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The Modulation of Calcium Currents by the Activation of mGluRs

Functional Implications

Alessandro Stefani*[*,1,2] Antonio Pisani,[1] Nicola B. Mercuri,[1] and Paolo Calabresi[1]

[1]Clinica Neurologica, Università di Tor Vergata, 00133 Rome,
[2]IRCCS Clinica Santa Lucia, Via Ardeatina 308, 00143 Rome, Italy

Abstract

Glutamatergic transmission in the central nervous system (CNS) is mediated by ionotropic, ligand-gated receptors (iGluRs), and metabotropic receptors (mGluRs). mGluRs are coupled to GTP-binding regulatory proteins (G-proteins) and modulate different second messenger pathways. Multiple effects have been described following their activation; among others, regulation of fast synaptic transmission, changes in synaptic plasticity, and modification of the threshold for seizure generation. Some of the major roles played by the activation of mGluRs might depend on the modulation of high-voltage-activated (HVA) calcium (Ca^{2+}) currents. Some HVA Ca^{2+} channels (N-, P-, and Q-type channels) are signaling components at most presynaptic active zones. Their mGluR-mediated inhibition reduces synaptic transmission. The interference, by agonists at mGluRs, on L-type channels might affect the repetitive neuronal firing behavior and the integration of complex events at the somatic level. In addition, the mGluR-mediated effects on voltage-gated Ca^{2+} signals have been suggested to strongly influence neurotoxicity. Rather different coupling mechanisms underlie the relation between mGluRs and Ca^{2+} currents: Together with a fast, membrane-delimited mechanism of action, much slower responses, involving intracellular second messengers, have also been postulated. In the recent past, the relative paucity of selective agonists and antagonists for the different subclasses of mGluRs had hampered the clear definition of the roles of mGluRs in brain function. However, the recent availability of new pharmacological tools is promising to provide a better understanding of the neuronal functions related to different mGluR subtypes. The analysis of the mGluR-mediated modulation of Ca^{2+} conductances will probably offer new insights into the characterization of synaptic transmission and the development of neuroprotective agents.

Index Entries: HVA Ca^{2+} conductances; neurotransmission; neuroprotection; rat; metabotropic receptors.

*Author to whom all correspondence and reprint requests should be addressed.

Introduction

About ten years ago, it was suggested that glutamate, the endogenous fast transmitter at the majority of excitatory synapses in the central nervous system (CNS), was also responsible for slow responses that involved intracellular signaling cascades. It was shown that glutamate stimulated phospholipase C (PLC) in cultured striatal and cerebellar granule cells as well as in cultured astrocytes and hippocampal slices (Sladeczek et al., 1985; Nicoletti et al., 1986a,b; Pearce, 1986). The glutamate-mediated stimulation of PLC, which produces mobilization of internal Ca^{2+} and formation of diacylglicerol and inositol triphosphate (IP3), was dependent on the activation of a new class of glutamate receptors, named metabotropic glutamate receptors (mGluRs). Their existence was confirmed in the *Xenopus* oocytes model (Sugiyama, 1987) and finally demonstrated by receptor-cloning experiments (for a review, *see* Pin and Duvoisin, 1995). Differently from ligand-gated receptors, mGluRs are coupled to G-proteins and modulate diverse transduction mechanisms. Besides the stimulation of the phosphoinositide (PI) hydrolysis, the activation of the mGluR subclasses has been linked to the inhibition of the forskolin-activated adenylate cyclase (AC) (Tanabe et al., 1993). Furthermore, second messengers other than IP3, DAG, or cyclic adenosin-monophosphate (cAMP) are affected by mGluRs, as reviewed by recent papers (Schoepp, 1994; Pin and Duvoisin, 1995). Finally, mGluRs are good candidates for direct channel modulation via membrane-delimited G-protein-mediated pathways (Hille, 1994).

The availability of selective agonists, such as 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) and 1S, 3R-ACPD (Palmer et al., 1989; Manzoni et al., 1990), and the recent synthesis of more selective compounds have contributed to the definition and characterization of mGluRs. As many as eight mGluRs have been cloned (Nakanishi, 1992; Schoepp and Conn, 1993; Hollman and Heinemann, 1994). They are divided in three groups, according to their amino acid sequence, pharmacological profile, and putative transduction mechanism. Group I, which comprises mGluR1 and mGluR5, is characterized by the stimulation of the PI hydrolysis/Ca^{2+} signal transduction (Masu et al., 1991). Group I mGluRs are well-characterized pharmacologically: The potency rank order for agonists at group I mGluRs is quisqualate (Quis) > glutamate > ACPD. Group II mGluRs, comprising mGluR 2 and mGluR 3, are mainly defined by the phospholipase A (PLA)-mediated reduction of the forskolin-induced intracellular cAMP. At group II receptors, cycloglycines as DCG-IV and CCG-1 (and glutamate) were shown to be more potent than Quis (Hayashi et al., 1992). Group III mGluRs (mGluR 4, 6, 7, and 8), similarly to group II, are linked to PLA cascade, and thus reduce the forskolin-stimulated cAMP formation. However, they are mainly identified for their sensitivity to L-2-amino-4-phosphonobutyric acid (L-AP4) and L-serine-o-phosphate (L-SOP) and relative insensitivity to ACPD (Tanabe et al., 1993; Saugstadt et al., 1994; Kinzie, 1995).

