec while the cell was held steadily at a given potential. The *straight line* in the *right panel* (intercepting the x-axis at +4 mV) was fit by linear regression.

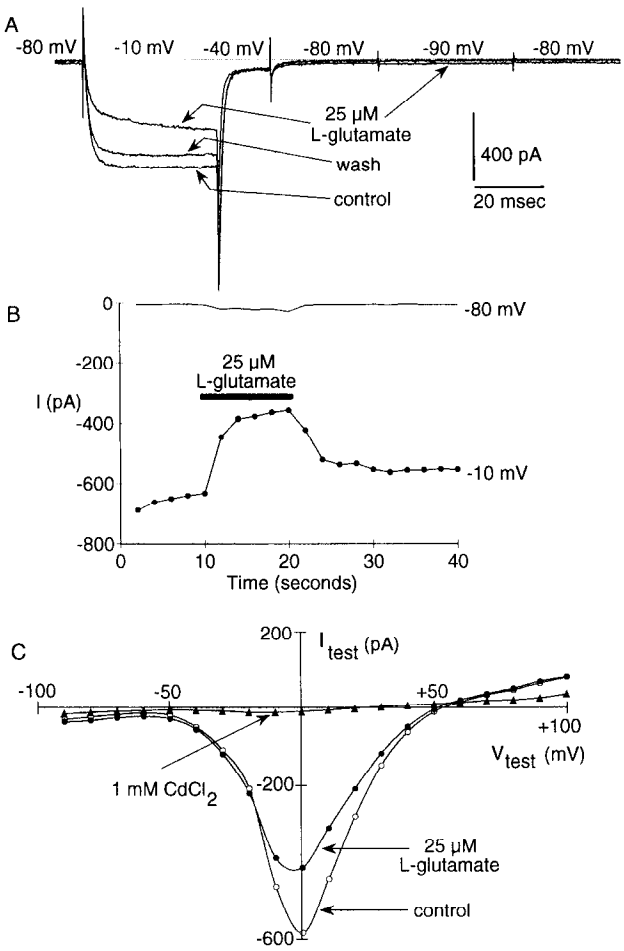

Figure 2. L-Glutamate inhibition of Ca channel current. *A*, Whole-cell currents recorded in control, 4 sec after application of 25 μM L-glutamate, and 10 sec after removal of L-glutamate. The *broken line* is drawn at 0 current. *B*, Time course of Ca channel inhibition by 25 μM L-glutamate; same cell as in *A*. The pulse pattern shown in *A* was repeated every 2 sec. Holding current was measured at −80 mV. Ca channel current was measured 10 msec after stepping to −10 mV (*circles*). *C*, Current–voltage relationship of depolarization-activated current in the absence (*solid circles*) and presence (*open circles*) of 25 μM L-glutamate and after blocking Ca channels with 1 mM $CdCl_2$ (*solid triangles*). Holding potential, −80 mV.

standard internal solution containing 108 mM TEACl, we confirmed that 25 μM CNQX completely blocked currents activated by 25 μM L-glutamate at potentials ranging from −80 to +80 mV (see Figs. 1*B*, 4).

Concentration–response experiments. Concentration–response relations for agonists were obtained by applying a set of five to seven different agonist concentrations. In most experiments, each concentration was applied for ~10 sec; the full set of applications was usually repeated more than once in the same cell with similar or identical results. Saturating concentrations normally bracketed each full set of agonist applications to control for change in response with time. The suppression of Ba current by a concentration of agonist was measured as the percentage inhibition of Ba current, normalized by the inhibition produced in the same cell by a saturating concentration of the agonist.

Compounds. L-Glutamate, L-aspartate, kainate, *N*-methyl-D-aspartate, ibotenate, glycine, ±-baclofen, 2-chloroadenosine (2-Cl-adenosine), guanosine-5′-triphosphate (GTP), and guanosine-5′-*O*-(3-thiophosphate) (GTP-γ-S) were obtained from Sigma. Synthetic (+)-quisqualate was obtained from Research Biochemicals Inc. (Waltham, MA). 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX), *RS*-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), ±-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (racemic *t*-ACPD), 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD), 1*R*,3*S*-1-aminocyclopentane-1,3-dicarboxylic acid (1*R*,3*S*-ACPD), L-2-amino-4-phosphonobutyric acid (L-AP4), and L-2-amino-3-phosphonopropionic acid (L-AP3) were obtained from Tocris Neuramine (Bristol, UK). Another batch of L-AP3 was the kind gift of Dr. D. Schoepp (Lilly Research Laboratories, Indianapolis, IN). Nimodipine was a generous gift of Dr. Alexander Scriabine (Miles Laboratories, West Haven, CT). (+)-S-202-791 was a gift of Dr. R. P. Hof (Sandoz, Basel, Switzerland). Synthetic ω-conotoxin GVIA (ω-CgTx-GVIA) and somatostatin were obtained from Peninsula Laboratories (Belmont, CA).

Results

Glutamate inhibition of Ba currents

Whole-cell currents were recorded using Ba as the charge carrier and TEA as the predominant internal and external cation. Under these ionic conditions, L-glutamate–gated conductances were greatly reduced, and modulation of Ca channel current could be clearly distinguished from activation of background current. Figure 2 illustrates the effect of 25 μM L-glutamate on Ba current in hippocampal pyramidal neurons. In the experiment illustrated in Figure 2, *A* and *B*, Ba current was evoked every 2 sec by a 50 msec voltage step from −80 to −10 mV. Application of 25 μM L-glutamate resulted in a rapid inhibition of Ba current that reached steady-state in 4 sec (Fig. 2*B*); in this cell, 25 μM L-glutamate caused a 40% reduction in the inward current measured 10 msec after the step to −10 mV, when control Ba current was maximal. In 22 cells tested with the protocol in Figure 2, *A* and *B*, 25 μM L-glutamate caused a mean reduction in Ba current of 26 ± 2% (range, 15–45%). In addition to depressing the Ba current, L-glutamate induced a slow phase of activation not present in control, an effect previously seen with other transmitters that inhibit Ca channels (e.g., Forscher and Oxford, 1985; Marchetti et al., 1986; Ikeda and Schofield, 1989; Kasai and Aosaki, 1989). The effect of L-glutamate rapidly reversed on return to control solution, with significant reversal within 2 sec and steady-state achieved in 8 sec (Fig. 2*B*). In most cells, reversal was complete (Figs. 3–6); when it was not (e.g., Fig. 2*B*), the decline in current seemed consistent with "rundown" seen under control conditions.

In some cells (including that in Fig. 2*A*,*B*), L-glutamate produced a small increase in the holding current at −80 mV due to activation of glutamate receptor channels, presumably passing small currents carried by Ba and possibly TEA. Since this current reverses slightly positive to 0 mV (Fig. 1*B*), activation of these channels would produce an enhanced inward current at −10 mV, so this clearly cannot account for the reduction in inward Ca channel current at −10 mV caused by L-glutamate. In principle, the current induced by conventional glutamate receptors could partly counteract the reduction in inward Ca channel current at −10 mV caused by L-glutamate, but with these ionic conditions the effect would be very small. For example, in the cell shown in Figure 2, *A* and *B*, the inward current induced at −80 mV is −15 pA, and since L-glutamate-activated currents have a roughly linear current–voltage relation, reversing near +5 mV (Fig. 1), the inward current induced at −10 mV would be about −3 pA, negligible compared to the control current of −640 pA or to the reduction of 280 pA caused by L-glutamate. Also, although L-glutamate reduced the inward Ca channel current elicited by stepping to −10 mV in every neuron tested, in many neurons effects on holding current were too small to be detectable even at −80 mV (e.g., Fig. 3).

