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Facilitatory Coupling between a Glutamate Metabotropic Receptor and Dihydropyridine-Sensitive Calcium Channels in Cultured Cerebellar Granule Cells

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The effect of metabotropic glutamate receptor activation on Ca dihydropyridine (DHP)-sensitive channels recorded in the presence of 1 μM Bay K 8644 was examined on cultured cerebellar granule cells using the patch-clamp technique in the cell-attached configuration. Bath-applied agonist (*trans*-ACPD, 1*S*,3*R*-, and 1*R*,3*S*-ACPD isomers, and glutamate or quisqualate in the presence of CPP and CNQX) evoked an increase in Ca channel activity with a variable latency of 8.9 ± 8.6 sec in 40% of the recorded cells. Neither L-CCG1, L-AP3, L-AP4, nor AMPA or NMDA activated Ca channels. Two dihydropyridine-sensitive channels present in this cell type were activated by *trans*-ACPD: the classical 24 pS L-type channel and a smaller-conductance 7 pS channel. The effect was shown to be mediated by neither intracellular Ca^{2+} nor a pertussis toxin (PTX)–sensitive G protein. Interestingly treatment with BAPTA-AM increased the number of responding patches and the activity was more sustained throughout the drug application. After overnight PTX treatment, activation of the Ca channels persisted even after washout of the agonist. These results indicate that mGluR1/mGluR5 probably mediate the facilitation of dihydropyridine-sensitive Ca channels.

[Key words: Ca^{2+} current, glutamate metabotropic receptor, Ca channel facilitation, cerebellum, G protein, patch clamp]

Glutamate is a major neurotransmitter in the mammalian CNS. It activates two families of receptors, the ionotropic (AMPA, kainate, and NMDA) and the metabotropic glutamate receptors (mGluRs). Among the metabotropic receptor family, six receptor subtypes have been cloned (Houamed et al., 1991; Masu et al., 1991; Tanabe et al., 1992) and their pharmacology and coupling mechanisms characterized (Schoepp et al., 1990; Schoepp and Conn, 1993). The mGluR1 and mGluR5 subtypes are positively coupled to phospholipase C and lead to inositol triphosphate (IP_3) synthesis. When expressed in Chinese hamster ovary (CHO) cells, mGluR1 also induces arachidonic acid release and activates adenylate cyclase (Aramori and Nakanishi, 1992). mGluR2, mGluR3, mGluR4, and mGluR6 inhibit the forskolin-stimulated accumulation of intracellular cyclic adenosine 3′,5′-monophosphate (cAMP) (Nakanishi, 1992; Tanabe et al., 1992). An uncloned receptor with different pharmacology is also negatively coupled to adenylate cyclase in cultured striatal cells (Prézeau et al., 1992).

We previously observed that mGluR agonists activate a Ca^{2+}-dependent K channel of large conductance (the big K channel) in mouse cerebellar granule cells maintained in culture (Fagni et al., 1991). This result was obtained in Ca^{2+}-free medium and, therefore, corroborated previous biochemical studies showing that stimulation of mGluRs increases phosphoinositide production (Sladeczek et al., 1985; Nicoletti et al., 1986), which, in turn, releases Ca^{2+} from intracellular stores (Sugiyama et al., 1987). However, we observed that increasing the external Ca^{2+} concentration up to 5 or 10 mM enhanced the effect of the mGluR agonists (Fagni et al., 1991). This finding suggested that, in addition to the IP_3 system, mGluR stimulation might also induce, directly or indirectly, a Ca^{2+} influx.

It was therefore interesting to examine a possible mGluR modulation of voltage-dependent Ca channels as a mechanism for this Ca^{2+} influx in cerebellar granule cells. Of the various types of Ca channels that have been described in central neurons (for review, see Tsien et al., 1991; Ellinor et al., 1993), T-type (low-threshold, fast inactivating) Ca channels have been found to be absent from cerebellar granule cells (De Waard et al., 1991). In addition, since big K channels were recorded at relatively depolarized membrane potentials, and in a sustained manner, this suggests that if any voltage-dependent Ca channel was involved in the regulation of big K channels under these conditions, it could only correspond to mainly the high-threshold noninactivating Ca channels. In these neurons, two types of dihydropyridine (DHP)-sensitive channels display this property: L-type large-conductance channels (Slesinger and Lansman, 1991; Forti and Pietrobon, 1993), and a smaller-conductance 7 pS channel (Bossu et al., 1994).

We found that mGluR agonists effectively induced opening of both DHP-sensitive calcium channels. For the L-type Ca channel, we show that this effect was not blocked but enhanced following pretreatment with pertussis toxin (PTX) or BAPTA-AM, indicating that the excitatory ACPD effect was apparently mediated neither by a PTX-sensitive G protein nor by intracellular Ca^{2+}. These observations are suggestive of an additional

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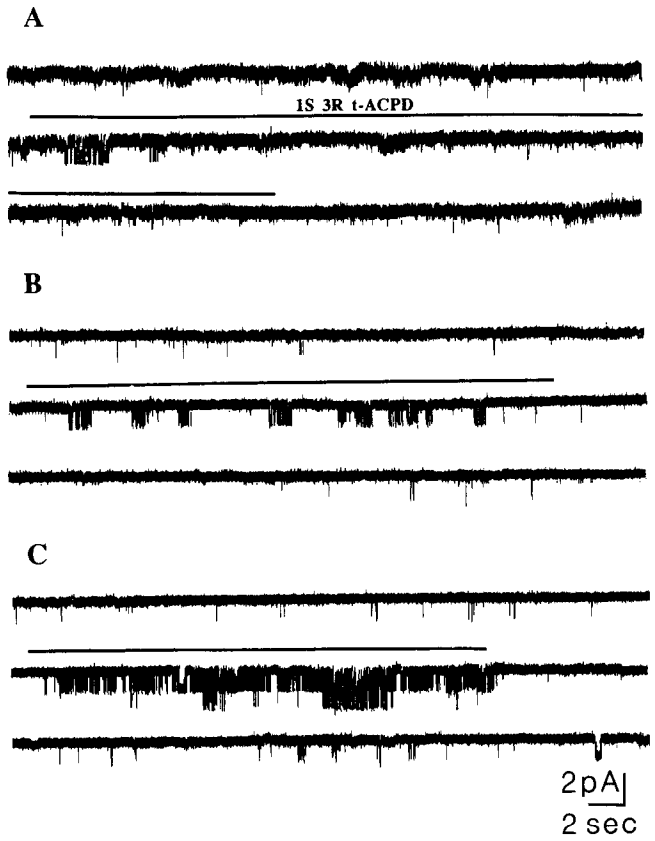

