G protein-coupled receptors mobilize neuronal signaling cascades which until now have not been shown to depend on the state of membrane depolarization. Thus we have previously shown that the metabotropic glutamate receptor type 7 (mGlu7 receptor) blocks P/Q-type Ca$^{2+}$ channels via activation of a G$_i$ protein and PKC, in cerebellar granule cells. We show here that the transient depolarizations used to evoke the studied Ca$^{2+}$ current were indeed permissive to activate this pathway by a mGlu7 receptor agonist. Indeed, sustained depolarization to 0 mV was sufficient to inhibit P/Q-type Ca$^{2+}$ channels. This effect involved a conformational change in voltage-gated sodium channel independently of Na$^+$ flux, activation of a pertussis toxin-sensitive G-protein, inositol trisphosphate formation, intracellular Ca$^{2+}$ release, and PKC activity. Subliminal sustained membrane depolarization became effective in inducing inositol trisphosphate formation, release of intracellular Ca$^{2+}$ and in blocking Ca$^{2+}$ channels, when applied concomitantly with the mGlu7a receptor agonist, d,L-amino-phosphonobutyrate. This synergistic effect of membrane depolarization and mGlu7 receptor activation provides a mechanism by which neuronal excitation could control action of the mGlu7 receptor in neurons.

The excitatory neurotransmitter, glutamate, mediates its effects by activating ionotropic and metabotropic (mGlu) receptors. Eight genes encoding mGlu receptors have been identified and classified into three groups. The group I (mGlu1 and mGlu5 receptors) activates PLC through a G$_i$ protein, whereas group II (mGlu2 and mGlu3 receptors) and group III (mGlu4, mGlu6, mGlu7, and mGlu8 receptors) are coupled to G$_i$/G$_o$ proteins (1). These receptors are widely distributed throughout the mammalian brain (2–5), but only the mGlu7 receptor subtype is almost exclusively localized at presynaptic sites (6–8).

Group II and III mGlu receptors, and particularly the mGlu7 receptor subtype, inhibit synaptic transmission (1, 9). Thus in vitro studies have shown that stimulation of mGlu7 receptors decreases release of glutamate in cerebellar cultures (10) and GABA in striatal cultures (11), promoting neuroprotection and excitotoxicity, respectively. Moreover, we have recently shown that mGlu7 receptors selectively block P/Q-type Ca$^{2+}$ channels in neurons (12), and these channels control transmitter release (13). Together these studies suggest that mGlu7 receptors play an important role in the modulation of synaptic transmission.

Recent studies have pointed out that in the rat locus coeruleus, group III mGlu receptor agonists down-regulate high but not low frequency synaptic activity (14), suggesting that this receptor action depends on the state of neuronal depolarization. Moreover, a voltage-dependent activation of a G$_i$ protein has recently been shown in rat brain synaptoneurosomes (15). In light of these results, and because the mGlu7 receptor is coupled to a G$_i$ protein, this receptor is a potential candidate for mobilization of both voltage- and G$_i$ protein-dependent events in neurons. We therefore investigated, in cultured cerebellar granule cells, the effect of membrane depolarization on this receptor signaling. We have previously shown that the mGlu7 receptor-induced blockade of P/Q-type Ca$^{2+}$ channels is independent of a direct action of G$_i$ protein $\beta\gamma$ subunits on the Ca$^{2+}$ channels, but results from IP$_3$ formation and PKC activation (12). Here we show a synergistic effect of the receptor activation and membrane depolarization.

EXPERIMENTAL PROCEDURES
Neuronal Culture Preparation and Transfection—Primary cultures of mouse cerebellar granule cells were prepared as previously described (16). Since these cultured neurons do not express functional native group III mGlu receptors in the soma (12), they were transfected with an expression plasmid containing the mGlu7a receptor protein, using a method described elsewhere (17). As only 10–20% neurons were transfected using this method, the mGlu7a receptor was co-transfected with the transfection marker, green fluorescent protein (GFP), for single cell electrophysiological recording and intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) measurement.