Figure 2*C* illustrates the current–voltage relation for a neuron in the absence and presence of 25 μM L-glutamate. As expected for an effect on Ca channels, the changes in Ba current produced by L-glutamate were largest at the voltages (near 0 mV) where

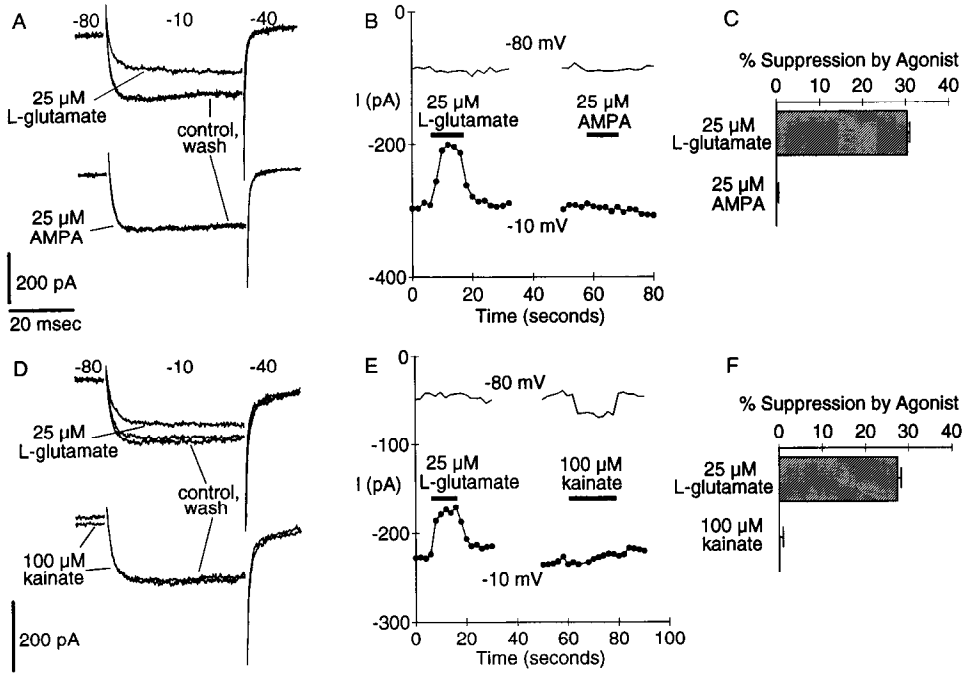

Figure 3. Lack of effect of AMPA/kainate receptor agonists. *A*, Inhibition of whole-cell Ba current by 25 μM L-glutamate but not 25 μM AMPA. *B*, Effects of L-glutamate and AMPA on steady current at −80 mV and on current elicited by a step to −10 mV; same neuron as in *A*. Pulses from −80 to −10 mV were elicited every 2 sec. *C*, Pooled results in four cells. L-Glutamate at 25 μM was applied to every cell before and after testing the effects of AMPA. *D* and *E*, Inhibition of Ba current by 25 μM L-glutamate but not 100 μM kainate. Pulses from −80 to −10 mV were elicited every 2 sec. *F*, Collected results in four cells. L-Glutamate at 25 μM was applied to every cell before and after testing the effects of kainate. All currents were elicited by steps to −10 or 0 mV from −80 mV.

the Ca channel current was most pronounced and the current gated by L-glutamate is smallest (Fig. 1*B*).

We conclude that the reduction in inward current elicited by depolarization represents modulation of Ca channel current, and is not an artifact due to activation of current through glutamate-gated channels.

Receptor pharmacology

Figure 3 compares the effect of L-glutamate with that of kainate and AMPA, agonists at non-NMDA glutamate receptors (O'Brien and Fischbach, 1986; Verdoorn and Dingledine, 1988; Zorumski and Yang, 1988; Patneau and Mayer, 1990, 1991). Neither AMPA nor kainate had a significant effect on Ca channel current, even when tested in cells with reproducible responses to L-glutamate (Fig. 3). The effects of kainate offered an especially clear distinction between activation of current through non-NMDA receptor channels and the modulation of Ca channel current; kainate induced a substantial inward current at −80 mV (Fig. 3*D*,*C*) but had no effect on the Ca channel current at −10 mV. [The larger effects of kainate, when compared to L-glutamate or AMPA, on background current are expected, since kainate activates currents with much larger steady-state components than L-glutamate or AMPA, both of which induce rapidly desensitizing currents (Kiskin et al., 1986; Patneau and Mayer, 1990, 1991).]

CNQX, an antagonist at non-NMDA receptors (Honore et al., 1988; Verdoorn et al., 1989), had no effect on the ability of L-glutamate to depress Ca channel current (Fig. 4), even when present at 25 μM, a concentration that completely inhibited L-glutamate activation of background current in the same cell (Fig. 4; see also Fig. 1*B* and Materials and Methods).

These results, summarized in Figures 3, *C* and *F*, and 4*B*, show that the reduction in Ca channel current by L-glutamate is mediated by a receptor with pharmacological sensitivities different than non-NMDA (kainate/AMPA) receptors.

NMDA receptors also do not mediate the effects of L-glutamate on Ca channel current. L-Aspartate, an agonist at NMDA receptors, had no effect on Ca channel current (Fig. 5*A*–*C*). NMDA (100 μM) also had no effect on Ca channel current (Fig.

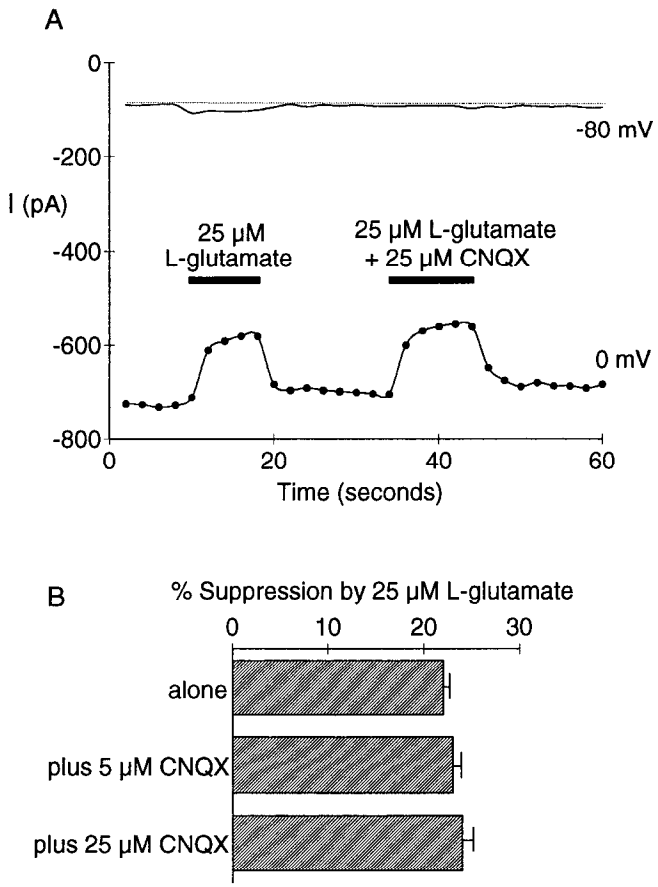

Figure 4. Lack of effect of CNQX on L-glutamate suppression of Ca channels. *A*, Action of 25 μM L-glutamate on whole-cell Ba current in the absence or presence of 25 μM CNQX. Glutamate produced a small increase in holding current at −80 mV, which was blocked by CNQX. Pulses from −80 to 0 mV were applied every 2 sec. *B*, Collected results; CNQX was tested in eight cells at 5 μM and in five cells at 25 μM. L-Glutamate at 25 μM was applied alone to every cell before and after testing the effects of CNQX. Currents elicited by a step from −80 to either −10 or 0 mV.

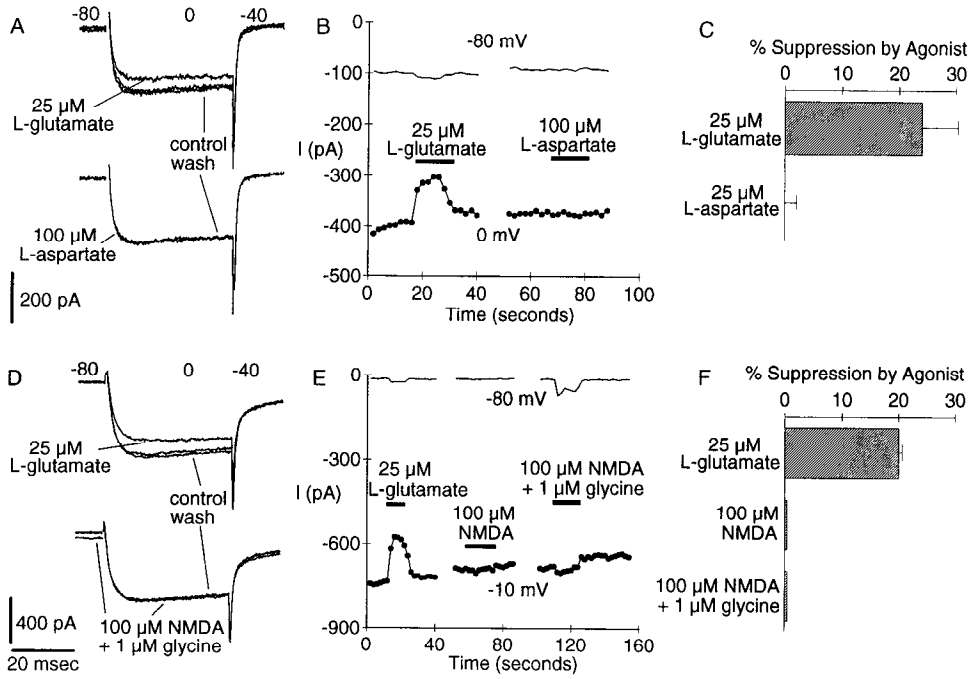

Figure 5. Lack of effect of NMDA receptor agonists. *A* and *B*, Inhibition of Ba current by 25 μM L-glutamate but not 100 μM L-aspartate. Pulses from −80 to 0 mV were elicited every 2 sec. *C* and *D*, Inhibition of Ba current by 25 μM L-glutamate but not 100 μM NMDA, either in the absence or presence of 1 μM glycine. NMDA evoked a small increase in holding current when applied together with glycine; the small apparent increase in Ba current at −10 mV is likely due to current carried by Ba through NMDA receptor-gated channels. Pulses elicited every 2 sec.