Figure 1. Effect of 100 μM 1*S*,3*R*-ACPD on Ca channel activity: cell-attached recordings obtained from three different mouse neurons bathed in physiological NaCl solution. The patch pipette potential was held at −60 mV (patch membrane potential = 0 mV, assuming cell resting membrane potential of −60 mV). *Horizontal lines* represent agonist applications. Note the different patterns of Ca channel activity: an initial burst followed by a period of quiescence in *A*, successive burst separated by periods of quiescence in *B*, sustained activity in *C*. Calibration applies to *A*–*C*.

negative feedback normally exerted by both a PTX-sensitive G protein and Ca ions on this channel (Sayer et al., 1992; Fagni et al., 1993; Sahara and Westbrook, 1993).

Some of these results have been presented in abstract form (Bossu et al., 1993).

Materials and Methods

Cell preparation. Granule cells were isolated from the cerebellum of newborn mice or rats (PN 5–7), plated on poly-L-ornithine–coated petri dishes and grown in culture for 7–10 d as described elsewhere (Fagni et al., 1991, for mouse; De Waard et al., 1991, for rat). Culture media contained a relatively high concentration of KCl (30 mM) to reduce glial cell outgrowth and improve neuronal survival.

Electrophysiology. Unitary currents were recorded using the cell-attached configuration of the patch-clamp technique; the external recording medium was free of added Ca^{2+} and either contained 0.1 mM EGTA, 140 mM NaCl, 4 mM $MgCl_2$, 5 mM KCl, 10 mM HEPES-Tris, 0.3 μM tetrodotoxin, and 10 mM glucose, pH 7.4 (mouse), or consisted of 140 mM K gluconate, 10 mM EGTA-KOH, 20 mM HEPES-KOH, 2 mM $MgCl_2$, and 10 mM glucose, pH 7.4 (rat). Patch pipettes were filled with an 110 mM $BaCl_2$/10 mM HEPES-Tris solution and (except where otherwise noted) contained 1 μM Bay K 8644 to optimize unitary Ca^{2+} current recordings.

Recordings were performed at steady-state membrane potentials since these conditions, used to record mGluR-induced big K channel activation (Fagni et al., 1991), previously indicated mGluR-enhancement of Ca influx. Patch potentials given in the text were estimated assuming cell resting potentials of −60 mV and 0 mV for cells bathed in the NaCl and K gluconate solutions, respectively.

Data analysis. Unitary current recordings were stored on videotape and analyzed off line. For analysis, current traces were filtered at 1.5 kHz with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA), and sampled at 5 kHz on a 20 MHz PC 386DW computer. The threshold for detecting opening and closing transitions was set at 50% of the open level of each event, rejecting all events that did not exceed 0.5 msec (i.e., less than two sampled points). Data were then converted to values corresponding to open times, closed times, and amplitudes. These values provided a way of calculating open probability (NP_o), amplitude and open time distributions using the PCLAMP 5.5 software of Axon Instruments (Foster City, CA). Values of NP_o were divided by the maximum number of open levels (N) observed in each patch during the drug application, thus giving the value of the open probability (P_o) for single channels. Mean amplitudes of unitary currents were calculated by fitting a Gaussian distribution, by the least squares method, to amplitude distribution histograms. Sums of decaying exponentials were fitted by least squares to each open time histogram. Data were expressed as means ± SD from n cells, and Student's t tests were used for statistical analysis.

Drug applications. Drug solutions were prepared in the external solution (pH 7.4) and applied outside the recorded patch of membrane (i.e., in the incubation bath), in the immediate vicinity of the cell, by means of a fast perfusion system described elsewhere (Fagni et al., 1991). Briefly, this consisted of a multibarrel system providing constant flow of solutions that could be switched from control to drug-containing solution in less than 30 msec. For some specific experiments, cells were incubated overnight with PTX (500 ng/ml) or for 30 min with BAPTA-AM (50 μM) previously prepared at 6 mM in DMSO.

Materials. Glutamate receptor agonists AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate), NMDA (*N*-methyl-D-aspartate), *trans*-ACPD (*trans*-1-amino-cyclopentyl-1,3-dicarboxylate), L-AP3 [L(+)-2-amino-3-phosphonopropionic acid], and L-AP4 [L(+)-2-amino-4-phosphonobutyric acid]; and antagonists CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and CPP [(±)(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid] were purchased from Tocris Neuramin (UK). BAPTA-AM [bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, tetra(acetoxymethyl)-ester] was from Calbiochem, and pertussis toxin (PTX), from List Biochemical Laboratories (USA). L-CCG1 [(2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)glycine] was a gift of Dr. H. Shinozaki (The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan) and the 1*S*,3*R*- and 1*R*,3*S*-ACPD isomers were gifts of Dr. K. Curry (University of British Columbia, Department of Physiology, Vancouver, Canada).

Results

Activation of Ca channels by mGluR agonists

When the patch pipette potential was maintained at depolarized potentials (−30 mV up to 0 mV), bath application of the selective mGluR agonist 1*S*,3*R*-ACPD (100 μM) (Fig. 1) or the racemic *trans*-ACPD evoked an increase in Ca channel activity in rodent cerebellar neurons, with no difference found between cells from rat or mouse. The agonist-induced increase in Ca channel activity was variable between patches, both in terms of onset latency and pattern of channel opening. Latency varied from less than 1 sec to 25 sec (mean 8.9 ± 8.6 sec, n = 15). Responses varied from an initial burst-like activity interrupted by periods of quiescence in the continued presence of agonist (Fig 1*A*,*B*), to a response that was sustained for the entire period of agonist application (30 sec to 2 min; Fig. 1*C*).

An excitatory effect occurred in the presence of 1 μM Bay K 8644. As illustrated, *trans*-ACPD added to the bath induced an increase Ca channel activity, regardless of whether the patches were recorded in conditions where the cell was hyperpolarized (NaCl solution; Fig. 1) or depolarized (isotonic K gluconate solution; e.g., Fig. 2*A*).