Electrophysiological Recordings—Barium currents were recorded using the whole cell configuration of the patch clamp technique, at room temperature, from mGlu7a/GFP-expressing cerebellar granule cells, after 9 ± 1 days in vitro. The bathing medium contained (mM): BaCl$_2$ (20), HEPES (10), tetrathylammonium acetate (10), glucose (15), and Na acetate (120), adjusted to pH 7.4 with Na-OH and 330 mM with Na acetate. Drug solutions were prepared in this medium and pH of the solutions was readjusted to 7.4 with NaOH. The NMDA receptor-channel blocker, MK-801 (1 $\mu$M), was added to all the solutions in order to avoid activation of this receptor by the $\eta$-isofrom of d$_{1}$-AP$_4$.2 We also added TTX (0.3 $\mu$M), 6-Cyano-7-nitroquinoline-2,3-dione (100 $\mu$M), and 7-(hydroxyimino)cycloprop[a]chromen-1a-carboxylate ethyl ester (CPCCOEt 250 $\mu$M), in order to block Na$^+$ flux through VGSCs and release of glutamate, as well as activation of ionotropic glutamate receptors and endogenous mGlu receptors. Indeed, cultured cerebellar granule neurons do not express functional native mGlu5 receptors (17–19). Patch pipettes had resistances of 3–5 M$\Omega$ when filled with the following internal solution (mM): Cs-acetate (100), CsCl (20), MgCl$_2$ (2), HEPES (10), glucose (15), EGTA (20 mM), Na$_2$ATP (2 mM), and cAMP (1 mM), adjusted to pH 7.2 with CsOH and 300 mM with Cs-acetate. In some experiments, intracellular EGTA was replaced by BAPTA. Re-

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1 The abbreviations used are: IP$_3$, inositol trisphosphate; GFP, green fluorescent protein; [Ca$^{2+}$], intracellular [Ca$^{2+}$]; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N,N'-tetraacetic acid; TTX, tetrodotoxin; PTX, pertussis toxin; VGSC, voltage-gated sodium channel.

2 L. Fagni and M. Lafon-Cazal, unpublished observation.
cordings started at least 5 min after breaking the membrane patch and stabilization of evoked Ba$^{2+}$ current (IBa).

Barium currents were evoked using voltage-clamp pulses of 500 ms duration, from a holding potential of −80 mV to a test potential of 0 mV, at a rate of 0.1 Hz, except when specified in the text. Membrane resistance was measured by applying hyperpolarizing pulses (−70 mV) amplitude and 55 ms duration, from a holding potential of −80 mV. Current signals were recorded using an Axopatch 200 amplifier (Axon Instruments, Foster City, CA), filtered at 1 kHz with an 8-pole Bessel filter and sampled at 3 kHz on a Pentium II PC computer. Analyses were performed using the pClamp6 program of Axon Instruments. Barium currents were measured at their peak amplitude and expressed as mean ± S.E. of the indicated number (n) of experiments.