5*D*–*C*), even when applied together with 1 μM glycine (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988), to allow for activation of NMDA receptor-gated cation channels.

The ability of L-AP4 to mimic the actions of L-glutamate was also examined. In 10 cells that responded to L-glutamate (31 ± 4.1% reduction of Ca channel current), 100 μM L-AP4 had no effect (1.3 ± 0.7% reduction).

The metabotropic glutamate receptor that mediates IP_3 (inositol-1,4,5-trisphosphate) formation responds to L-glutamate, but not kainate, AMPA, or NMDA, and is insensitive to block by CNQX (Sladeczek et al., 1985; Sugiyama et al., 1987, 1989; Palmer et al., 1988; Schoepp and Johnson, 1988; Manzoni et al., 1990), matching the results shown so far for L-glutamate inhibition of Ca channel current. Three other analogs of L-glutamate that act as agonists at metabotropic receptors are quisqualate, ibotenate, and *t*-ACPD (Sladeczek et al., 1985; Nicoletti et al., 1986a,b; Sugiyama et al., 1987; Palmer et al., 1988; Schoepp and Johnson, 1988; Manzoni et al., 1990; Houamed et al., 1991; Masu et al., 1991). Quisqualate is also a non-NMDA receptor agonist, while ibotenate also acts as an agonist at NMDA receptors. *t*-ACPD is a selective agonist at the metabotropic glutamate receptor (Palmer et al., 1988; Manzoni et al., 1990); 1*S*,3*R*-ACPD is thought to be the active enantiomer in racemic *t*-ACPD (Irving et al., 1990).

Ibotenate, *t*-ACPD, and (+)-quisqualate all mimicked the ability of glutamate to cause a rapid and reversible inhibition of the Ca channel current (Fig. 6*A*). Although there was steady rundown of the Ca channel current in the course of this experiment, each agonist suppressed about the same fraction of whole-cell current. (+)-Quisqualate and L-glutamate evoked a small increase in holding current, consistent with activation of non-NMDA receptors, while holding current was not affected by ibotenate (which was applied in the absence of glycine) or *t*-ACPD. The lack of effect of *t*-ACPD on holding current is consistent with the idea that it acts selectively at metabotropic glutamate receptors, at least at submillimolar concentrations (Palmer et al., 1988; Manzoni et al., 1990). The suppression of Ca channel current by ACPD was stereoselective. As shown in Figure 6*B*, 1*S*,3*R*-ACPD suppressed whole-cell Ba currents by 30% while an equal concentration of 1*R*,3*S*-ACPD was without effect. These agonist sensitivities are consistent with mediation of Ca channel inhibition by a metabotropic glutamate receptor.

Figure 7 shows the concentration–response relations for the four agonists. Each agonist was tested at a range of concentrations applied to a single cell, and the inhibition was expressed as a fraction of the maximal inhibition produced in the cell by a saturating concentration, tested in bracketing applications at the beginning and end of the series (e.g., Fig. 7*B*). The relative affinities of metabotropic agonists, estimated from EC_{50} values, were (+)-quisqualate (0.11 μM) > L-glutamate (1.6 μM) > ibotenate (7.8 μM) > *t*-ACPD (48 μM). In addition to being the most potent, (+)-quisqualate differed for the other agonists in having a more shallow dose–response curve. The active enantiomer in racemic *t*-ACPD, 1*S*,3*R*-ACPD, had an EC_{50} of 15.5 μM (data not shown; seven determinations in four cells).

Saturating concentrations of all four agonists produced about the same percentage reduction in current. In 13 cells in which all four agonists were applied sequentially, a saturating concentration of quisqualate (8 μM) produced a 22 ± 2.5% inhibition; the corresponding values for L-glutamate (at 40 μM), ibotenate (at 200 μM), and *t*-ACPD (at 1 mM) were 24 ± 2.6%, 24 ± 4.0%, and 20 ± 2.5%, respectively.

L-AP3 has been reported to block noncompetitively the phosphoinositide hydrolysis mediated by the activation of metabotropic glutamate receptors (Schoepp and Johnson, 1989; Schoepp et al., 1990a,b). We tested the possibility that L-AP3 might noncompetitively antagonize L-glutamate suppression of Ca channel current. L-AP3 at 1 mM had no significant effect on Ca channel current (0.9 ± 0.1% reduction; $n = 10$) when applied alone. L-AP3 at 1 mM also had no significant effect on the suppression of Ca channel current produced by 25 μM L-glutamate. In 10 cells, 25 μM L-glutamate produced a 21 ± 2% reduction of Ca channel current while 25 μM L-glutamate plus 1 mM L-AP3 produced a 20 ± 4.2% reduction. It appears that L-AP3 does not noncompetitively antagonize the suppression of Ca channels produced by L-glutamate. Because responses to

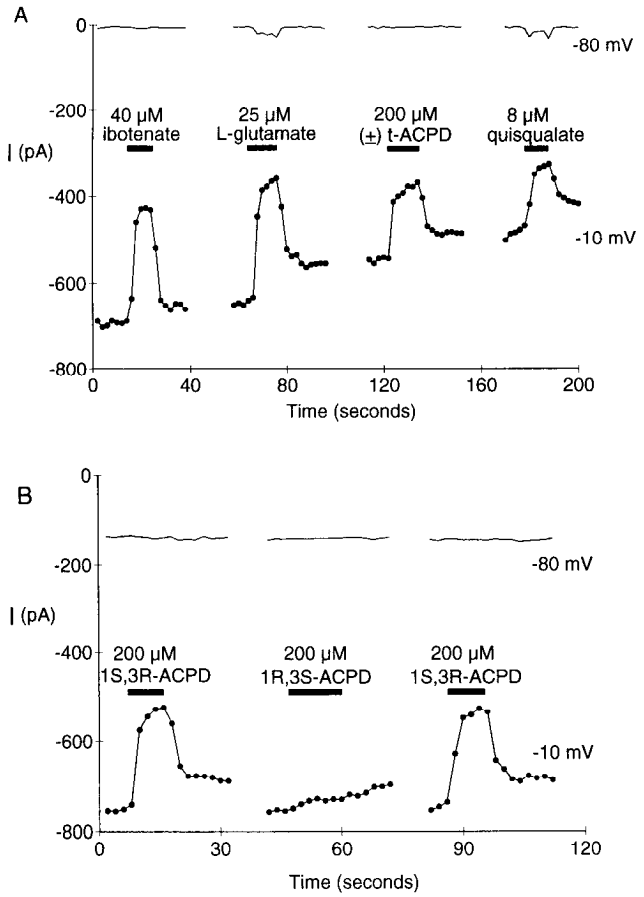

Figure 6. Metabotropic glutamate receptor agonists mimic the inhibition of whole-cell Ba current by L-glutamate. *A*, Effects of 40 μM ibotenate, 25 μM L-glutamate, 200 μM racemic *t*-ACPD, and 8 μM (+)-quisqualate on Ba current in the same neuron. In this cell, both L-glutamate and quisqualate evoked a small increase in holding current, consistent with their agonist actions at non-NMDA receptor channels. Ibotenate did not alter the holding current at −80 mV, presumably because no glycine was present (see Fig. 5). Pulses from −80 to −10 mV were elicited every 2 sec. *B*, Comparison of the effects of two enantiomers in racemic *t*-ACPD, 1*S*,3*R*-ACPD, and 1*R*,3*S*-ACPD. Steps from −80 to 0 mV were delivered every 2 sec.