In the last decade, as molecular biologists have provided crucial insights into the structure and pharmacology of mGluRs, a great effort has also been made in trying to elucidate which functions the different mGluRs targeted in central neurons. Well beyond the goals of the present article is the review of the myriad of events that the activation of mGluRs presumably causes, as elegantly described by recent commentaries (Schoepp et al., 1990; Pin and Duvoisin, 1995; Schoepp, 1994; Ben-Ari and Aniksztejn, 1995). We will focus on a specific mGluR action, the modulation of HVA Ca^{2+} conductances. This represents a widespread target of mGluR modulation in many different areas of the CNS (Lester and Jahr, 1990; Nawy and Jahr, 1990; Sayer et al., 1992; Swartz and Bean, 1992; Trombley and Westbrook, 1992; Sahara and Westbrook, 1993; Swartz et al., 1993; Chavis et al., 1994, 1995; Hay and Kunze, 1994; Rothe et al., 1994; Stefani et al., 1994, 1996; Ikeda et al., 1995; Choi and Lovinger, 1996). First, we will examine the mGluR-mediated

modulation of N-type channels and its impact on transmitter release. Then we will analyze the mGluR-induced changes on the L-type, dihydropyridine (DHP)-sensitive Ca^{2+} channels. An important issue will be to examine the type of coupling mechanisms underlying the modulatory action of mGluRs: Some evidence is consistent with a fast, membrane-delimited, G-protein coupled mechanism; other research indicates a slower mechanism involving second messenger cascades. We will try to correlate, when possible, the mGluR-related effects to a particular subgroup of mGluRs. In conclusion, we will highlight some functional implications related to the mGluRs effects on Ca^{2+} channels.

The Activation of mGluRs Modulates HVA Ca^{2+} Currents

The multiple types of HVA Ca^{2+} channels present on central neurons may be distinguished by means of a pharmacological approach. In order to suppress the Ca^{2+} currents through L-, N-, P-, and Q-type Ca^{2+} channels, selective blockers are available; respectively, DHP antagonists, ω-conotoxin GVIA (ω-CgTx), funnel-web toxin or ω-Agatoxin-IVA (ω-AgTx, at low nanomolar concentrations), and ω-CgTx MVIIC. Many different neuronal functions might be related to the modulation of these conductances.

N, P, and Q Ca^{2+} channels are well-known to regulate, at the axon terminals of many central synapses, the Ca^{2+} influx that leads to transmitter release (Turner et al., 1993). The synaptic release in the CA3/CA1 hippocampal pathway of the rat, for instance, is triggered by N- and Q-type channels, as demonstrated by the full suppression of the intrahippocampal field potential by the concomitant application of ω-CgTx IVA and ω-CgTx MVIIC (Wheeler et al., 1994). In striatum, the corticostriatal release of endogenous glutamate is occluded by ω-CgTx, supporting the prominent role of N-type channels in this system (Calabresi et al., 1994). In other central pathways, like olfactory neurons or dorsal root ganglion (DRG) cells, the role of P-type channels seems more relevant, as suggested by the ability of ω-AgTx to almost eliminate excitatory postsynaptic miniature potentials (EPSCs) (Takahashi and Momiyama, 1993; Kimura et al., 1995). Thus, it seems interesting to investigate whether glutamate, by interfering with mGluRs, effectively modulates these Ca^{2+} channels and dampens synaptic transmission in several areas of the CNS.

Inhibition of ω_3-CgTx-Sensitive Channels and Regulation of Synaptic Transmission

The hypothesis that glutamate might affect, via G-protein-activated mechanisms, Ca^{2+} conductances was first investigated by Lester and Jahr (1990). In primary cultures of hippocampus, they showed that Quis and glutamate (but not AMPA) selectively depressed Ca^{2+} currents when neurons were dialyzed with GTP-γS, the nonhydrolizable analog of GTP. This pioneering study suggested the prominent involvement of L-type Ca^{2+} channels. Yet, it indicated a novel approach to the analysis of glutamate responses in the CNS and it was suddenly followed by other observations that focused the mGluR-mediated inhibition of N-type currents.

In pyramidal neurons acutely isolated from the CA3 region of the rat hippocampus, Swartz and Bean (1992) showed that t-ACPD and its active stereoisomer, 1S,3R-ACPD, reduced, in a rapid and reversible way, the HVA Ca^{2+} currents driven by the activation of N-type channels. The prevalent involvement of N-channels was demonstrated by the fact that ω-CgTx prevented the modulation by ACPD. Also, Sahara and Westbrook (1993) showed that agonists at mGluRs reduced barium (Ba^{2+}) currents in 5–7-d-old cultured hippocampal cells. Although a significant part of the modulation appeared to be dependent on L-channels blockade *(see below)*, the largest part of the observed mGluR-mediated inhibition of Ca^{2+} currents was ascribed to N-type channels, being sensitive to ω-CgTx. Additional evidence in favor of the modulation of N-type conductances came from other recent works.