Inositol Phosphate (IP) Synthesis Measurements—The procedure to measure IP accumulation in neurons was adapted from a one previously described (20). Briefly, 1-week-old cerebellar cultures were incubated for 14 h in culture medium containing 2 $\mu$Ci/ml myo-[3H]inositol (23.4 Ci/mmol, Invitrogen, Paris, France). Cells were then washed 3 times and incubated for 1 h at 37 °C in 1 ml of HEPES saline buffer (146 mM NaCl, 4.2 mM KCl, 0.5 mM MgCl$_2$, 0.1% glucose, 20 mM HEPES, 0.3 μM TTX, 10 μM MK801, 100 μM 6,7-Dinitroquinoxaline-2,3-dione, and 250 μM CPPCOCeT, pH 7.4) supplemented with 1 unit/ml glutamate pyruvate transaminase (Roche Molecular Biochemicals, Meylan, France) and 2 mM pyruvate (Sigma, St. Quentin-Fallavier, France). In experiments where [KCl] was elevated to 30 or 100 mM, the same respective amounts of NaCl were removed from the saline buffer solution. This induced the membrane steady-state depolarizations to −40 and −10 mV, respectively, at 20 °C, assuming an intracellular K$^{+}$ concentration of 140 mM. Cells were then washed again with the same saline buffer, and LiCl was added to a final concentration of 10 mM. The agonist was applied 15 min later and left for 5 min. The reaction was stopped by replacing the incubation medium with 0.5 ml of perchloric acid (5%) on ice. Supernatants were recovered and IP purified on Dowex columns. Total radioactivity remaining in the membrane fraction was counted after treatment with 10% Triton X-100, 0.1 N NaOH for 30 min and used as a standard. Results were expressed as the ratio of [H]IP formation over radioactivity present in the membranes. Experiments were performed in triplicates for statistical analysis. Similar results were obtained in mGlur7 receptor-transfected and nontransfected cultures. Note that only data obtained from transfected cultures are presented here. Given that only 10−20% neurons were transfected with our method, this indicated that majority of D,L-AP4-transfected cultures resulted from activation of the native mGlur7 receptors (12).

Voltage-dependent action of D,L-AP4 on IBa. Barium currents were evoked by 500-ms depolarization pulses applied at a frequency of 0.1 Hz. The graph represents the current amplitude relative to the current average amplitude measured before period 1. During period 1, membrane potential was held for 1 min at −80 mV, in the absence of depolarization pulse. Traces are representative IBa recorded at different times of the experiment indicated by the arrows. Note that D,L-AP4 inhibited IBa when applied concomitantly with (period 2), but in the absence of (period 1) depolarization pulses. Similar results were obtained in 5 other neurons.

RESULTS

Voltage-dependent Blockade of IBa by mGlur7 Receptors—Stimulation of mGlur7 receptors by D,L-AP4 (500 μM) had no effect on somatic whole cell IBa, in nontransfected cerebellar granule cells, which was consistent with the presynaptic location of the receptor in neurons (12). Therefore experiments were performed in cultured cerebellar granule cells transfected with a mGlur7 receptor expression plasmid. The receptor was expressed in both cell body and neurites which allowed us to study its effect on somatic IBa. In mGlur7 receptor-transfected cerebellar granule cells application of the receptor agonist, D,L-AP4 (500 μM), at a steady state potential of −80 mV, did not affect IBa evoked at the end of the application (mean ± S.E. = 1 ± 1% inhibition; n = 6; Fig. 1, period 1). In contrast, application of D,L-AP4, combined with transient and repetitive depolarizations (which evoked IBa), induced a progressive and potent inhibition (39 ± 2%; n = 6) of the current that last upon wash out of the agonist (Fig. 1, period 2) (12). These results indicated that mGlur7 receptors inhibited Ca$^{2+}$ channels under conditions where cerebellar granule cells were transiently and repetitively depolarized, but not in resting neurons.

Sustained Depolarization Inhibited P/Q-type Ca$^{2+}$ Channels—Because inhibition of Ca$^{2+}$ channels by mGlur7 receptors was dependent on transient and repetitive depolarization, we examined the effect of membrane depolarization per se (in the absence of receptor agonist) on the channel activity. To mimic physiological activity, we stimulated neurons using 20-ms depolarization pulses, from −80 to 0 mV. When applied at a frequency of 0.1 Hz, these stimulations evoked IBa of stable amplitude over a period of at least 45 min (Fig. 2A, open circles). Increasing the frequency of the depolarization pulses up to 25 Hz decreased IBa amplitude (Fig. 2A, points). The frequency-dependent decrease in amplitude of the current was reversible immediately after cessation of the tetanic stimulation if the train of depolarization pulses last for less than 30 s (Fig. 2A, J). On the other hand, a 1-min train of transient depolarizations induced a long lasting inhibition of the current (Fig. 2A, 2). To quantify this blockade we replaced the

FIG. 1. Voltage-dependent action of D,L-AP4 on IBa. Barium currents were evoked by 500-ms depolarization pulses applied at a frequency of 0.1 Hz. The graph represents the current amplitude relative to the current average amplitude measured before period 1. During period 1, membrane potential was held for 1 min at −80 mV, in the absence of depolarization pulse. Traces are representative IBa recorded at different times of the experiment indicated by the arrows. Note that D,L-AP4 inhibited IBa when applied concomitantly with (period 2), but in the absence of (period 1) depolarization pulses. Similar results were obtained in 5 other neurons.