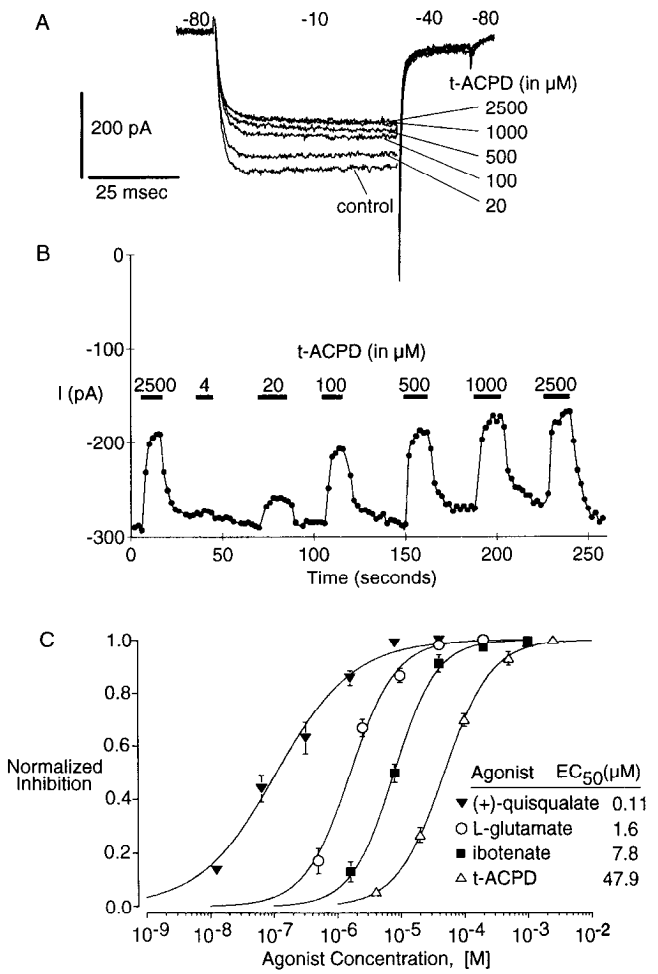

Figure 7. Concentration–response relations for inhibition of whole-cell Ba current by metabotropic glutamate receptor agonists. *A* and *B*, Inhibition of Ba currents by concentrations of racemic *t*-ACPD from 4 μM to 2.5 mM. There was little change in the response to a saturating concentration (2.5 mM) of *t*-ACPD bracketing the other concentrations. Voltage steps from −80 to 0 mV were delivered every 2 sec. *C*, Collected data for the concentration–response relations for quisqualate, L-glutamate, ibotenate, and *t*-ACPD. Experiments like that in *A* and *B* were done in four to six cells for each agonist. The amount of inhibition produced by each concentration was expressed as a fraction of the effect produced by a saturating concentration of the agonist in the same cell. Data points show mean ± SEM for a total of 11 determinations in six cells for each concentration of (+)-quisqualate, 6 determinations in four cells for L-glutamate, 8 determinations in five cells for ibotenate, and 11 determinations in four cells for *t*-ACPD. *Smooth curves* are drawn according to the following: normalized inhibition = $1/[1 + (EC_{50}/[\text{agonist}])^n]$, with EC_{50} = 0.11 μM, n = 0.71 for (+)-quisqualate; EC_{50} = 1.62 μM, n = 1.2 for L-glutamate; EC_{50} = 7.8 μM, n = 1.3 for ibotenate; and EC_{50} = 47.9 μM, n = 1.2 for racemic *t*-ACPD. The active enantiomer 1*S*,3*R*-ACPD had an EC_{50} of 15.5 μM with a slope (n) of 1.12 (seven determinations in four cells; data not shown).

half-maximal concentrations of agonists can be rather small, we used concentrations of L-glutamate that were nearly saturating (25 μM; see Fig. 7), so we cannot rule out a weak competitive action of L-AP3.

What types of Ca channels does the metabotropic receptor inhibit?

Hippocampal pyramidal neurons have multiple types of high-threshold Ca channels (Takahashi et al., 1989; Fisher et al., 1990; Toselli and Taglietti, 1990; Mogul and Fox, 1991; O'Dell and Alger, 1991; Regan et al., 1991; Thompson and Wong, 1991; Mintz et al., 1992). At the whole-cell level, different components of high-threshold current are probably best distinguished from each other using pharmacological tools, since it is likely that currents carried by distinct channels overlap in their voltage dependence and kinetics, as in peripheral neurons (Plummer et al., 1989; Regan et al., 1991). In agreement with a previous report using guinea pig CA3 pyramidal neurons (Mogul and Fox, 1991), we find that rat CA3 pyramidal neurons have a relatively large component of high-threshold current (~25–40%) inhibited by dihydropyridine blockers. The dihydropyridine-sensitive current flows though high-conductance L-type channels detected at the single-channel level (Mogul and Fox, 1991; data not shown). We find that the N-type channel blocker ω-CgTx-GVIA blocks about 25–45% of the high-threshold current in CA3 neurons (e.g., Figs. 8*C*, 11). Like most other neurons (Regan et al., 1991), hippocampal CA3 neurons also possess high-threshold current that is resistant to saturating concentrations of dihydropyridines and ω-CgTx-GVIA. A small fraction of this current can be blocked by ω-Aga-IVA, a peptide toxin from spider venom that is a selective blocker of P-type channels

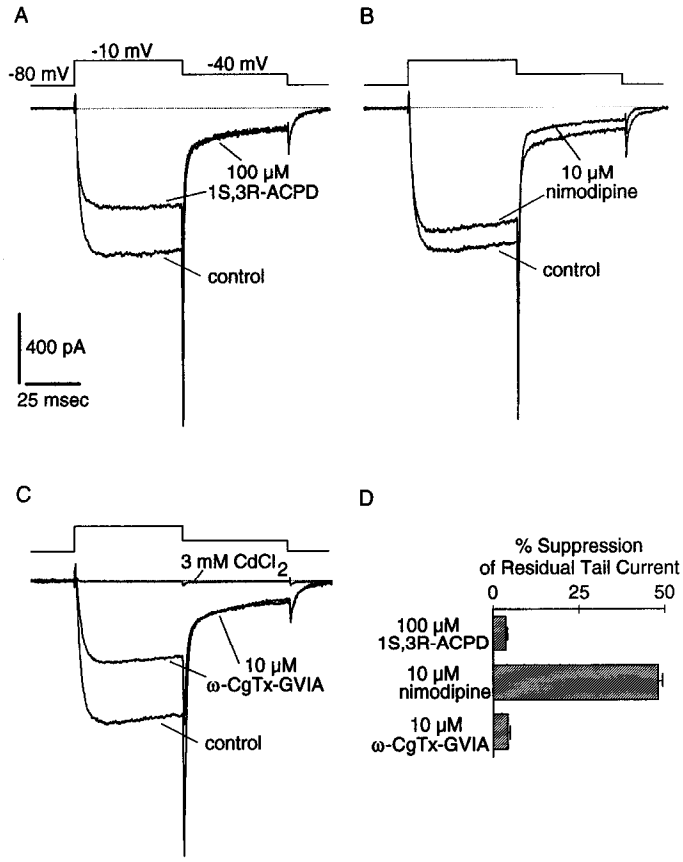

Figure 8. Lack of effect of 1*S*,3*R*-ACPD on dihydropyridine-sensitive tail currents. *A*, Whole-cell Ba currents recorded in control and 6 sec after application of 1*S*,3*R*-ACPD. *B*, Current from the same cell as in *A* before and 15 sec following the application of 10 μM nimodipine. *C*, Same cell as in *A* and *B* before and after application of either 10 μM ω-CgTx-GVIA or 3 mM $CdCl_2$. *D*, Pooled results from 11 neurons showing the effects of 1*S*,3*R*-ACPD, nimodipine, and ω-CgTx-GVIA on the current measured 30 msec after repolarization to −40 mV from a test potential of either 0 or −10 mV. The holding potential in all cells was −80 mV. The mean suppression of peak test current by 100 μM 1*S*,3*R*-ACPD was 27 ± 2.7%. No low-threshold current was seen in any of these cells when stepping from −110 mV to −50 mV. All currents are shown after leak subtraction using current elicited by a step from −80 mV to −90 mV.