Application of *trans*-ACPD (100 μM) or its isomers (100–500 μM) was effective in eliciting an increase in Ca channel activity,

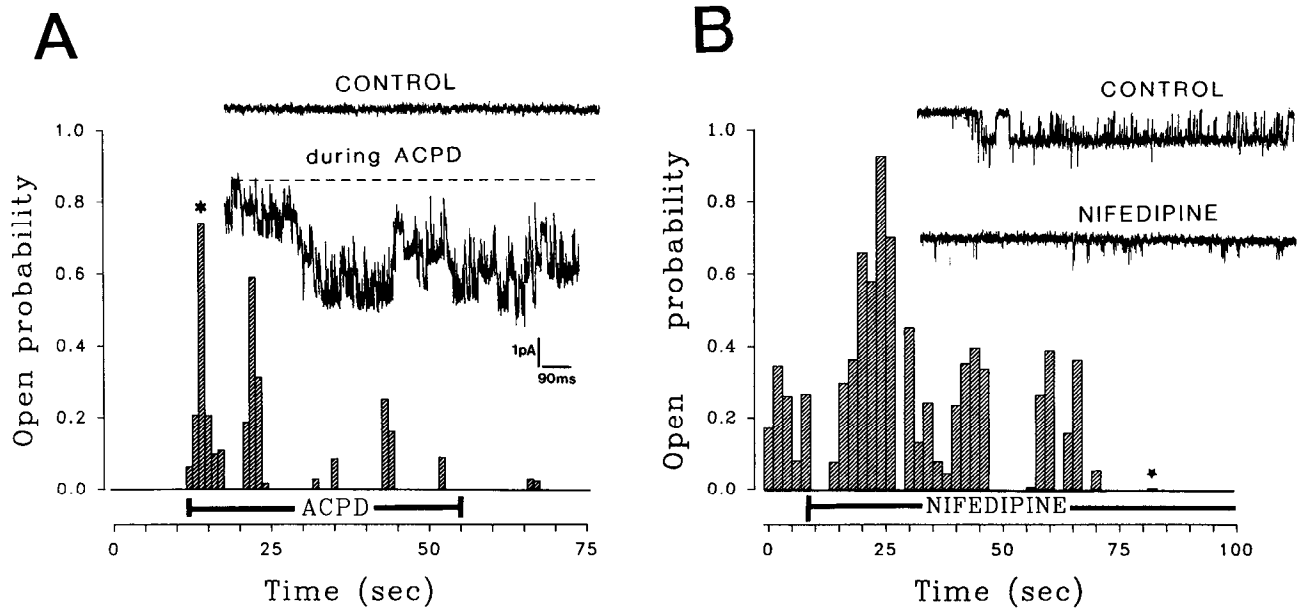

Figure 2. Activation of L-type channel by *trans*-ACPD: patch yielding unitary Ca channel activity. *A* and *B* show open probability histograms as a function of recording time for unitary Ca channel activity in control and during drug application. *A*, Ca channel activity is induced by 100 μM *trans*-ACPD (applied during *bar*) at a −30 mV holding potential at which no Ca channel activity was recorded in control conditions (amplitude of single events = −1.45 pA; note the presence of three channels on the patch). *B*, On the same patch, the same Ca channel is now activated by depolarizing the membrane potential to −20 mV, and 1 μM nifedipine (applied during *bar*) blocks this activity (mean amplitude of elementary events = −1.10 pA). *Insets* illustrate traces obtained in control conditions (*upper traces*) and in the presence of the drug (*lower traces*), taken at the times indicated by the *asterisks*. Cell attached conditions on a rat granule cell bathed in isotonic K gluconate solution.

increasing open probability P_o from 0.015 ± 0.02 to 0.52 ± 0.2 (n = 3) for *trans*-ACPD and from 0.03 ± 0.03 (n = 8) to 0.62 ± 0.24 (n = 5) with 1*R*,3*S*-ACPD, and to 0.64 ± 0.06 (n = 5) with 1*S*,3*R*-ACPD, respectively (Fig. 3*A*,*B*) within the initial drug-induced burst of activity. If the P_o in the presence of *trans*-ACPD is calculated over the entire duration of drug application, it is reduced because of the silent periods to 0.062 ± 0.03 (n = 4). The nonselective mGluR agonists glutamate (1 mM) and quisqualate (50 μM), in the presence of 10 μM CPP and/or 50 μM CNQX to block ionotropic glutamate receptors, also increased the Ca channel activity, increasing open probability from 0.03 ± 0.06 (n = 8) to 0.75 ± 0.14 (n = 8) and 0.58 ± 0.23 (n = 5), respectively, again during the initial bursts. Examples of such effects are shown in Figure 4. The tested mGluR agonists (both enantiomers and racemic *trans*-ACPD, glutamate, and quisqualate) were effective in increasing Ca channel activity in 13 of 31 patches (42%) that displayed Ca channel openings before agonist application. Neither L-AP3 (1 μM, n = 5), L-AP4 (1 μM, n = 10), L-CCG1 (1 μM, n = 10), nor AMPA (50 μM, n = 5) or NMDA (100 μM, n = 5) in the presence of the ionotropic receptor antagonists activated Ca channels (data not shown).

Note that in all cases where basal activity was observed, application of a glutamate metabotropic agonist did not modify the amplitude of the activity. This suggests that the metabotropic agonist had no effect on the membrane potential.

Attempts to characterize the ACPD response on macroscopic Ba currents in whole-cell recording conditions failed in most cases, although *trans*-ACPD was found to increase the whole-cell I_{Ba} in a small number of cells (data not shown). This suggests that the intracellular messenger involved in this response is readily diffusible and lost during dialysis of the cell by the recording pipette solution.