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applied at a frequency of 0.1 Hz. The following figures, IBa was evoked by 500-ms depolarization pulses but not long (2) high frequency depolarization pulses. In this and the following figures, IBa was evoked by 500-ms depolarization pulses applied at a frequency of 0.1 Hz. The graph represents IBa amplitude measured as described in the legend to Fig. 1. During periods 1, 2, and 3, membrane potential was held for 1 min at −80 mV (1) or 0 mV (2 and 3), in the absence of transient depolarization pulses. In A and B, traces are representative of IBa recorded at different times of the experiment (arrows). Similar results as those in A and B were obtained in 5 other neurons. C and D, voltage (C) and time (D) dependence of the depolarization-induced inhibition of IBa. Each value is the mean ± S.E. of at least five experiments. E, representative IBa traces recorded before (control) and after a 1-min period of steady-state membrane potential at −80 and 0 mV. Original traces (on the left) were normalized (on the right) for better comparison of their inactivation kinetics. Note that although of smaller amplitude, IBa recorded after a steady-state depolarization to 0 mV displayed similar inactivation kinetics as control IBa.

FIG. 2. Depolarization-induced IBa inhibition. A, amplitude of whole cell IBa evoked by 20-ms depolarization pulses applied at a frequency of 0.1 Hz (open circles) or 25 Hz (points). Note the decrease in amplitude of the current evoked by high, but no low frequency depolarization pulses. This decrease in amplitude was reversible after short (1), but not long (2) high frequency depolarization pulses. B, in this and the following figures, IBa was evoked by 500-ms depolarization pulses applied at a frequency of 0.1 Hz. The graph represents IBa amplitude measured as described in the legend to Fig. 1. During periods 1, 2, and 3, membrane potential was held for 1 min at −80 mV (1) or 0 mV (2 and 3), in the absence of transient depolarization pulses. In A and B, traces are representative of IBa recorded at different times of the experiment (arrows). Similar results as those in A and B were obtained in 5 other neurons. C and D, voltage (C) and time (D) dependence of the depolarization-induced inhibition of IBa. Each value is the mean ± S.E. of at least five experiments. E, representative IBa traces recorded before (control) and after a 1-min period of steady-state membrane potential at −80 and 0 mV. Original traces (on the left) were normalized (on the right) for better comparison of their inactivation kinetics. Note that although of smaller amplitude, IBa recorded after a steady-state depolarization to 0 mV displayed similar inactivation kinetics as control IBa.

high frequency train of stimulation by a single square pulse depolarization of equivalent duration. Thus maintaining the cell for 1 min at a steady state potential of −80 mV did not alter IBa evoked after this resting period (0 ± 1% inhibition, n = 6, Fig. 2B, 1). On the other hand, holding the cell for 1 min at 0 mV inhibited IBa evoked after cessation of this steady state depolarization period (40 ± 2% inhibition; n = 6, Fig. 2B, 2). This effect was voltage- (Fig. 2C) and time-dependent (Fig. 2D). After the sus-

tained depolarization period, neither membrane resistance (not shown), nor IBa inactivation kinetics (Fig. 2E) were significantly altered, indicating that inhibition of IBa did not result from change in passive properties of the membrane or biophysical properties of the Ca2+ channels. We interpreted the reversible decrease in IBa amplitude observed after a short train of stimulations or sustained depolarization shorter than 10 s, as a classical Ca2+ channel inactivation. On the other hand, we tentatively interpreted the long lasting decrease induced by longer depolarization trains, or sustained steady state depolarization, as a pure Ca2+ channel inhibition.