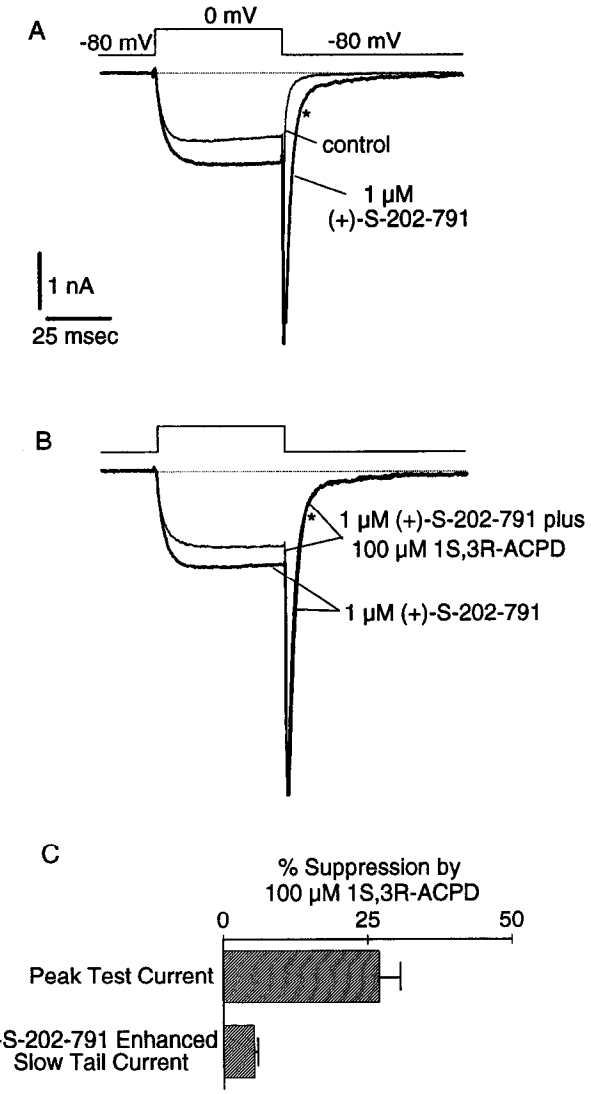

Figure 9. Slow tail currents enhanced by dihydropyridine agonists are not suppressed by 1*S*,3*R*-ACPD. *A*, Whole-cell Ba currents recorded in control and 16 sec after application of (+)-S-202-791. *B*, Currents from the same cell as in *A* before and 6 sec following the application of 1*S*,3*R*-ACPD while continuously in the presence of (+)-S-202-791. *C*, Pooled results from five neurons showing the effects of 1*S*,3*R*-ACPD on (+)-S-202-791 slow tail currents and peak test currents. Slow tails were measured at 5 msec (denoted by *asterisk* on current records) after repolarization to −80 mV from a test potential of 0 mV. The holding potential in all cells was −80 mV. All currents are shown after leak substraction by extrapolation from negative potentials. The *broken line* represents zero current.

(Mintz et al., 1992); based on sensitivity to ω-Aga-IVA, an average of only 14% of the high-threshold current in CA3 pyramidal neurons is contributed by P-type channels (Mintz et al., 1992). While none of these pharmacological tools are perfect (in particular, it remains to be seen whether individual channel types are completely blocked by saturating concentrations of the agents), they have been shown to target fractions of high-threshold current that show little overlap in sensory, cerebellar Purkinje, superior cervical sympathetic, and CA1 pyramidal neurons (Aosaki and Kasai, 1989; Takahashi et al., 1989; Mogul and Fox, 1991; Regan et al., 1991; Mintz et al., 1992). We focused on examining the actions of 1*S*,3*R*-ACPD on L-type and N-type channels.

Figures 8–10 illustrate three independent experimental approaches designed to address whether L-type channels are modulated by activation of the metabotropic glutamate receptor. First, in most CA3 pyramidal neurons, when the voltage was stepped to near 0 mV and then repolarized to −40 mV, deactivation of current was incomplete at −40 mV; with further repolarization to −80 mV, the current deactivated completely with a slow tail current having a time constant of ~3 msec. Both the residual current at −40 mV and the slow tail current at −80 mV are partially blocked by nimodipine but are insensitive to ω-CgTx-GVIA, suggesting that these current components are carried at least in part by L-type channels (Fig. 8*A–C*). We tested whether 1*S*,3*R*-ACPD suppressed these current components. In 11 neurons, application of 100 μM 1*S*,3*R*-ACPD caused a 27 ± 2.7% inhibition of the test current measured at 0 mV while having almost no effect (3.7 ± 1.3% inhibition) on the residual current measured 30 msec after repolarization to −40 mV (see Fig. 8). In these same neurons, 10 μM nimodipine blocked the residual current at −40 mV by 48 ± 2.7%, while 10 μM ω-CgTx-GVIA was without much effect (4.3 ± 1.9% decrease). The slow tail current present on repolarization from

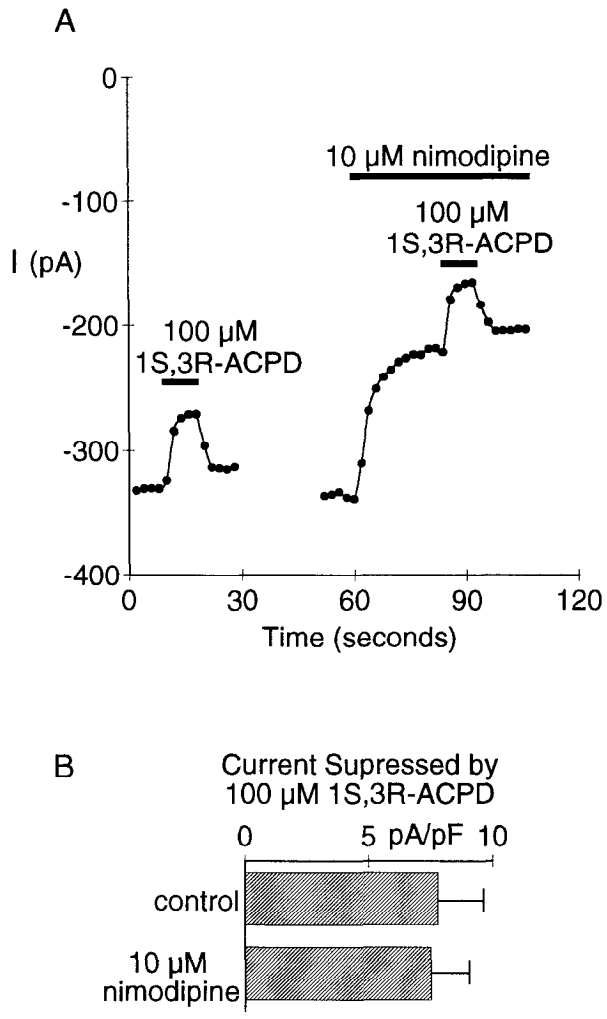

Figure 10. Dihydropyridine antagonists do not occlude 1*S*,3*R*-ACPD suppression of whole-cell Ba currents in CA3 pyramidal neurons. *A*, Time course of the effects of 1*S*,3*R*-ACPD on Ba currents before and after application of nimodipine. Ba current weres evoked by depolarizations to 0 mV. Holding potential was −80 mV. Test pulses were given every 2 sec. *B*, Pooled data from six neurons for the mean current (normalized to the cell capacitance) suppressed by 100 μM 1*S*,3*R*-ACPD before and after block of the nimodipine-sensitive fraction of whole-cell Ba current. Mean nimodipine block was 31 ± 2.3%. 1*S*,3*R*-ACPD suppressed 19 ± 1.1% of the whole-cell Ba current before and 25 ± 1.1% after nimodipine block.

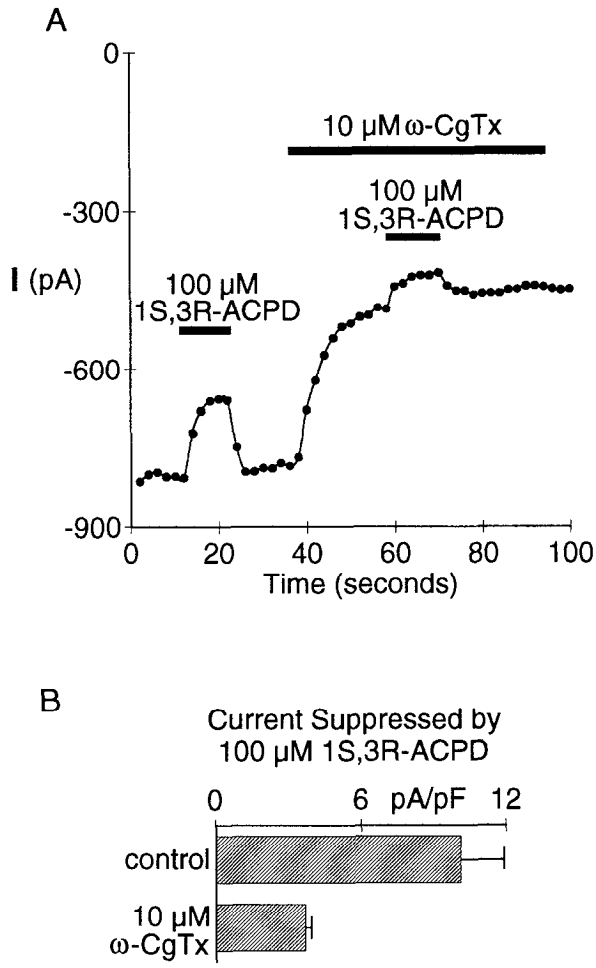

Figure 11. 1*S*,3*R*-ACPD suppresses ω-CgTx-GVIA–sensitive Ca channels. *A*, Time course of the effects of 1*S*,3*R*-ACPD on Ba currents before and after application of ω-CgTx-GVIA. Ba currents were evoked by depolarizations to 0 mV. Holding potentials was −80 mV. Test pulses were given every 2 sec. *B*, Pooled data from six neurons for the mean current (normalized to the cell capacitance) suppressed by 100 μM 1*S*,3*R*-ACPD before and after block of the ω-CgTx-GVIA–sensitive fraction of whole-cell Ba current. Mean ω-CgTx-GVIA block was 36.3 ± 1.7%. 1*S*,3*R*-ACPD suppressed 23 ± 3.9% of the whole-cell Ba current before and 12.9 ± 1.9% after ω-CgTx-GVIA block.