In cultures of visceral sensory neurons, ω-CgTx strongly attenuated the mGluR-induced decrease of HVA Ca^{2+} currents (Hay and Kunze, 1994). A complete occlusion of the ACPD response by ω-CgTx was also obtained by our group in acutely isolated striatal neurons (Stefani et al., 1994). A large reduction of HVA Ca^{2+} currents by mGluR agonists was finally reported in retinal cells (Rothe et al., 1994). In this report, either depressant or potentiating effects were observed as a consequence of mGluR activation. Interestingly, both responses were occluded by ω-CgTx (and not by nifedipine).

All these observations confirmed that in several areas of the CNS, N-type Ca^{2+} channels are a major target of mGluR modulation. To what extent such a modulation implies changes in the synaptic transmission will now be examined.

A good example of inhibition of the transmitter release by agonists at mGluRs is the mammalian hippocampus. As already discussed, both glutamate and t-ACPD reduced HVA Ca^{2+} currents from either acutely isolated or cultured CA3 neurons (Swartz and Bean, 1992; Sahara and Westbrook, 1993). These findings consistently matched the conclusions drawn from extracellular and intracellular recordings in hippocampal slices. In the report by Manzoni and coauthors (1994), ACPD caused a large depression in the field EPSP in hippocampal slices, at both the synapses in CA1 and at the mossy fiber synapses in CA3. First evidence for hippocampal involvement of mGluRs in the inhibition of excitatory transmission was obtained by Baskys and Malenka (1991). They showed that the mGluR-activation depressed excitatory postsynaptic potentials (EPSCs), that this action was developmentally regulated, because the synaptic transmission was maximally depressed during the first postnatal month, and that this inhibition was not related to postsynaptic effects of 1S,3R-ACPD on potassium (K^+) conductances or changes in postsynaptic AMPA responses.

In striatum, we have shown that the activation of mGluRs decreases the cortically evoked synaptic potentials (Calabresi et al., 1992a). Noticeably, low micromolar concentrations of mGluRs agonists (<30 μ*M*) reduced the EPSP caused by endogenous glutamate without affecting postsynaptic membrane properties or the cellular sensitivity to exogenously applied glutamate (Calabresi et al., 1992a). Similar results were previously shown by Lovinger (1991), who described a dose-dependent depression by ACPD on intrastriatal excitation. It was also demonstrated that agonists at the subgroup III mGluRs (L-AP4) produced a clear inhibition of the corticostriatal signals (Calabresi et al., 1992a, 1993). Lovinger failed to detect any appreciable L-AP4-mediated modulation of excitatory synaptic transmission and interpreted such a discrepancy in terms of differences in the stimulation route (intrastriatal instead of cortico- or calloso-striatal stimulation). The possible modulation of GABAergic transmission was recently analyzed by our group (Stefani et al., 1994). The mGluR-induced depression of intrastriatal GABAergic transmission was correlated to mGluR-mediated efficacy in reducing HVA Ca^{2+} currents. Although this study focused 1S,3R-ACPD responses (presumably on group II receptors), unpublished results from our laboratory confirm that L-AP4 depresses Ca^{2+} conductances in both striatal and pyramidal cortical cells.

Very interesting results, although conflicting, have been collected in rat neocortex. Swartz and coauthors (1993) observed that 1S,3R-ACPD strongly inhibited N-type Ca^{2+} currents in cortical neurons. These authors also found that corticostriatal synaptic transmission was mainly mediated by N-type Ca^{2+} currents. In addition, it has been shown that ACPD dose-dependently reduced the corticostriatal EPSP, supporting a strong correlation between the modulation of the Ca^{2+} signals in the cortical cell bodies (and probably in the corticostriatal axon terminals) and the resulting synaptic potentials (Calabresi et al., 1992a; Lovinger and McCool, 1995).

In the dorsal frontoparietal neocortex of young rats, the mGluR-mediated decrease of whole-cell Ca^{2+} currents was resistant to ω-CgTx but occluded by the DHP antagonist nifedipine (Sayer et al., 1992). However, in adult neurons

of rat neocortex the exposure to multiple mGluRs agonists reversibly reduced both excitatory and inhibitory components of the evoked postsynaptic potentials (PSPs) (Burke and Hablitz, 1994). Moreover, since 1S,3R-ACPD did not decrease the amplitude of spontaneous PSPs and enhanced paired pulse facilitation (PPF), it was postulated as a mGluR-mediated reduction of transmitter release (Burke and Hablitz, 1994). Recently published reports add new observations to this fascinating debate: On one side, Choi and Lovinger (1996) showed that, at least in young pyramidal neurons isolated from deeper layer of neocortex, the mGluR-mediated modulation of HVA Ca^{2+} currents was sustained by group I and group II agonists. On the other hand, we have found that l-AP4 and l-SOP are significantly active in reducing the same conductances as full development occurs (Stefani et al., 1996a,b). The pharmacological implications of these results, although apparently conflicting, are beyond the goals of this article (for age-dependency of the responses, however, *see later*).