Characterization of the Ca channel positively coupled to mGluR

The channel activated by *trans*-ACPD was characterized both by its pharmacology and its conductance. On the cell illustrated

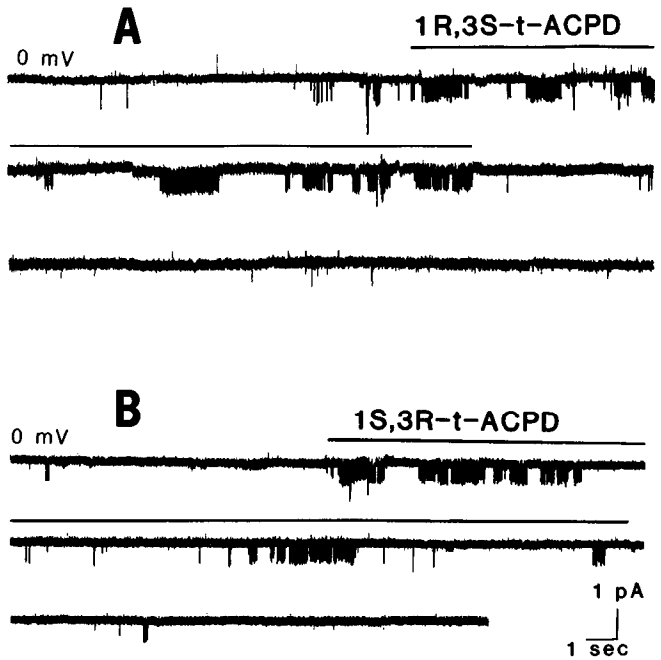

Figure 3. Effects of 500 μM ACPD isomers on Ca channel activity. *Horizontal lines* represent the indicated agonist applications. *A* and *B* were obtained from two different cell-attached patches at 0 mV membrane potential, from mouse granule cells bathed in physiological NaCl solution.

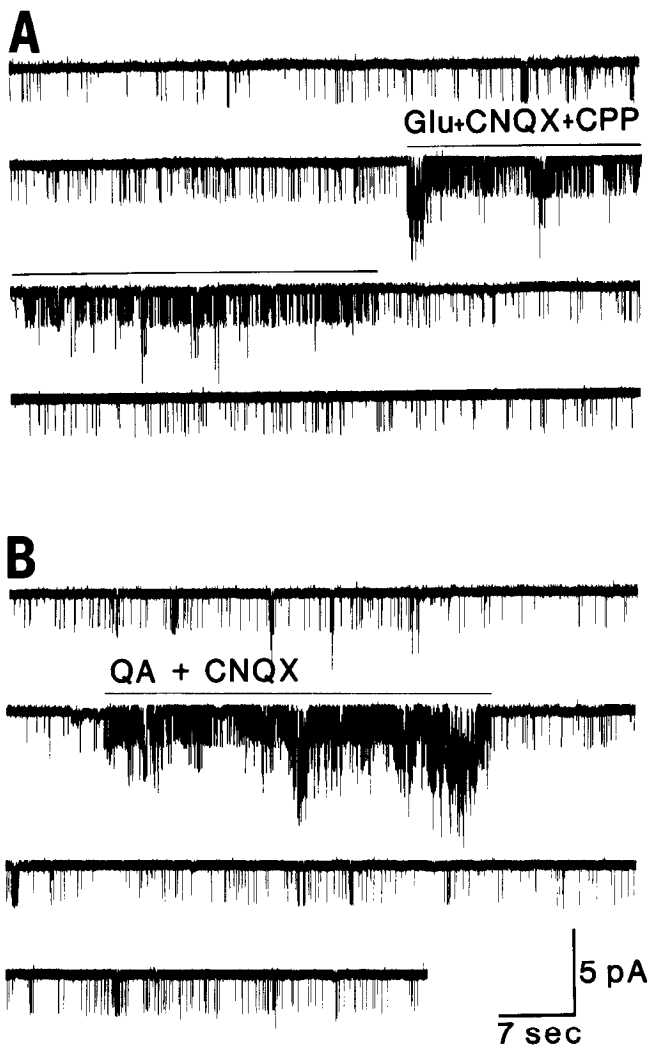

Figure 4. Effects of nonselective glutamate agonists on unitary Ca channel activity at −30 mV. Metabotropic activity was selected in *A* by coapplication of 50 μM CNQX and 10 μM CPP with 1 mM glutamate and in *B* by 50 μM CNQX with 50 μM quisqualate. Recordings were obtained from the same cell-attached patch, which also responded to 500 μM 1*S*,3*R*-ACPD, from mouse granule cells bathed in physiological NaCl solution.

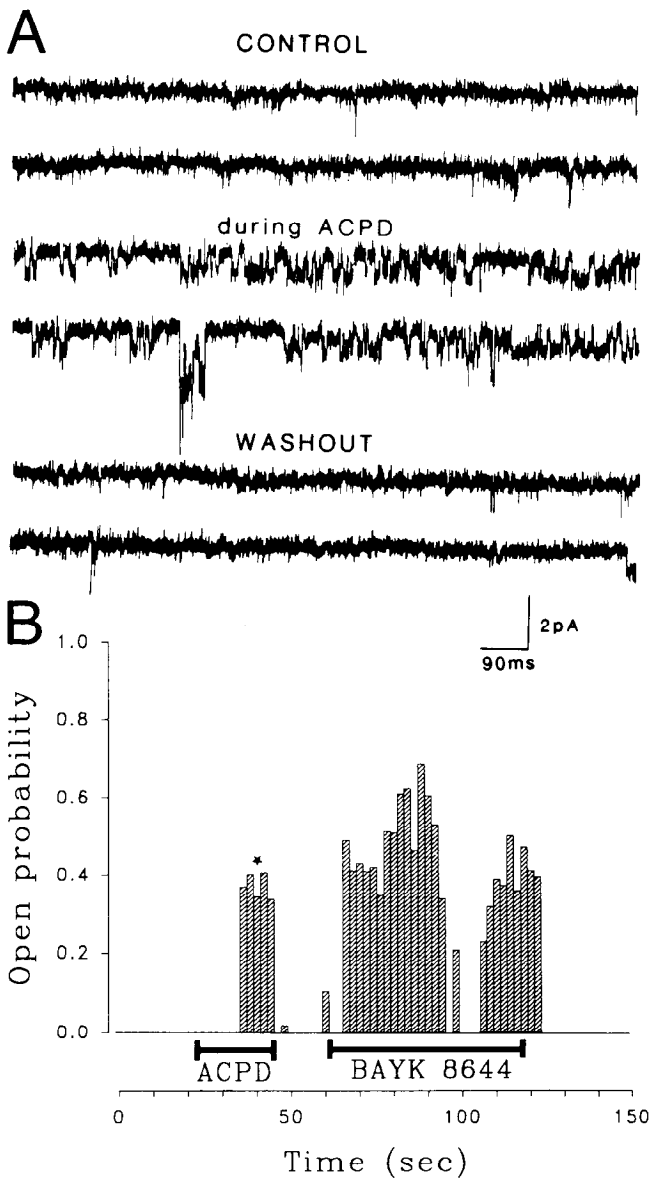

Figure 5. Effects on *trans*-ACPD on the 7 pS DHP-sensitive Ca channel activity. *A*, Unitary currents (0.7 pA mean amplitude) recorded from a cell-attached patch at a steady-state patch potential of −10 mV, during application of 100 μM *trans*-ACPD. Note that this channel activity corresponds to a 7 pS DHP-sensitive Ca channel. Recordings were obtained from a rat granule cell bathed in isotonic K gluconate solution and without Bay K 8644 in the pipette solution. *B*, Open probability histogram as a function of recording time for the same patch as in *A*. *Asterisk* points to the recording period illustrated in *A*. Note also the pronounced increase in P_o during application of 1 μM Bay K 8644 to the cell body.