The following data supported this hypothesis. First, IBa evoked after a sustained depolarization to 0 mV (Fig. 2B, 2), although of smaller amplitude, still displayed similar inactivation kinetics (Fig. 2E). Second, IBa inhibition was mediated through intracellular messengers. Thus, inhibition of IBa induced by sustained depolarization was abolished by PTX, the PKC inhibitor GF109203X, intracellular dialysis of the IP3 receptor antagonist heparin, or intracellular Ca2+ chelator BAPTA (Fig. 3A). Moreover, KCl-induced depolarization increased basal IP accumulation, in a PTX-dependent manner (Fig. 3B). Taken together these results suggested that inhibition of Ca2+ channels induced by sustained depolarization was distinct from their classical voltage-dependent inactivation, since it involved a Gα protein, intracellular Ca2+, and PKC. We then searched for the voltage sensor of this inhibitory pathway. Since voltage-induced inhibition of IBa was blocked by PTX (Fig. 3A), the voltage-sensitive step of this phenomenon should be either upstream Gα protein activation, or the Gα protein itself. Previous studies have shown the existence of a Gα protein activation through a conformational change of VGSCs, regardless of the Na+ current (15). Since cerebellar granule cells express functional somatic VGSCs (~145 pA/pF Na+ current) (17), we examined whether these channels were in-
volved in the depolarization-induced inhibition of Ca\(^{2+}\) channels. Since experiments were performed in the presence of TTX, the effects observed here were obviously independent of Na\(^+\) flux through VGSCs. The R-4-[3-(4-diphenylmethyl-1-piperazinyl)-2-hydroxypropoxy]-1H-indole-2-carbonitrile (R-DPI 201–106; 50 \(\mu\)M, 10 min), a drug that blocks VGSC conformational changes (22), also blocked the voltage-induced inhibition of IBa (2 ± 5% inhibition, \(n = 8\), Fig. 4A). Interestingly, this drug has been shown to block depolarization induced activation of G\(_o\) protein in synaptoneuroses (15). The potent and selective VGSC activator, veratridine, shifted the voltage-induced inhibition of IBa toward more negative potentials (Fig. 6B, filled triangles). The S-DPI 201–106 (50 \(\mu\)M, 10 min treatment), which mimics the action of veratridine on VGSC (22), also mimicked the effect of veratridine on IBa inhibition (data not shown). In the absence of sustained depolarization, neither veratridine nor S- or R-DPI 201–106 significantly altered IBa (1 ± 2, 2 ± 4, and 2 ± 1% inhibition, respectively; \(n = 10\) for both conditions). These results suggested that VGSCs were the voltage sensors that triggered the depolarization-induced inhibition of Ca\(^{2+}\) channels.

We then explored which type of Ca\(^{2+}\) channel was inhibited by the sustained depolarization. After application of \(\omega\)-agatoxin-IVA (250 nM), the toxin-resistant IBa was not significantly affected by subsequent sustained depolarization (38 ± 2% inhibition with \(\omega\)-agatoxin-IVA; 2 ± 0.4% inhibition with depolarization, \(n = 7\), Fig. 4B) and vice versa (40 ± 1.8% inhibition with depolarization; 2.5 ± 1.2% inhibition with subsequent \(\omega\)-agatoxin-IVA application, \(n = 8\), Fig. 4C). The Ca\(^{2+}\) channels inhibited by sustained depolarization were therefore of the P/Q-type.

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**Fig. 4. Membrane depolarization-mediated inhibition of P/Q-type Ca\(^{2+}\) channels involved activation of Na\(^+\) channels.** Same as in the legend of Fig. 2B, but in a R-DPI treated neuron (A) or untreated neurons (B and C). The square pulses indicate 1 min steady-state depolarizations to 0 mV. Aga, \(\omega\)-agatoxin-IVA (250 nM). For each panel, similar results were obtained in at least seven other experiments.