In neurons of the nodose ganglia, the modulation of HVA Ca^{2+} currents might be responsible for the well-known presynaptic depression of glutamate EPSP in the nucleus tractus solitarius (NTS). Microinjection of 1S,3R-ACPD decreased the EPSP evoked from NTS, suggesting that mGluRs are present on the presynaptic terminals at the level of the NTS (Hay and Kunze, 1994). As pointed out by Hay and Kunze (1994), it is crucial, in correlating the modulation of Ca^{2+} signals to the transmitter release, to show evidence that mGluRs and the specific modulated Ca^{2+} channels are indeed present at visceral afferent synaptic terminals in the NTS. This has been the case for NTS neurons (Hay and Kunze, 1994).

Mitral cells of the accessory olfactory bulb provide glutamate to neighboring granule cells and to output pathways, among which is the laterant perforant pathway to the dentate gyrus. It has been shown that agonists acting at group III mGluRs, namely L-AP4, strongly attenuated HVA Ca^{2+} currents in mitral cells (Trombley and Westbrook, 1992). In that study, it was not attempted to determine the relative contribution of N-type or L-type Ca^{2+} channels to the observed mGluR group III-mediated modulation However, it has to be emphasized that the same report analyzed the whole-cell recorded EPSPs from pairs of bulb mitral neurons. Significantly, the EPSPs were indeed decreased (by almost 50%) by L-AP4 in the low micromolar range. All these observations have provided a robust evidence of the efficacy of mGluRs agonists in acting as presynaptic regulators through the modulation of voltage-gated HVA Ca^{2+} currents.

mGluRs on L-Type, Dihydropyridine-Sensitive HVA Ca^{2+} Channels

mGluR activation is known to modulate not only ω-CgTx-sensitive Ca^{2+} channels, but also L-type DHP-sensitive channels. The pioneering observations by Lester and Jahr (1990) indicated L-type channels as the primary target of mGluR modulation in hippocampal cultures. This finding was confirmed by Sahara and Westbrook (1993). In fact, these authors have observed that low micromolar concentrations of nifedipine might prevent part of the mGluR-induced inhibition of HVA Ca^{2+} currents. However, they have also reported a modulatory effect by N-type channels *(see above)*.

In acutely isolated neurons from the neocortex of young rats, Sayer and coauthors (1992), as previously mentioned, described ACPD inhibitory responses that were occluded by nifedipine. In order to further validate the involvement of L-type currents, they showed that the activation of mGluRs strongly inhibited the long-lasting tail unmasked by adding a DHP agonist (Sayer et al., 1992). It should be mentioned that also in our preparations (freshly isolated cortical neurons from adult neocortex), part of the observed group III mGluR-mediated modulation was blocked by DHP-antagonists (Stefani et al., 1996b). Another structure in which the involvement of L-channels in the mGluR-induced changes of the Ca^{2+} currents has been extensively documented is

the cerebellum (Chavis et al., 1994, 1995). Chavis and coauthors, in a series of elegant experiments performed on cultured cerebellar granule cells, made clear the possibility that agonists at mGluRs might produce either facilitation or inhibition of L-type channels, depending on the activated mGluR subtype. In fact, mGluR activation opened DHP-sensitive channels through the activation of group I receptors (mGluR 1/5); on the other hand, DCG-IV, presumed to act selectively on mGluR 2/3 (group II), diminished the L-currents in the same neurons.

The possibility that glutamate, through mGluRs, decreases the Ca^{2+} fluxes driven by voltage-gated L-channels has also been postulated in cochlear neurons (Lachica et al., 1994). In this recent study, ACPD mimicked glutamate in reducing the Ca^{2+} response evaluated in fura-2 loaded neurons of the nucleus magnocellularis. This action was suggested as potentially preventing the accumulation of Ca^{2+} that eventually would cause cellular damage.

The mGluR-mediated actions on L-type currents might have several implications on the functional activity of central neurons. L-channels are supposed to have a great impact on a variety of cellular functions, including repetitive firing behavior (particularly in conditions of overwhelming excitation), activation of Ca^{2+}-dependent K^+ conductances, regulation of intracellular Ca^{2+} stores, dendritic release, and gene expression (Howe and Surmeier, 1995). In all these conditions, it is conceivable that the activation of mGluRs plays complex roles. Chavis and coauthors (1995), for instance, have suggested that the simultaneous mGluR-induced depression and facilitation of L-currents in cerebellar granule cells, together with the well-known modulation of K^+ conductances, is not incidental (Fagni et al., 1993; Chavis et al. 1995). They observed that Ca^{2+} and K^+ channel proteins, as well as the mGluRs, are closely associated in the granule cell membrane (Chavis et al., 1995). They suggested that, because of their vicinity, a sort of functional triplet, constituted by an mGluR in the proximity of depolarizing Ca^{2+} channels and hyperpolarizing Ca^{2+}-gated K^+ channels, might play rather important roles in controlling the cell firing. A good example of this is the mGluR-mediated burst-firing mode in dorsolateral septal nuclei (Zheng and Gallagher, 1992). This activity is indeed blocked by Ca^{2+} channel blockers.