in Figure 2, when the membrane potential was maintained just below the threshold for activation of the channel (−30 mV in this case), *trans*-ACPD evoked bursting activity at the beginning of drug application (see induced changes in P_o on lower graph in Fig. 2*A*). Activity of such a channel could be increased by further depolarization to −15 mV (Fig. 2*B*), and abolished by an external application of the DHP antagonist, nifedipine (1 μM). The conductance of this channel, estimated between −35 and −10 mV, was equal to 24 pS (data not shown). The mean chord conductance of the Ca channel activated by the various mGluR agonists was 25 ± 3 pS (n = 11), in the absence and presence of mGluR agonists. Finally, this channel displayed two open times characterized by the following time constants τ_1 = 0.77 ± 0.25 msec and τ_2 = 4.62 ± 0.90 msec with a weight ratio W_1/W_2 of 1.59 ± 0.57 (n = 8). These kinetic properties were similar to those obtained in the absence of drug (τ_1 = 0.80 ± 0.06 msec, τ_2 = 4.88 ± 0.85 msec, $_w1/W_2$ = 2.15 ± 1.48, n = 7) (data not shown). These properties are characteristic of the classical L-type Ca channels identified in cerebellar granule cells (Feltz et al., 1990; Slesinger and Lansman, 1990, 1991; Forti and Pietrobon, 1993).

In addition to this classical L-type Ca channel activity, cerebellar granule cells possess a 7 pS Ca channel that is also sensitive to DHPs (Feltz et al., 1990; Bossu et al., 1994). In four patches where this activity was recorded in isolation, we observed that it was also increased during *trans*-ACPD applications (Fig. 5). This effect was not characterized further.

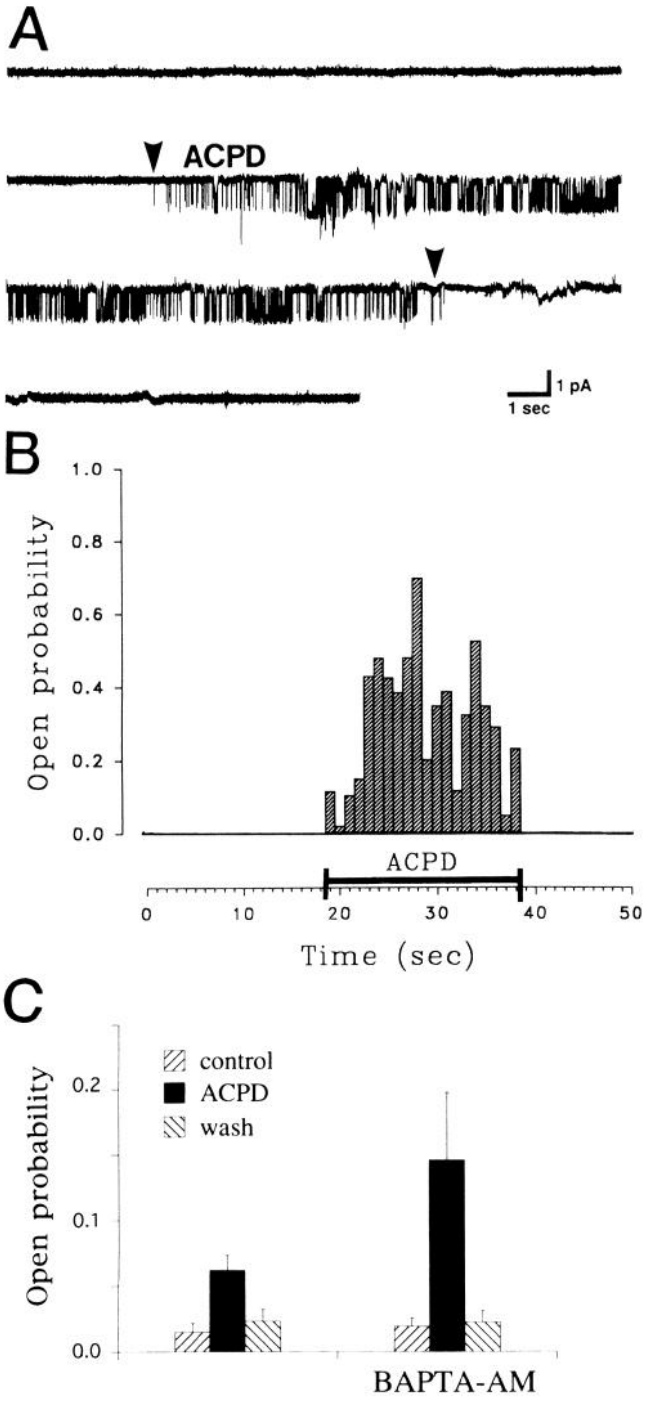

Figure 6. Effect of BAPTA-AM on the activation of Ca channel by mGluR: continuous cell-attached recording obtained from a rat cerebellar neuron previously loaded with BAPTA-AM. *A,* Successive traces of the activity recorded in control conditions and during an application of 100 μM *trans*-ACPD (*between arrowheads*), recorded with the cell bathed with isotonic potassium gluconate. The patch membrane potential was −5 mV. *B,* Corresponding open probability histogram. Note that, in this condition, the activity is maintained through the ACPD application. *C,* Comparison of the ACPD effect without and with a BAPTA-AM preincubation. Open probability was measured on eight cells in each case that displayed a similar activity before ACPD application. Height of each column shows mean value in each case, and error bars, the corresponding SE.