−40 to −80 mV was also partially inhibited by nimodipine but not by ω-CgTx-GVIA or 1*S*,3*R*-ACPD.

Similar results were obtained for dihydropyridine agonist–enhanced tail currents in five neurons. Figure 9 shows that 1 μM (+)-S-202-791 greatly enhanced a slow tail current component elicited when repolarizing from 0 mV to −80 mV. As in other neurons, enhancement of the slow tail current by the dihydropyridine agonist can likely be attributed to long openings of L-type Ca channels induced by the drug (Nowycky et al., 1985; Plummer et al., 1989). 1*S*,3*R*-ACPD at 100 μM suppressed 27 ± 4.5% of the test current measured at 0 mV while having little or no effect on the slow tail current components (5.3 ± 1.4% inhibition; see Fig. 9), suggesting that agonist-enhanced L-type channels are not affected by 1*S*,3*R*-ACPD. Finally, we tested whether the dihydropyridine antagonist nimodipine could occlude the suppression of high-threshold current by 1*S*,3*R*-ACPD. In six neurons, 10 μM nimodipine blocked 31 ± 2.3% of the Ba current elicited by stepping from −80 to near 0 mV. The Ca channel current suppressed by 1*S*,3*R*-ACPD (expressed as pA of current per pF of cell capacitance) was measured before and after nimodipine block. 1*S*,3*R*-ACPD at 100 μM suppressed 7.8 ± 2.0 pA/pF of current prior to nimodipine application and 7.5 ± 2.3 pA/pF of current after block by nimodipine (Fig. 10), suggesting that almost none of the current suppressed by 1*S*,3*R*-ACPD could be blocked by nimodipine.

These three results all suggest that L-type current is not suppressed by metabotropic glutamate receptor agonists.

Figure 11 shows that ω-CgTx-GVIA and 1*S*,3*R*-ACPD act on the same fraction of Ca channel current in CA3 pyramidal neurons. In six neurons, 10 μM ω-CgTx-GVIA blocked 36.3 ± 1.7% of the Ba current elicited by stepping from −80 to near 0 mV. 1*S*,3*R*-ACPD (100 μM) suppressed 10.2 ± 2.0 pA/pF of current prior to ω-CgTx-GVIA application and 3.7 ± 0.3 pA/pF of current after block by ω-CgTx-GVIA (see Fig. 11). We conclude that the major portion of the current suppressed by 1*S*,3*R*-ACPD is of the N type.

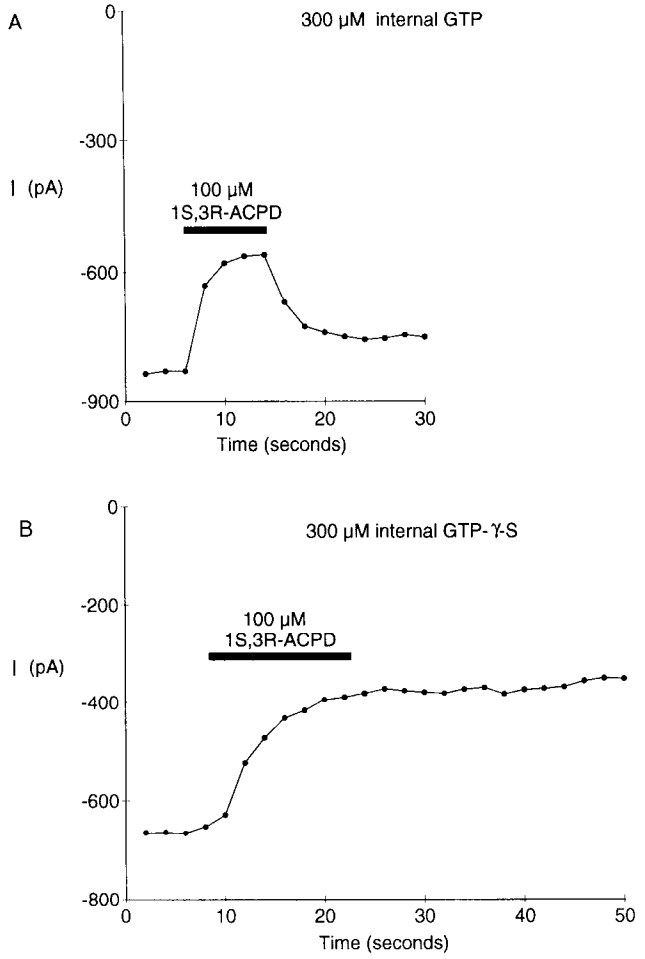

Figure 12. A G-protein couples metabotropic glutamate receptors to Ca channels. *A*, Time course for the effect of 1*S*,3*R*-ACPD on whole-cell Ba current in a cell dialyzed for 11 min with 300 μM GTP. *B*, Same experiment as in *A* but for a different cell dialyzed with 300 μM GTP-γ-S for 13 min. The irreversibility of suppression in the presence of GTP-γ-S was seen in nine other neurons. Ba current were evoked by depolarizations to −10 mV. Holding potential was −80 mV. Test pulses were given every 2 sec.

While the suppression of current by 1*S*,3*R*-ACPD was dramatically less after block of ω-CgTx-GVIA–sensitive current, the inhibition by 1*S*,3*R*-ACPD was not completely occluded. Approximately 60–70% of the whole-cell current in these cells can be accounted for by dihydropyridine-sensitive (L-type) and ω-CgTx-GVIA–sensitive (N-type) channel populations. In most CA3 cells, the P-type channel blocker ω-Aga-IVa blocks little of the remaining 30–40% of the high-voltage-activated current (Mintz et al., 1992). We tested the ability of ω-Aga-IVa to block Ca channels in CA3 cells under the conditions of the present study (with 25 mM $BaCl_2$ rather than the 5 mM $BaCl_2$ used by Mintz et al., 1992). In five cells, 200 nM ω-Aga-IVa blocked only 5 ± 1% of the whole-cell Ca current. In four additional CA3 cells, 200 nM ω-Aga-IVa produced no quantifiable block. Because of the small effect of ω-Aga-IVa, it was impossible to determine if the small fraction of P-type current present in some cells could be modulated by 1*S*,3*R*-ACPD. It seems clear that P-type current could account for only a very small fraction, if any, of the Ca channel current modulated by the metabotropic glutamate receptor.

Coupling between receptor and channel involves G-proteins

We tested for the involvement of a G-protein in the coupling between metabotropic glutamate receptors and Ca channels in CA3 pyramidal neurons by comparing cells dialyzed with GTP and its nonhydrolyzable analog, GTP-γ-S. In neurons dialyzed with 300 μM GTP, the suppression of Ca channel current by 100 μM 1*S*,3*R*-ACPD was readily reversible (Fig. 12). In contrast, if cells were dialyzed for 10–15 min with 300 μM GTP-γ-S, the suppression of Ca channel current by 1*S*,3*R*-ACPD was irreversible (Fig. 12; $n = 9$). This suggests that a G-protein is involved in the coupling between receptor and channel.

Neurons express numerous distinct G-protein subunits, some of which are sensitive to ribosylation by islet-activating protein (pertussis toxin) (Ui, 1984). We compared the ability of pertussis toxin to block the coupling between several different neurotransmitter receptors and Ca channels in these neurons. Somatostatin, baclofen, and 2-Cl-adenosine were tested along with 1*S*,3*R*-ACPD, as they have been reported to couple via pertussis toxin–sensitive G-proteins to Ca channels in numerous different types of neurons (Holz et al., 1986; Lewis et al., 1986; Gross et al., 1989; Ikeda and Schofield, 1989; Sah, 1990; Kleuss et al., 1991; Scholz and Miller, 1991a,b). We tried to use pertussis toxin on acutely dissociated CA3 cells with two different approaches. First, the A protomer was included in the patch pipette at concentrations of 2 μg/ml in the presence of 2 mM nicotinamide adenine dinucleotide and 4 mM ATP. In four cells dialyzed for 20–60 min with A protomer, 1*S*,3*R*-ACPD, baclofen, 2-Cl-adenosine, and somatostatin still inhibited Ca channel current, with no diminution in response during the recording. Second, the holoenzyme of pertussis toxin (1 μg/ml) was applied to hippocampal slices for 5 hr at 37°C (the longest that slices remained viable at this temperature). In three cells treated in this manner, no significant reduction in response to 1*S*,3*R*-ACPD, baclofen, 2-Cl-adenosine, or somatostatin was seen when compared to control cells. Neither of these approaches showed a significant effect of pertussis toxin on baclofen, 2-Cl-adenosine, or somatostatin responses, previously reported to be sensitive to the toxin. We conclude that pertussis toxin probably was not active under the conditions we used it, and our results leave open the possibility that the suppression of Ca channels by metabotropic glutamate receptors is pertussis toxin sensitive.