mGluRs Modulate Ca^{2+} Conductances Through Different Transducing Mechanism

Membrane-Delimited Pathway

A fast modulation of HVA Ca^{2+} currents by mGluRs agonists has been extensively documented and characterized by its sensitivity to pertussis toxin (PTX), indicating that mGluRs are coupled to G-proteins of the Gi family (Hille, 1994). It is noteworthy recalling that many transmitters depress Ca^{2+} currents in a PTX-sensitive manner (Lipscombe et al., 1989; Toselli et al., 1989). This action usually is not mediated by changes of cyclic nucleotide metabolism or other conventional second messengers. In this kind of modulation, it is assumed that a component of the agonist-activated G-protein directly interacts with the $\alpha 1$ subunit of the Ca^{2+} channel to decrease the probability of its opening (Hille, 1994). The clearcut demonstration that a membrane-delimited pathway underlies the observed modulation is achieved only by on-cell patch experiments. In this experimental condition, in fact, agonist applied in the bath to the entire cell surface is unable to depress the current of the Ca^{2+} channels isolated in the pipet. In addition, a membrane-delimited mechanism develops and recovers very fast and is frequently voltage-dependent. Moreover, it does not commonly require the presence of external Ca^{2+} or mobilization of internal cations, since it reflects a direct change of the modes of gating of the membrane channel. We will examine some reports consistent with this kind of mGluR-mediated modulation.

Trombley and Westbrook (1992) showed that the L-AP4-induced fast inhibition of HVA Ca^{2+}

currents in cultured neurons of the olfactory bulb required a G-protein-mediated mechanism through different procedures. First, the modulation was blocked by the removal of magnesium, which is believed to be necessary for the activation of G-proteins. Second, when GTP-γS was substituted for GTP in the patch pipet, the L-AP4 response could not recover. Finally, the incubation of olfactory bulb neurons in PTX prevented the L-AP4-mediated action on Ca^{2+} currents. Also in cultured hippocampal cells, GTP-γS made the inhibition irreversible (Trombley and Wesbrook, 1992). Since the Ca^{2+} chelator BAPTA did not affect the observed responses, the authors postulated that modifications of free intracellular Ca^{2+} were not required. The inclusion of staurosporine, a broad spectrum kinase inhibitor, prevented the L-AP4 inhibition of the whole-cell Ca^{2+} currents, suggesting that Ca^{2+}-dependent kinases were not significantly involved.

Another good example of fast, membrane-delimited mechanism of action was shown by Swartz and Bean (1992). When CA3 hippocampal cells were dialyzed with GTP-γS, the suppression of Ca^{2+} currents by 1S,3R-ACPD became irreversible. More interestingly, these authors clearly demonstrated a spatial restricted pathway for the mGluR action on Ca^{2+} currents. First, they obtained Ba^{2+} currents from outside-out patches that were rapidly and reversibly inhibited by 1S,3R-ACPD as well as by baclofen and adenosine (well-known modulators of PTX-sensitive membrane-delimited pathways). Moreover, in the cell-attached configuration, ACPD applications were without significant effect, when applied outside the patched membrane.

The exemplary work by Sahara and Westbrook (1993) indeed showed that part of the mGluR-induced modulation occurred through a fast kinetic membrane-delimited pathway. This assumption was drawn by the observation that: A large modulation could take place in the presence of high intracellular Ca^{2+} chelators, and that the ACPD responses had a very rapid onset and recovery. However, it must be reminded that, in the same neurons, t-ACPD had also the ability to stimulate rather different responses, characterized by slow kinetics and the putative involvement of signaling cascades *(see below)*.

In striatum, Stefani and coauthors (1994) showed that ACPD and 1S,3R-ACPD reduced HVA Ca^{2+} currents through a rather fast and G-protein-mediated mechanism; again, it can be emphasized that the mGluR-mediated depression of HVA Ca^{2+} currents in striatum recovered completely and quickly and was not dependent on substitution of Ba^{2+} with Ca^{2+} itself.

Intracellular Second Messengers

As already mentioned, in hippocampal neurons, together with a fast component of Ca^{2+} channel inhibition, a slower ACPD-mediated inhibition was also observed (Sahara and Westbrook, 1993). The latter was strongly dependent on the steady-state intracellular free Ca^{2+} concentration (although the release from Ca^{2+} intracellular stores was not a critical factor, since the response was not clearly antagonized by caffeine or thapsigargin). Time constant of both onset and recovery of this slow inhibition were in the order of minutes.