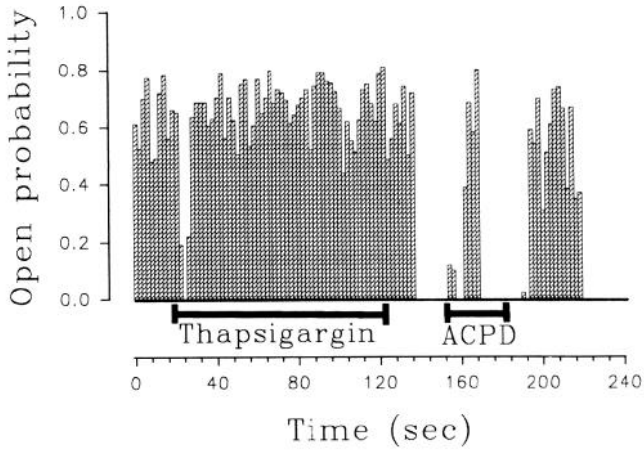

Figure 7. Effect of *trans*-ACPD on Ca channel open probability following thapsigargin treatment: patch containing a single L-type channel activity, recorded from a rat cell pretreated with BAPTA-AM (50 μM for 30 min) and then bathed in isotonic K gluconate solution. In this condition, the open probability histogram of a −1.28 pA elementary event induced at 0 mV as a function of time shows a delayed effect of thapsigargin (1.5 μM applied during *bar*) since blockade of the Ca channel activity only occurred after its washout, and this effect was reversible. Note that *trans*-ACPD (100 μM) is still efficacious though stores have been depleted.

Effect of internal Ca^{2+}

Since stimulation of mGluRs coupled to phosphoinositides increases $[Ca^{2+}]_i$ (Sladeczek et al., 1988, for a review) and because intracellular Ca^{2+} has been shown to control Ca^{2+} currents in GH3 cells (Kalman et al., 1988), cultured sensory neurons (Bossu et al., 1989) and ventricular myocytes (Yue et al., 1990), we examined whether the action of *trans*-ACPD on the studied Ca channel was affected by internal Ca^{2+}. Cells were pretreated with the membrane-permeant Ca^{2+} chelator BAPTA-AM to buffer $[Ca^{2+}]_i$. After the BAPTA-AM treatment, *trans*-ACPD (100 μM) was found to activate the Ca channel increasing open probability from 0.023 ± 0.016 in control to 0.15 ± 0.13 (n = 8) during the entire ACPD application. Moreover, the number of patches responding was found to increase to 80% versus 40% in the absence of BAPTA (12 of 15 cells tested from two separate cultures of rat cells) and the enhanced activity was more sustained during the *trans*-ACPD application (Fig. 6*A,B*). The latter was reflected by the higher increased open probability when measured over the entire ACPD application period: 0.15 ± 0.13 (n = 8) compared to 0.062 ± 0.03 (n = 8) without BAPTA pretreatment (Fig. 6*C*). This enhancement of activity observed in the presence of BAPTA suggests that Ca^{2+} released from IP_3-sensitive stores is not the messenger involved in the activation of the L-type channels. On the contrary, this suggests that internal calcium exerts an inhibitory control on the effect of *trans*-ACPD on this channel.

The next question addressed was whether or not the level of calcium in the IP_3-sensitive stores exerts a control on the influx of Ca^{2+} through L-type channels. To test this idea, stores were first depleted by external application of thapsigargin, and then *trans*-ACPD was applied. Following application of 1.5 μM thapsigargin, an irreversible blockade of the L-type channel activity was observed as expected from an increased internal Ca^{2+} concentration. Under this condition, *trans*-ACPD was not able to induce Ca channel activity (n = 6 not illustrated). To prevent an inhibitory thapsigargin effect mediated by Ca ions, cells were

