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The C Terminus of the Metabotropic Glutamate Receptor Subtypes 2 and 7 Specifies the Receptor Signaling Pathways*

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Julie Perroy[‡], Gustavo J. Gutierrez[§], Vincent Coulon[¶], Joel Bockaert[‡], Jean-Pilippe Pin[‡],
and Laurent Fagni[¶]

From the [‡]CNRS, UPR 9023, CCIPE, 34094 Montpellier Cedex 5, France, [§]EMBL, Meyerhofstrasse 1, Heidelberg D-69117, Germany, and [¶]Institut de Génétique Moléculaire de Montpellier, UMR 5535 CNRS, 1919 Route de Mende, 34293 Montpellier Cedex 5, France

There is accumulating evidence that the specificity of the transduction cascades activated by G protein-coupled receptors cannot solely depend on the nature of the coupled G protein. To identify additional structural determinants, we studied two metabotropic glutamate (mGlu) receptors, the mGlu2 and mGlu7 receptors, that are both coupled to G_o proteins but are known to affect different effectors in neurons. Thus, the mGlu2 receptor selectively blocks N- and L-type Ca²⁺ channels via a protein kinase C-independent pathway, whereas the mGlu7 receptor selectively blocks P/Q-type Ca²⁺ channels via a protein kinase C-dependent pathway, and both effects are pertussis toxin-sensitive. We examined the role of the C-terminal domain of these receptors in this coupling. Chimeras were constructed by exchanging the C terminus of these receptors and transfected into neurons. Different chimeric receptors bearing the C terminus of mGlu7 receptor blocked selectively P/Q-type Ca²⁺ channels, whereas chimeras bearing the C terminus of mGlu2 receptor selectively blocked N- and L-type Ca²⁺ channels. These results show that the C terminus of mGlu2 and mGlu7 receptors is a key structural determinant that allows these receptors to select a specific signaling pathway in neurons.

The physiological actions of most neurotransmitters are mediated by activation of both ionotropic and G protein-coupled receptors (GPCRs¹ or metabotropic receptors). For instance, the metabotropic receptors activated by the excitatory neurotransmitter, glutamate, play crucial roles in synaptic plasticity, neurotoxicity, as well as neuroprotection. These effects result from a large variety of regulations such as direct mediation of glutaminergic synaptic transmission or modulation of neurotransmitter release or membrane excitability (1). Transduction of extracellular signals to intracellular responses via GPCR

includes stimulation of the receptor, activation of a trimeric G protein, and subsequent mobilization of specific effectors. The regions of the receptor involved in G protein activation have been studied extensively (2). The second intracellular loop of mGlu receptors is responsible for the selective recognition of the G protein α subunit (3, 4), whereas all other receptor intracellular segments control the coupling efficacy (4).

Eight genes encoding mGlu receptors have been identified and classified into three groups, according to their sequence and intracellular signaling. The group I mGlu receptors (mGlu1 and -5) are coupled to G_q-like proteins and activate phospholipase C, whereas the group II (mGlu2 and -3) and group III (mGlu4 and -6–8) receptors are coupled to G_i/G_o proteins and inhibit cAMP formation (5, 6). The signaling cascades of mGlu receptors seem, however, to be far more complex than these consensual pathways. These receptors can indeed activate cGMP phosphodiesterase (7), phospholipase D (8), phospholipase A₂ (9), and mitogen-activated protein kinase cascades (10). Through these pathways, the mGlu receptors can control ionic channel activities (11–13). Thus it is remarkable that mGlu receptors activating the same group of G proteins inhibit distinct Ca²⁺ channel subtypes (14–16). A series of studies (17–20) have suggested that G protein β subunits may be essential for the specific coupling of various metabotropic neurotransmitter receptors to Ca²⁺ channels, but others (21–24) have shown that the same G protein $\beta\gamma$ subunit combination can inhibit different Ca²⁺ channel subtypes and vice versa. The mGlu receptors have even been shown to activate directly effectors and generate cellular responses independently of G protein activation (25). This large variety of mGlu receptor actions together with the limited number of functional G protein subunit combinations (26) suggest that the signaling cascade specificity of a given GPCR cannot arise solely from the nature of the activated G protein. The aim of the present study was to examine whether receptor structural determinants are involved in the specificity of GPCR signaling.