Other recent works have supported the possibility that the mGluR-mediated modulation of HVA Ca^{2+} currents requires the activation of second messenger pathways. Sayer and coauthors were the first to show a rather slow ACPD-mediated modulation of HVA Ca^{2+} currents that was strongly attenuated, although not entirely abolished, by substituting external Ca^{2+} with Ba^{2+}. This Ca^{2+} dependency seemed to support the idea that the ACPD response was secondary to Ca^{2+} release from intracellular stores, or that the mGluR-mediated reduction in HVA Ca^{2+} currents (mainly L-type) required a Ca^{2+}-mediated Ca^{2+}-channel inhibition. Yet, intracellular BAPTA up to 20 m*M* did not antagonize the response, and thapsigargin was not able to significantly affect the inhibition (Sayer et al., 1992). These observations highlight a common problem: Although in some preparations a transduction mechanism must be involved, it turns out not to be easy to define it. In the works by Chavis and coau-

Table 1
mGluR-Mediated Modulation of HVA Ca^{2+} Currents

Channel[a]	Structure	Effect	mGluR[b]	Reference
L	Hippocampal neurons	Reduction	G-I (high sens. to Quis)	Lester and Jahr, 1990
N	Hippocampal neurons	Reduction	G-II?	Swartz and Bean, 1992
N/L	Hippocampal neurons	Reduction	G-II? G-III (?/l-AP4 as agonist)	Sahara and Westbrook, 1993
N	Cortical neurons	Reduction	G-II?	Swartz et al., 1993
N	Cortical neurons	Reduction	G-I and G-II (Quis- and DCG-IV as agonists)	Choi and Lovinger 1996
N/L	Cortical neurons	Reduction	G-III (l-AP4 as agonist)	Stefani et al., 1996
L/N	Cortical neurons	Reduction	G-I (high sen. to Quis)	Sayer et al., 1992
N/L	Olfactory bulb neurons	Reduction	G-III (l-AP4 as agonist)	Trombley and Westbrook, 1992
N	Striatal neurons	Reduction	G-II?	Stefani et al., 1994
N	Visceral neurons	Reduction	G-II	Hay and Kunze, 1994
L	Cerebellar granule	Reduction	G-II (DCG-IV as agonist)	Chavis et al., 1994
L	Cerebellar granule	Increase	G-I	Chavis et al., 1994
L	Cochlear neurons	Reduction	G-II?	Lachica et al., 1994
L	Retinal cells	Reduction/ increase	G-III/G-II	Rothe et al., 1994
N	Transfected DRG cells	Reduction	G-II	Ikeda et al., 1996

[a]The calcium channel subtypes were schematically distinguished in N and L, because the vast majority of the reported observations were performed with N- and L-type channel antagonists. At present, however, different groups are testing the possibility that P-type and Q-type calcium channels are also modulated by mGluR agonists.
[b]G-I, G-II, and G-III means Group-I, Group-II and Group-III, respectively.

thors, a great effort is made in trying to elucidate the signaling pathways connected to mGluR-mediated effects (Chavis et al., 1994, 1995). It has to be remembered that these authors have mainly chosen to analyze cell-attached recordings, the most suitable configuration to study the involvement of a second messenger system. However, no conclusive evidence could be postulated. Also, in the work dealing with ACPD-mediated inhibitory responses, Chavis and colleagues recognized as troublesome the identification of the signaling pathway involved. They assumed that the mGluR-mediated reduction of L-channel activity was subserved by mGluR II subgroup. In fact, Quis was unable to inhibit the conductance, glutamate was equally potent as 1S,3R-ACPD (and not more, as you would expect from agonists preferentially activating group I mGluRs), L-CCGI was the most potent agonist, L-aminophosphonobutyrate (L-APB) was not effective, and PTX at rather low concentrations completely obscured the responses. But again, which transduction mechanism occurs? The ACPD modulation was not affected by IBMX, and high concentrations of intracellular cAMP (up to 1 m*M*) did not modify the L-CCGI response, which made unlikely the direct inhibition of AC. Also phorbol esters, known to modulate HVA Ca^{2+} channels, and to activate PKC, did not modify the mGluR-mediated inhibitory effect. The authors were confident that the very long latency of the effects they showed was consistent with the modulation of a diffusible second messenger, although they could not unequivocally characterize it (Chavis et al., 1994). A hypothesis they raised was a direct block of the channel done by a Go/Gi protein.

Functional Implications

In this article, we have briefly outlined the main studies concerning the mGluR-mediated actions on HVA Ca^{2+} currents, reviewed in Table 1. Until recently, the pharmacological analysis of the mGluR-mediated response has