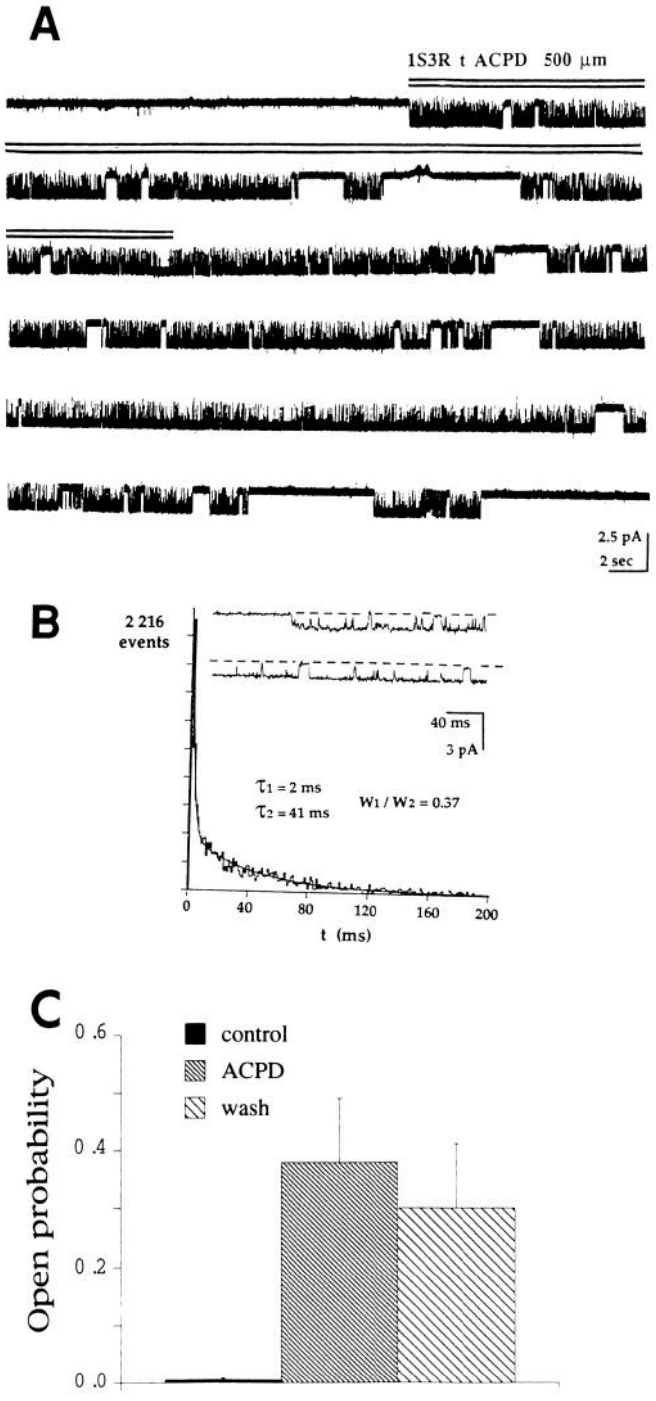

Figure 8. Effects of PTX on the action of *trans*-ACPD. *A* and Ca channel activity at a steady-state membrane potential of 0 mV and recorded from a cell pretreated with 500 ng/ml PTX. *B*, Characteristics of the open times of the channel activity shown in *A*. The *dashed line* represents the closed state of the channel. The open time histogram fitted the following double exponential: $y = 670 \exp(-t/2) + 1863 \exp(-t/41)$. Recordings were obtained from a mouse granule cell bathed in physiological NaCl solution. *C* shows the open probability after PTX treatment estimated in eight cells (where the holding potential was set at a just subthreshold value to elicit a Ca channel activity in control conditions) during ACPD application and 1 min after wash. Note by comparison with Figure 5 that the ACPD effect is now prolonged. Height of each column shows mean value, and error bars, SD.

pretreated by BAPTA-AM. In this case ($n = 10$), the effect of thapsigargin on the Ca channels was less potent and delayed, and now reversible (Fig. 7). In these conditions, *trans*-ACPD was still able to increase Ca channel activity to an extent similar to that observed without thapsigargin pretreatment. This suggests that the ACPD response is not controlled by the Ca^{2+} level in the stores.

Effect of pertussis toxin on the Ca channel

Finally, an attempt was made to characterize the G protein–mediating this response. The effects of preincubating the cells with PTX (500 ng/ml) overnight, were studied on the action of both *trans*-ACPD enantiomers. A similar PTX-treatment has previously been shown to result in ADP-ribosylation of G_i and G_o in neurons (Prézeau et al., 1992). This treatment did not block the action of either 1*S*,3*R*-ACPD or 1*R*,3*S*-ACPD (Fig. 8). The PTX treatment even enhanced the effects of ACPD in two ways: activation of the Ca channels persisted during washout of the agonist (Fig. 8*A*,*C*) and the mean open time significantly increased ($\tau_1 = 3.72 \pm 1.9$ msec, $\tau_2 = 23 \pm 11$ msec, $W_1/W_2 = 10.07 \pm 0.8$, n = 5 (Fig. 8*B*; compared to $\tau_1 = 0.77 \pm 0.25$ msec and $\tau_2 = 1.62 \pm 0.90$ msec, W_1/W_2 of 1.59 ± 0.57, $n = 8$, in control conditions). The PTX pretreatment also increased to 73% (16 of 22 patches) the proportion of patches responding to ACPD (instead of 42%). This indicates that PTX-sensitive G proteins mediate an inhibitory control in this system.

Discussion

Our results show that glutamate receptors are positively coupled to Ca channels in cerebellar granule cells maintained in culture. In this cell type, various Ca channels have been shown to be present. We show that this positive coupling exists between glutamatergic receptors and a complex family of DHP-sensitive channels. The pharmacology of the effect indicates that solely mGluRs are involved.

Mediation of glutamate-induced activation of Ca channels by a metabotropic receptor

Glutamate is known to exert a positive control on voltage-dependent Ca^{2+} entry, mainly through the depolarizing effects mediated by ionotropic receptors. We describe here a glutamate-enhanced Ca channel activity that was observed when the membrane potential of the cells was zeroed by bathing the cells in isotonic K^+. Further, when recordings were made when the cell membrane potential was polarized by bathing the cells in NaCl, the amplitude of the activated channel was identical before and during agonist application, thus excluding the possibility that this activation resulted from depolarization of the cell. The hypothesis of a direct action on Ca channels is ruled out by the fact that, in the cell-attached recording configuration, the recorded channel is isolated from the bath by the tight seal between the membrane and the patch pipette. Taken together, these observations imply that a diffusible second messenger was involved in Ca channel activation by a metabotropic receptor.

Our results suggest that mGluR1 and/or mGluR5 are the receptor(s) transducing the response. This hypothesis is first supported by our pharmacological observations. Quisqualic acid is the most active agonist on mGluRs coupled to phospholipase C and almost inactive on mGluRs negatively coupled to adenylate cyclase (Manzoni et al., 1990; Nakanishi, 1992). In our experiments, quisqualate activated Ca channels. By contrast,

neither L-CCG1 nor L-AP4 mimicked this facilitatory effect. Also, both 1*S*,3*R*- and 1*R*,3*S*-ACPD equally activated these channels, at concentrations that have been shown to stimulate inositol phosphate formation and increase $[Ca^{2+}]_i$ (Manzoni et al., 1993). Third, the involvement of mGluRs inhibiting cAMP production (i.e., mGluR2, mGluR3, and mGluR4; Nakanishi, 1992) is unlikely since cAMP has been shown to upregulate Ca channels in neurons (Gray and Johnston, 1987; Chetkovich et al., 1991) and because of the PTX insensitivity of the presently observed ACPD effects.

Activation of DHP-sensitive Ca channels

In cerebellar granule cells, various Ca channels have been identified (Feltz et al., 1990; Slesinger and Lansman, 1990). The family of dihydropyridine-sensitive channels, not inactivated by a steady-state depolarization, is complex; it includes, in addition to a series of L-type (24 pS) channels with distinct gating properties (Slesinger and Lansman, 1991; Forti and Pietrobon, 1993), a small 7 pS channel (Bossu et al., 1994). We show that glutamatergic metabotropic receptors are positively coupled to both the small and the large-conductance Ca channels (see also Zegarra-Moran and Moran, 1993, for such a facilitatory effect of glutamate on the Ca^{2+} L-type channel in the same cells). An increase in Ca^{2+} current following stimulation of mGluRs has also been recently observed in chick dorsal root ganglion and rat hippocampal neurons (Mironov and Lux, 1992). Due to the steady-state depolarization protocol that was employed in the present experiments, similar effects on the other types of Ca channels present in these neurons would have escaped our investigations. Our experiments show that mGluRs can be positively coupled to L-type channels, in addition to the negative coupling previously described (Sayer et al., 1992; Sahara and Westbrook, 1993).