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**Fig. 5. Mutual occlusion of D,L-AP4- and depolarization-induced inhibition of Ca\(^{2+}\) channels.** Amplitude of IBa was expressed as described in the legend to Fig. 1. D,L-AP4 was applied before (A) or after (B) a 1-min steady-state depolarization to 0 mV (square pulse). Note the nonadditive inhibitory effects of voltage and D,L-AP4.

**Synergistic Action of mGlu7 Receptors and Membrane Depolarization on Ca\(^{2+}\) Channel Inhibition—**Since the mGlu7 receptor and membrane depolarization shared common mechanisms, inhibition of IBa by these factors should be mutually occluded. This hypothesis was confirmed by the following experiments. We have seen here above (Fig. 1) that in mGlu7 receptor-transfected cerebellar granule cells, application of the receptor agonist, D,L-AP4 (500 \(\mu\)M), during repetitive and transient depolarizations, progressively inhibited IBa (39 ± 2% inhibition; \(n = 6\)). Interestingly, the amount of IBa remaining after this action of D,L-AP4 was not significantly affected by subsequent sustained depolarization (36 ± 3% inhibition with D,L-AP4; 3 ± 2% inhibition with depolarization; \(n = 5\), Fig. 5A). Conversely, after sustained depolarization, D,L-AP4 application (associated with repetitive transient depolarizations) did not further inhibit the remaining IBa (39 ± 3% inhibition with depolarization; 1 ± 1% inhibition with D,L-AP4; \(n = 6\); Fig. 5B). These results indicated a mutual occlusion of the effects of sustained depolarization and mGlu7 receptor activation.

We then assessed whether or not voltage and mGlu7 receptor could act synergistically to inhibit Ca\(^{2+}\) channels. Transient and successive depolarization pulses applied at 0.1 Hz frequency (Fig. 1), or a 1-min steady-state depolarization to −40 mV (Fig. 6, A, J, and B, filled circles), did not per se affect IBa. Similarly to these subliminal stimulations, D,L-AP4 applied at a steady state potential of −80 mV did not alter IBa (Figs. 1 and 6, B, open circles). However, a 1-min steady-state depolarization to −40 mV, combined with a D,L-AP4 application inhibited IBa by 20% (Fig. 6, A, 2, and B, open circles). These results indicated a synergistic inhibitory effect of mGlu7 receptors and membrane depolarization on IBa. We verified that this synergistic effect involved VGSCs. Application of the VGSC blocker, R-DPI 201–106, antagonized the action of D,L-AP4 applied concomitantly with transient depolarization pulses (7 ± 3% inhibition; \(n = 5\)). Moreover, co-application, but not separate applications, of veratridine and D,L-AP4, at a membrane potential of −60 mV, significantly inhibited IBa (Fig. 6B, filled and open triangles). These results indicated that the mGlu7 receptor action depended on activation of VGSCs.

This synergistic effect of mGlu7 receptor and membrane depolarization was also observed on the receptor intracellular signaling cascade. Thus, basal IP level was not affected by 30 mM KCl (equivalent to −40 mV depolarization), nor by D,L-AP4 alone, but increased when KCl and D,L-AP4 were co-applied (Fig. 6C). The synergistic action of KCl and D,L-AP4 was also evident on evoked intracellular Ca\(^{2+}\) release. Thus, D,L-AP4 or 30 mM KCl alone did not induce any significant Ca\(^{2+}\) response, but together evoked marked and reversible intracellular Ca\(^{2+}\) release (Fig. 6D). These results indicated that coincident sub-
liminal membrane depolarization and mGlu7 receptor activation were required for the synthesis of IP \(_3\) and intracellular Ca\(^{2+}\) release.

It is worth noting that a high concentration (100 mM) of KCl (equivalent to a steady-state depolarization to \(-10\) mV) induced a similar IP response as a co-application of 100 mM KCl and D,L-AP4 (Fig. 6C). This result was consistent with the nonadditive inhibitory effects of a sustained depolarization to 0 mV and D,L-AP4 application on IBa (Figs. 5 and 6, open and filled circles).