been hampered by the lack of selective compounds. Some correlations, however, between effect on Ca^{2+} conductances and the specific involved subclass of mGluRs might be proposed (Table 1). Evidence against the involvement of the group I mGluR in the modulation of HVA Ca^{2+} currents has been shown by different authors. The inhibition of voltage-gated Ca^{2+} currents in adult hippocampus and striatum is not sensitive to L-AP3 (Swartz and Bean, 1992; Stefani et al., 1994). Therefore, it can be presumed that changes in the PI turnover do not underlie the observed responses. In addition, in fura-2 loaded retinal ganglion cells, none of the used agonists (Quis, t-ACPD) affected Ca^{2+} release from intracellular stores (Rothe et al., 1994); also this observation argues against the activation of the PLC pathway. On the other hand, the inhibition of HVA Ca^{2+} currents observed in cortical regions seemed to support a group I receptor activation (Lester and Jahr, 1990; Sayer et al., 1992). Also, the slow modulation described by Sayer and coauthors (1992) was obscured by Ba^{2+} in place of Ca^{2+}, which implies the requirement of Ca^{2+}-dependent events. However, L-AP3 up to 1 m*M* was not a good antagonist of this response. In other words, although some features of the modulation seemed to indicate a precise involvement of PLC, no clearcut demonstration could be obtained. Choi and Lovinger (1996) reported that Quis and DCG-IV have a strong modulatory effect on HVA, suggesting that both group I and group II are involved in the mGluR-mediated response in young pyramidal neurons *(see later)*. Approaching the same responses with more selective antagonists and by means of on-cell recordings (which do not disrupt the intracellular milieau) will probably help in clarifying these questions. Concerning mGluR III subgroup responses, it must be stressed that L-AP4 was in fact identified as a specific ligand for a new glutamate receptor in the early 1980s (Forster and Fagg, 1984; Koerner and Cotman, 1991). Moreover, it was recognized that L-AP4 acts through G-proteins (Nawy and Jahr, 1990) as an "autoreceptor-like agonist" at the presynaptic glutamate receptors to decrease glutamate release. Unequivocally, L-AP4 has been shown to reduce HVA Ca^{2+} signals in cultured olfactory bulb neurons (Trombley and Westbrook, 1992), cultured retinal ganglion cells (Rothe et al., 1994), and, but in a very small population, in cultured hippocampal neurons (Sahara and Westbrook, 1993). Furthermore, the finding that L-AP4 decreased the corticostriatally evoked EPSP (Calabresi et al., 1992a, 1993) was recently supported by the L-AP4-mediated modulation of HVA Ca^{2+} currents on adult cortical neurons (Stefani et al., 1996a). Conflicting results on the roles played by group III mGluRs arise from recent studies. In particular, a strong age-dependency seems to underlie the modulation exerted by this subclass of receptors (Vazquez et al., 1995; Choi and Lovinger, 1996; Stefani et al., 1996b).

It should be noted, however, that many of the studies regarding the mGluR-induced modifications of HVA Ca^{2+} currents have suggested the involvement of group II receptors (Swartz and Bean, 1992; Sahara and Westbrook, 1933; Chavis et al., 1994; Rothe et al., 1994; Stefani et al., 1994; Ikeda et al., 1995; Lachica et al., 1995). Moreover, in some areas, evidence emerged in favor of a simultaneous activation of more subclasses of mGluRs (group I and group II in Choi and Lovinger, 1996, group II and III in Stefani et al., 1996a). Conclusive findings are far from being obtained. Ikeda and coworkers (1995), by injecting with mGluR2 cRNA DRG neurons (which normally do not express this receptor), unmasked an mGluR-mediated inhibition of HVA Ca^{2+} currents (N-type). By providing a means of investigating the interaction between an unambiguously identified receptor subtype and a neuronal ion channel, they have demonstrated that mGluR2 couples to N-type Ca^{2+} channels via a PTX-sensitive pathway (Ikeda et al., 1995). However, it will be more interesting to investigate analogous responses in structures in which mGluRs are indeed operative *in situ*.

We have reported evidence in favor of a modulatory effect, by mGluR agonists reducing Ca^{2+} currents, on the synaptic release of both excitatory and inhibitory responses. Yet,

how the synaptic transmission is really affected in vivo is a crucial matter with regard to the potential capability of mGluRs to modify the "synaptic strength." In other words, to what extent does the activation of mGluRs change the concomitant stimulation of iGluRs by the endogenous transmitter?

It might be hypothesized that in those structures in which the affinity of the endogenous ligand for mGluRs is very high, even low concentrations of glutamate (barely effective in activating the postsynaptic glutamate channels), might actually downregulate the transmitter release. If this is the case, we could speculate that in conditions of very "intense" synaptic activation (and/or under excitotoxic conditions), the depression of the vescicular transmitter release, obtained by the mGluR-mediated reduction of the Ca^{2+} currents, would limit the undesirable consequences of such an overwhelming exposure of the postsynaptic neuron to glutamate. However, this scenario is conceivable mainly when fast, membrane-delimited responses occur. In fact, it is rather unlikely that mGluR-mediated slow responses, involving the activation of second messengers, can be consistently activated by synaptically released glutamate. Nevertheless, in conditions of repeated, low-frequency stimulation, glutamate concentration in the synaptic cleft is thought to keep a low, but significant concentration for longer time, and thus is able to stimulate the mGluR-mediated responses.

When it comes to the control of cellular excitability, the functional interplay between iGluRs and mGluRs is influenced by many factors. In particular, the crosstalk between iGluRs and mGluRs seems to be influenced by age-related functional changes (Burke and Hablitz, 1994).