Heterogeneity of metabotropic responses

Two questions then arise: first, are the two types of control present on cerebellar granule cells, and second, is the activation of different receptors and/or distinct receptor–effector couplings involved? We show here that metabotropic glutamate receptors exert a facilitatory effect on Ca channels, probably mediated by mGluR1/mGluR5.

The PTX insensitivity implies that none of the G_i/G_o proteins are involved in the mGluR-enhanced Ca channel activity. A negative coupling between mGluRs and L-type Ca channels via a G_o-like protein is not excluded by the present study, however, and might account for the prolonged *trans*-ACPD stimulatory effects following PTX treatment. Such a PTX-sensitive inhibitory effect is involved when L-type Ca channels of cerebellar granule cells are *transiently* activated by step depolarizations (Fagni et al., 1993). In contrast to the facilitation effect described here, it is mimicked by L-CCG1 and is PTX sensitive (Chavis et al., in press). Also, inhibition of Ca^{2+} current following stimulation of mGluRs has been observed in hippocampal (Lester and Jahr, 1990; Swartz and Bean, 1992) and neocortical neurons (Sayer et al., 1992). The latter effect is likely coupled by a G_o-like protein. It has been clearly established that G_o, a pertussis toxin–sensitive G protein, negatively controls L-type Ca channels in neuroblastoma glioma hybrid cells (Hescheler et al., 1987) and anterior pituitary cells (Lledo et al., 1992), and may do so in adrenal chromaffin cells (Kleppisch et al., 1992; see Schultz et al., 1990, for a review). Brabet et al. (1988) have shown that G_o is expressed in cerebellar granule cells. The numerous mGluR subtypes already described make it probable that more than one G protein is involved in the regulation of the L-type Ca channels. This has already been shown to be the case for muscarinic receptors acting on N-type Ca channels (Beech et al., 1992). The present study and the work of Chavis et al. (in press) is the first report of two distinct effects resulting from the activation of glutamate metabotropic receptors on a single cell type.

Messengers involved in the metabotropic response

Both mGluR1 and mGluR5 are positively coupled to phospholipase C (Nakanishi, 1992) and are expressed in cerebellar granule cells (Abe et al., 1992; Bessho et al., 1993). Since whole-cell Ca^{2+} currents in cerebellar granule cells can be upregulated by the IP_3 pathway (De Waard et al., 1992), one must consider here the possibility that either IP_3 or an IP_3-induced release of intracellular Ca^{2+} may be involved in the presently observed response. Buffering intracellular Ca^{2+} with BAPTA did not block the coupling between mGluRs and Ca channels. Therefore, the action of mGluR agonists was not directly mediated by internal Ca^{2+}, suggesting a direct action of IP_3 on Ca channels. However, the involvement of another second messenger cannot be excluded.

Given the previously reported sensitivity of L-type Ca channels to intracellular Ca^{2+} (Kalman et al., 1988; Bossu et al., 1989; Yue et al., 1990) and the rise in $[Ca^{2+}]_i$ during mGluR agonist application (Fagni et al., 1991), one would expect Ca^{2+} released by mGluR1/mGluR5 stimulation to inhibit, rather than stimulate, these channels. Indeed, the potentiation of *trans*-ACPD effects following BAPTA-AM pretreatment could be explained by the removal of such an intracellular Ca^{2+}-mediated inhibition of Ca channels.

In a number of cell types, enhanced influx of Ca^{2+} is triggered or modulated by the emptying of intracellular stores (for a short review, see Clapham, 1993). Since *trans*-ACPD is known to release Ca^{2+} from internal stores, it is possible that the observed enhancement of Ca channel activity depends on the efflux of Ca^{2+} from, or the Ca^{2+} content of, these stores. Assuming that the thapsigargin treatment completely emptied the internal IP_3-sensitive stores, one would expect that thapsigargin should enhance the *trans*-ACPD-induced channel activity if the latter depends on the Ca^{2+} content of the stores. Since the thapsigargin treatment never directly provoked such an effect even following BAPTA-AM treatment, Ca^{2+} efflux per se from the store or the filling state of the stores is unlikely to be the signal responsible for stimulating the enhanced Ca channel activity.

Even though the second messenger responsible for Ca channel activation has not been identified, it seems to be produced in the vicinity of its target. This is suggested by the observation of delays of less than 1 sec between the time when drug application was started and the beginning of the response. Similar short delays have been observed for the coupling of muscarinic and glutamatergic metabotropic receptors and Ca channels (Beech et al., 1992; Bernheim et al., 1992; Sahara and Westbrook, 1993). The present facilitatory effect could rarely be observed in whole-cell recording conditions. The latter observation implies that the second messenger is highly diffusible. However, we cannot exclude the possibility that whole-cell recording conditions could have modified the characteristics of the L-type Ca current itself.

The present article describes a positive coupling between glutamatergic receptors and Ca channels. It therefore corroborates

previous observations suggesting that an influx of Ca^{2+} participates in the mGluR-induced increase in $[Ca^{2+}]_i$. Thus, increased external Ca^{2+} (Fagni et al., 1991; Abe et al., 1992) or depolarization with high external K^+ concentrations enhanced mGluR-induced increases in $[Ca^{2+}]_i$, while reduced $[Ca^{2+}]_o$ decreased mGluR-induced increases in $[Ca^{2+}]_i$ (Guiramand et al., 1991). This indicates that the Ca and K channel proteins and also the metabotropic receptor are closely associated in the membrane. A functional triplet, constituting an mGluR in the vicinity of depolarizing Ca channels and hyperpolarizing Ca^{2+}-gated K channels, could play an important role in the control of cell firing as suggested by the following observations. Membrane potential oscillations in sympathetic preganglionic neurons (Spanswick et al., 1992) and burst firing in dorsolateral septal nucleus neurons follow mGluR activation (Zheng and Gallagher, 1992). The latter response was inhibited by Ca channel blockers, suggesting that an influx of external Ca^{2+} was required for this mGluR-mediated activity. In synaptic regions, metabotropic mGluR have been shown to participate in processes of synaptic plasticity such as long-term potentiation (McGuinness et al., 1991; Bortolotto and Collingridge, 1992) and long-term depression (Ito and Karachot, 1990). Long-term changes in synaptic efficacy involve Ca^{2+}-dependent processes and, therefore, might also depend upon the presently observed coupling between mGluR and Ca channels.

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