Spatial restriction for coupling between receptor and Ca channels

In some instances the coupling between neurotransmitter receptors and Ca channels involves diffusible second messengers (Reuter, 1983; Fisher and Johnston, 1990; Bernheim et al., 1991a). In contrast, in other cases the coupling between receptor and channel is physically restricted (Forscher and Oxford, 1985; Lipscombe et al., 1989; Bernheim et al., 1991a). We investigated these possibilities by employing outside-out cell-free membrane patch and cell-attached patch techniques.

Outside-out patches containing many Ca channels could be obtained from CA3 pyramidal neurons in which rundown of current was small over the course of 10–15 min. Semimacroscopic Ba currents from outside-out patches were rapidly and reversibly suppressed by application of 1*S*,3*R*-ACPD, baclofen,

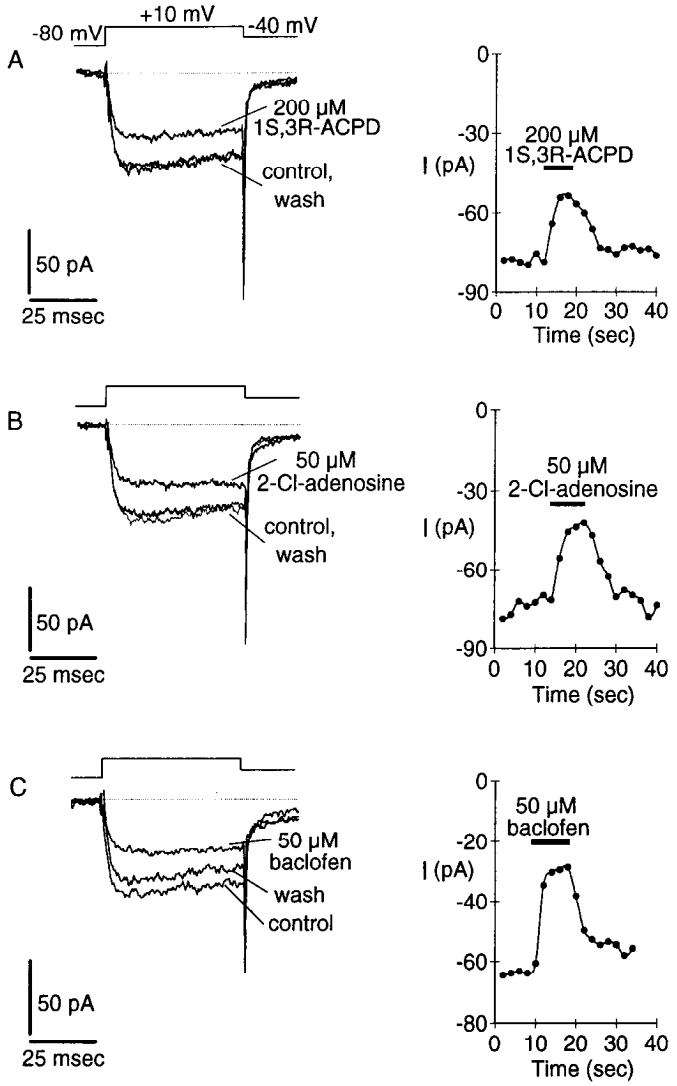

Figure 13. Suppression of macroscopic Ba currents in outside-out membrane patches. *A,* Effects of 1*S*,3*R*-ACPD on macroscopic Ba currents from a outside-out membrane patch. The patch was pulled from a CA3 pyramidal neuron 6 min prior to the time course shown. *B,* Effects of 2-Cl-adenosine on Ba currents from the same patch as in *A* but 7 min after patch formation. *C,* Effects of baclofen on Ba currents from the same patch as in *A* and *B* but 8 min after patch formation. In all cases, Ba currents were elicited by voltage steps from −80 to +10 mV given every 2 sec. Currents are shown after leak subtraction by extrapolating from negative potentials. In this patch, the leak currents at −80 mV were ~10 pA. *Broken lines* represent 0 current.

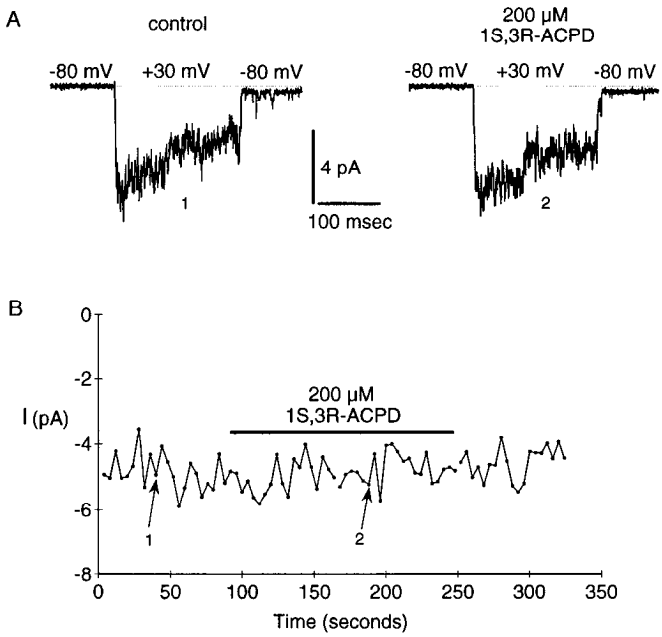

Figure 14. 1*S*,3*R*-ACPD does not suppress macroscopic Ba currents in cell-attached membrane patches when applied outside the patch membrane. *A,* Sample traces showing macroscopic Ba currents before and after application of 1*S*,3*R*-ACPD to the cell membrane outside the cell-attached patch. *B,* Time course of the effects of 1*S*,3*R*-ACPD on macroscopic Ba currents. Currents were evoked by voltage steps from −80 to +30 mV every 4 sec. 1*S*,3*R*-ACPD was applied for 2.5 min. Currents are shown after leak subtraction by extrapolating from negative potentials. In this patch, the leak currents at −80 mV were ~1 pA. *Broken lines* represent 0 current.

or 2-Cl-adenosine to the external face of the membrane (Fig. 13). In the patch shown in Figure 13, 1*S*,3*R*-ACPD, 2-Cl-adenosine, and baclofen were applied 6, 7, and 8 min following outside-out patch formation, respectively. In another patch, transmitter suppression of Ca current could be seen 15 min following patch formation. Similar results with these three transmitter analogs were seen in four other outside-out patches.

To determine if the coupling between metabotropic glutamate receptors and Ca channels involves a diffusible second messenger, we examined whether channels in cell-attached patches could be modulated by activation of receptors in the plasma membrane outside of the patch. Cell-attached patch recordings were made with 110 mM BaCl inside the pipette while the cell was bathed in a 145 mM K-gluconate solution to zero the membrane potential. Single-channel activity could routinely be seen in these patches. On occasion, many channels were present, giving semimacroscopic Ba currents having amplitudes of ~5–10 pA. Figure 14 shows a cell-attached patch recording from a CA3 pyramidal neuron. When voltage steps were evoked to +30 mV for 300 msec from a holding potential of −80 mV, Ba currents were about 5 pA, corresponding to ~10–20 Ca channels. Individual openings and reopenings could be seen in the tail after repolarization to −80 mV (Fig. 14*A*). After sampling macroscopic Ba currents for 2 min, 200 μM 1*S*,3*R*-ACPD was applied for 2.5 min to the cell membrane outside of the cell-attached patch. As shown in Figure 14, the application of 1*S*,3*R*-ACPD did not significantly alter the magnitude of the macroscopic Ba currents (4.7 ± 0.09 pA in control and 4.9 ± 0.08 pA in 1*S*,3*R*-ACPD). In two other cell-attached patches where macroscopic Ba currents could be measured, 1*S*,3*R*-ACPD application was also without significant effect (0.1% reduction in Ba current).

Other cell-attached patches contained only a few Ca channels. In four of these patches, Ca channel activity displayed short open times, bursting activity, and a conductance of 15–18 pS, even in the presence of 1 μM (+)-S-202-791. We tentatively designated this activity as N-type, possibly corresponding to the ω-CgTx-GVIA–sensitive fraction of whole-cell Ca channel current. In order to examine the effects of 1*S*,3*R*-ACPD, the chan-

nel activity was quantified by calculating the average current in 300 msec test pulses. In these four patches, the mean activity (stepping from −80 mV to either 0 or +10 mV) was 0.13 ± 0.03 pA in control solutions and 0.14 ± 0.04 pA after application of 200 μM 1*S*,3*R*-ACPD. In the presence of 1 μM (+)-S-202-791, three other patches displayed N-channel activity together with long 30 pS openings, presumably corresponding to L-channels. In these patches, the mean activity was 0.19 ± 0.03 pA in control solution and 0.25 ± 0.05 pA after application of 200 μM 1*S*,3*R*-ACPD.