One of the most stimulating issues is the long-lasting modification of synaptic efficacy. A regulatory mechanism mediated by mGluRs may indeed operate during repetitive synaptic stimulation. At present, the activation of mGluRs has been indicated as a crucial step in the induction and maintenance of long-term potentiation (LTP) and long-term depression (LTD) in hippocampus, cerebellum, olfactory cortex, and striatum (Aniksztejin et al., 1992; Calabresi et al., 1992b, 1995, 1996; Zheng and Gallagher, 1992; Bashir et al., 1993; Kaba et al., 1994; Linden, 1994; Nakanishi, 1994; O'Connor et al., 1994). We have provided evidence for the central role of PI-linked (group I) mGluRs in the formation of striatal LTD (Pisani et al., 1995; Calabresi et al., 1996). Furthermore, the Ca^{2+}-dependency of striatal LTD was supported by the finding that it is prevented by BAPTA or EGTA. This is in agreement with the idea that long-lasting changes in synaptic strength are related to postsynaptic Ca^{2+} entry, which in turn is largely influenced by the modulation of voltage-gated Ca^{2+} channels on postsynaptic somadendritic regions. As a consequence, the mGluR-induced modulation of HVA Ca^{2+} currents in neostriatum, albeit preferentially supported by the activation of mGluR subtypes not linked to PI hydrolysis (Stefani et al., 1994), would contribute to the occurrence, formation, and persistence of plastic changes of the synaptic transmission in the basal ganglia circuitry. Noticeably, the possibility that quickly occurring and slowly developing changes of synaptic transmission may influence each other is supported by the recent notion of "metaplasticity" (Bear, 1995). Thus, postsynaptic excitability, ruled by iGluRs and Ca^{2+}, would strongly affect the cellular disposition to each form of plasticity (Bear and Malenka, 1994). Although the cornerstones of this theory model (putative changes in the calcium affinity of CamKII kinases, "threshold modification" from net LTD to net LTP), are clearly beyond the scope of this article, it is supposed that the graded modification of postsynaptic Ca^{2+} may trigger both LTD (when Ca^{2+} is low) or LTP (when Ca^{2+} is high) (Bear and Malenka, 1994). Pharmacological studies are in progress in order to better define the exact contribution that distinct mGluRs, through the modulation of HVA Ca^{2+} currents, bring to the modifications of LTP/LTD (Calabresi et al., 1992b; Pisani et al., 1995).

The recent advance of cyclopropylglycine derivatives in the analysis of the pharmacology of mGluRs clarified some functional aspects of

the role played by these receptors in neuronal activity. In particular, much effort has been produced in the characterization of the function of mGluRs in excitotoxic damage. Preliminary, mostly biochemical, studies on cultured neurons had observed a potential block by ACPD on the NMDA-induced toxicity (Koh et al. 1991; Lombardi et al., 1994). At present, a neuroprotective efficacy against NMDA-mediated damage has been demonstrated either by blocking group I mGluRs (Buisson and Choi, 1995; Orlando et al., 1995), or by activating group II and group III mGluRs (Bruno et al., 1994, 1995b; Ambrosini et al., 1995; Buisson et al., 1996). Furthermore, by utilizing the antagonist of group I mGluR, S-4C3HPG, both Buisson and Choi (1995) and Orlando et al. (1995) showed that NMDA-induced toxicity was greatly attenuated.

Interestingly, in the quinolinic acid (NMDA agonist)-induced lesions in striatum (which resembles the neuropathological features of Huntington's disease) it was observed that decortication, by removing corticostriatal afferent glutamatergic afferents, prevented toxicity, but that coadministration of t-ACPD restored it (Bear et al., 1993). This effect seems to confirm a permissive role played by mGluRs (presumably group I) in the toxic damage caused by NMDA. In this regard, however, many factors contribute to the slow development of neuronal damage in the course of neurodegenerative diseases, among which either impairment of energy metabolism or modification in the voltage-dependency and/or permeability of iGluRs is supposed to increase neuronal vulnerability. In this respect, the mGluR-mediated inhibition of voltage-gated HVA Ca^{2+} channels that we have described might be viewed as a mechanism by which endogenous glutamate dampens its own transmission or, at the postsynaptic level, reduces the cell excitability and the depolarization-related events. Noticeably, in the report of Copani and others (1995), aside from agonists at group II and group III mGluRs, nimodipine and divalent inorganic blockers also strongly attenuated the NMDA-related toxicity, confirming that the block of Ca^{2+} signals may be a target for limiting toxicity. In order to delineate new strategies for the putative therapy of neurodegenerative processes, a combination of molecular agents should be encouraged. It seems conceivable that the concomitant utilization of proper mGluRs agonists together with low concentrations of calcium blockers might provide prevention with regard to overwhelming excitability or unbalanced Ca^{2+}-dependent events.

In conclusion, there is undoubtedly a renewed emphasis on trying to envision the modulation of Ca^{2+} currents by agonists at mGluRs as part of complex neuromodulatory functions. In order to better understand these actions, the availability of more selective pharmacological agents will be welcomed; it should also favor the integration of electrophysiological responses detected in isolated or cultured cells with evidence accumulated in "intact" systems, in which the endogenous transmission is preserved. The utilization of transgenic mice for selective group mGluRs will further facilitate the understanding of their roles in the central nervous system.

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