**DISCUSSION**

The present study shows that a long duration train of high frequency depolarization pulses, or a prolonged single steady state depolarization, can inhibit P/Q-type Ca\(^{2+}\) channels through a VGSC-G \(_o\) protein-PLC-dependent pathway. These results provide the first electrophysiological evidence for a voltage-dependent activation of a G-protein via VGSCs, independently of Na\(^{+}\) flux. We also show that subliminal depolarizations were permissive for activation of this signaling cascade by the mGlu7 receptor (Fig. 7).

Inhibition of the P/Q-type Ca\(^{2+}\) channel induced by sustained membrane depolarization was different from the classical voltage-mediated inactivation of these channels, since P/Q-type Ca\(^{2+}\) channel inactivation has been previously described to be reversible, virtually abolished when Ba\(^{2+}\) was used as charge carrier, and should not depend on G protein or PKC activation (23, 24).

Our results were consistent with previous biochemical studies showing a voltage-dependent interaction of VGSC with G\(_o\) protein in rat brain synaptoneurosome (15). Indeed, R-DPI 201–106, a drug that blocks depolarization-induced activation of G\(_o\) protein in this preparation (15), also blocked the depolarization-induced inhibition of Ca\(^{2+}\) channels in cerebellar granule cells. Although it is difficult to determine from the present study whether or not G\(_o\) protein activation occurred during activation or inactivation of VGSCs, studies in synaptoneurosome suggested that this may happen as long as depolarization lasts (15). We show here that voltage-dependent activation of G\(_o\) protein through VGSCs resulted in IP\(_3\) formation and intracellular Ca\(^{2+}\) release. Evidence for depolarization-induced IP\(_3\) formation has been previously reported in skeletal muscle (25), and hyperpolarization has been suggested to reduce agonist-induced generation of IP\(_3\) in rabbit mesenteric artery (26). Thus, the voltage-dependent activation of G\(_o\) protein and IP\(_3\) synthesis observed here may not be specific for cerebellar granule cells.
We found a synergistic action of VGSCs and mGlu7 receptors on IP$_3$ formation and intracellular Ca$^{2+}$ release. Voltage-dependent IP$_3$-mediated Ca$^{2+}$ release has also been reported in coronary artery during stimulation of metabotropic cholinergic receptors (27). Moreover, during stimulation of metabotropic purinergic receptors, membrane depolarization can stimulate the release of Ca$^{2+}$ from IP$_3$-sensitive stores, in rat megakaryocytes. Consistent with our findings, the voltage sensor of this effect has been proposed to be upstream the purinergic receptors (28).

Two alternative hypotheses can be proposed to explain the synergistic action of voltage and mGlu7 receptor in neurons. The mGlu7 receptors and VGSCs may share the same G$_o$ protein, or independently activate distinct G$_o$ proteins. Whatever the type of coupling to G$_o$ protein, a synergistic action of the receptor and VGSC was required to inhibit P/Q-type Ca$^{2+}$ channels. This provided a mechanism by which neuronal activity could promote glutamate-mediated metabotropic effects. It is likely that this mechanism occurs at the axon terminal, since mGluR7 (6–8), PKC activity (29), IP$_3$-sensitive Ca$^{2+}$ stores, and P/Q-type Ca$^{2+}$ channels (13, 30–32) are present at presynaptic sites and control neurotransmitter release (10, 11). Thus high but not low frequency axonal discharges promotes coincident activation of presynaptic mGlu7 receptors by the neurotransmitter glutamate and action potential-induced activation of VGSCs, in the axon terminal. This coincident events would allow their synergistic inhibitory action on presynaptic P/Q-type Ca$^{2+}$ channels and synaptic transmission. This hypothesis is consistent with the inhibitory effect of group III mGlu receptors on high but not low frequency synaptic transmission, observed in the rat locus coeruleus (14). It would also provide a mechanism by which mGlu7 receptor knockout mice develop epileptic seizures (33).

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