From the cell-attached experiments, we conclude that 1*S*,3*R*-ACPD applied to the cell membrane outside the patch did not suppress channels isolated in the patch. These results suggest that the coupling between metabotropic glutamate receptors, G-proteins, and Ca channels occurs in a physically restricted manner, and that a readily diffusible second messenger is not likely to be involved.

Discussion

Our results confirm the earlier reports by Lester and Jahr (1990) and Chernevskaya et al. (1991) that stimulation of glutamate receptors can suppress current through voltage-dependent Ca channels in hippocampal neurons.

Glutamate receptor type

In particular, our results support the suggestion by Lester and Jahr (1990) that the metabotropic glutamate receptor inhibits hippocampal Ca channels. In agreement with their results, quisqualate displayed agonist properties while NMDA, AMPA, and kainate were without effect, and (unlike quisqualate-activated cation channels) the response was not blocked by CNQX. The reversibility of the inhibition in our experiments allowed us to obtain concentration–response data for the agonists. The affinities (EC_{50}, half-maximal concentration) of the four agonists for inhibition of hippocampal Ca channels [(+)-quisqualate (0.11 μM) > L-glutamate (1.6 μM) > ibotenate (7.8 μM) > *t*-ACPD (48 μM)] are remarkably similar to those recently reported by Masu et al. (1991) for the ability of a cloned metabotropic receptor to stimulate Ca-activated Cl^- current when expressed in *Xenopus* oocytes [quisqualate (0.2 μM) > ibotenate (6 μM) > L-glutamate (9 μM) > *t*-ACPD (50 μM)]. In addition to being the agonist with highest affinity, quisqualate was unusual in having a more shallow concentration–response relationship (slope factor of 0.7) when compared to the other agonists (slope factors ranging from 1.1 to 1.3); this same difference can be seen in the concentration–response curves for the cloned metabotropic receptor (Masu et al., 1991). Interpretation of the dose–response data is not straightforward; the coupling of receptor to channel is certainly indirect for activation of the *Xenopus* oocyte Ca-activated Cl channel and involves at least a G-protein in the case of hippocampal Ca channels (Fig. 12). The comparison, however, leaves little doubt that a metabotropic glutamate receptor mediates the effect of L-glutamate in our experiments.

Although 1*S*,3*R*-ACPD is the least potent of the agonists (EC_{50} = 15 μM), in many ways it is the most useful because it has the best selectivity for activating the metabotropic receptor without affecting the glutamate receptors that directly activate cation channels (Palmer et al., 1988; Manzoni et al., 1990; Miller, 1991). The ability of 1*S*,3*R*-ACPD to inhibit Ca channels in hippocampal neurons has also been reported by Sahara and Westbrook (1991), who found partial antagonism by L-AP3; we did not observe noncompetitive antagonism by L-AP3, but our results do not rule out a weak competitive antagonism.

Our results are different than those of Chernevskaya et al. (1991), who reported a slowly developing, irreversible depression of Ca current by L-glutamate that could be mimicked by NMDA and L-aspartate, but not quisqualate. Although we saw no effect of NMDA receptor agonists, we focused on rapid, reversible effects of L-glutamate and its structural analogs. We typically applied agonists for about 10 sec, which may not be long enough to see the effect reported by Chernevskaya et al. (1991). Comparing the two sets of results leaves open the possibility that there are two pathways by which glutamate can depress Ca current in hippocampal neurons, produced by different receptor types.

Ca channel type

Norepinephrine, luteinizing hormone–releasing hormone, and neuropeptide Y modulate ω-CgTx-GVIA–sensitive (N-type) but not dihydropyridine-sensitive (L-type) channels in sympathetic neurons (Plummer et al., 1991; Boland and Bean, in press). Similar results were found for norepinephrine and GABA in chick dorsal root ganglion neurons (Cox and Dunlap, 1992). In dorsal raphe neurons, 5-HT also modulates ω-CgTx-GVIA–sensitive channels (Penington et al., 1991). In CA3 pyramidal neurons, the metabotropic glutamate receptor agonist 1*S*,3*R*-ACPD had no effect on dihydropyridine-sensitive channels (see Figs. 8–10). About two-thirds of the current modulated by 1*S*,3*R*-ACPD was sensitive to ω-CgTx-GVIA (Fig. 11), while the remainder was neither ω-CgTx-GVIA sensitive nor dihydropyridine sensitive.

Coupling of receptor to Ca channels

The speed and reversibility of the L-glutamate effect we have seen are similar to those of other transmitters that have been found to inhibit Ca channels. In fact, the effects of metabotropic glutamate receptor agonists are quite similar to those of ACh, 2-Cl-adenosine, and baclofen (Toselli and Lux, 1989; Scholz and Miller, 1991a,b; K. J. Swartz and B. P. Bean, unpublished observations; Fig. 13) on hippocampal neurons. After inhibition by L-glutamate, the current sometimes showed a slow phase of activation (e.g., Fig. 2*A*) that is typical of a number of other transmitters working through G-protein–linked receptors (Forscher and Oxford, 1985; Marchetti et al., 1986; Ikeda and Schofield, 1989; Kasai and Aosaki, 1989; Carbone and Swandulla, 1991; Penington et al., 1991). In the presence of internal GTP-γ-S, the Ca channel current suppression by 1*S*,3*R*-ACPD was irreversible and could be elicited only once, further suggesting the involvement of a G-protein in the coupling process.

The best-studied action of metabotropic glutamate receptors is the stimulation of phospholipase C and the subsequent actions of inositol phospholipids on intracellular Ca stores (Sladeczek et al., 1988; Schoepp et al., 1990b; Miller, 1991). It is unlikely that release of intracellular Ca stores is the message that links receptors to Ca channels in our experiments. Ba was used as the charge carrier, and intracellular Ca levels were buffered by 9 mM EGTA. In *Xenopus* oocytes, millimolar concentrations of EGTA abolish Ca-activated chloride currents brought about by activation of metabotropic glutamate receptors and subse-

quent release of Ca from intracellular stores (Manzoni et al., 1990; Masu et al., 1991). Furthermore, the suppression of Ca channel current by metabotropic glutamate receptor agonists was initiated and terminated rapidly, and could be elicited repeatedly with no diminution in response (e.g., Fig. 7*B*).

Our studies with cell-free outside-out patches and cell-attached patches suggest that the coupling between metabotropic glutamate receptors, G-proteins, and Ca channels is physically restricted and does not involve readily diffusible second messengers. While not directly tested in the present studies, a direct action of G-proteins on Ca channels (Yatani et al., 1987; Imoto et al., 1988), would be consistent with our results.

The fast and reversible inhibition we have studied is in contrast to the slower effects of glutamate in cultured hippocampal neurons previously reported by Lester and Jahr (1990), which had a requirement for internal or external Ca. Metabotropic glutamate receptor agonists have also been found to inhibit high-threshold Ca current in cortical neurons (Sayer et al., 1992); in contrast to our results, inhibition in cortical neurons required 2–5 min to reach completion and was selective for L-type Ca channels. We would not have detected such slow effects with the brief applications (~10 sec) we used in most experiments (although we did apply 1*S*,3*R*-ACPD for several minutes in making recordings from cell-attached patches, where we also saw no inhibition of L-type channels). Taking the results from different preparations together, it is possible that two pathways exist that couple metabotropic glutamate receptors to Ca channels, one that is fast and membrane delimited and acts primarily on N-type Ca channels, and another that is slow and acts primarily on L-type Ca channels. Such a scenario would be similar to the modulation of Ca channels in rat sympathetic neurons by muscarinic agonists, where distinct fast and slow pathways exist with differing selectivities for Ca channel types (Beech et al. 1991; Bernheim et al., 1991a,b).

Physiological significance

Our results show that a metabotropic glutamate receptor can modulate ω-CgTx-GVIA–sensitive Ca channels in CA3 pyramidal neurons through a physically restricted mechanism involving G-proteins. Synaptic transmission in rat hippocampus involves activation of ω-CgTx-GVIA–sensitive Ca channels (Kamiya et al., 1988). If metabotropic receptor modulation of Ca channels is operational at the presynaptic terminal, its activation would be expected to produce an inhibition of excitatory synaptic transmission. Concentrations of *t*-ACPD like those effective in inhibiting Ca channels have been found to suppress excitatory transmission at CA3–CA1 synapses (Baskys and Malenka, 1991) and at cortical–striatal synapses (Lovinger, 1991), suggesting that modulation of Ca channels by metabotropic glutamate receptors may be physiologically important for regulating the release of L-glutamate from excitatory synapses.

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