To address this issue we studied two GPCRs, the mGlu2 and mGlu7 receptor subtypes. These receptors are both coupled to G_i/G_o proteins but trigger different second messenger cascades, in cultured cerebellar granule cells. Thus, both receptors activate a pertussis toxin-sensitive G protein, but the mGlu2 receptor selectively blocks N- and L-type Ca²⁺ channels (15), whereas the mGlu7 receptor blocks the P/Q-type Ca²⁺ channels (27). Moreover, the effect of the mGlu7 (27) but not mGlu2 (15) receptor is PKC-dependent. To examine the possible role of the C-terminal intracellular domain of these receptors in specifying their transduction cascade, the coupling to N- and L- or P/Q-type Ca²⁺ channels of chimeric receptors bearing either the C-terminal domain of mGlu2 or that of mGlu7 was examined in cultured cerebellar granule cells. We found that the C

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¶ To whom correspondence should be addressed: CNRS, UPR 9023, CCIPE, 141 Rue de la Cardonille, 34000 Montpellier, France. Tel.: 33 467 142 945; Fax: 33 467 542 432; E-mail: fagni@bacchus.montp.inserm.fr.

¹ The abbreviations used are: GPCR, G protein-coupled receptor; mGlu, metabotropic glutamate; PKC, protein kinase C; DHPG, dihydroxyphenyl glycine; GFP, green fluorescent protein; DL-AP4, DL-amino-phosphonobutyrate; IP, inositol phosphate.

terminus of the mGlu7 receptor was necessary and sufficient to select a PKC-dependent inhibitory pathway specific to the P/Q-type Ca^{2+} channel, whereas the C terminus of the mGlu2 receptor was necessary and sufficient for a PKC-independent inhibition of N- and L-type Ca^{2+} channels. These findings reveal a new role of the C terminus of mGlu2 and mGlu7 receptors in specifying their respective transduction cascades downstream from the G protein activation.

EXPERIMENTAL PROCEDURES

Cell Culture—Primary cultures of cerebellar cells were prepared as described previously (28). Briefly, 1-week-old newborn mice were decapitated and the cerebellum dissected. The tissue was then gently triturated using fire-polished Pasteur pipettes, and the homogenate was centrifuged at 500 rpm. The pellet was resuspended and plated in tissue culture dishes previously coated with poly-L-ornithine. Cells were maintained in a 1:1 mixture of Dulbecco's minimum essential medium and F-12 nutrient (Life Technologies, Inc.) supplemented with glucose (30 mM), glutamine (2 mM), sodium bicarbonate (3 mM), HEPES buffer (5 mM), decomplexed fetal calf serum (10%), and 25 mM KCl to improve neuronal survival. One-week-old cultures contained 1×10^6 cells.

Plasmids and Transfection—The chimeric receptors were constructed as follows. The mGlu2/7 and mGlu7/2 chimeras were obtained by exchanging the C-terminal region proximal to the seventh transmembrane domain of the mGlu7a receptor (beginning with HPELN-VQKRR) with its homologous mGlu2 receptor domain (beginning with QPQKNVSSH) (see Fig. 2), using the polymerase chain reaction overlap method (29). The construction of the mGlu1/2 chimera containing the N-terminal extracellular domain of the mGlu1 receptor and the heptahelical and C-terminal regions of the mGlu2 receptor has been described previously (30). The mGlu1/2/7 chimera was constructed by replacing the fragment encoding the 3'-half of the mGlu1/2 chimera by that of the mGlu2/7 chimera, using the *Bsr*GI and *Xba*I restriction sites of the mGlu2 receptor heptahelical domain coding sequence and the pRK5 poly-linker, respectively.

The wild type and chimeric receptors were tested for their coupling to G protein using the method that we described previously (31). Briefly, inositol phosphate (IP) accumulation was measured in HEK-293 cells co-transfected with the tested receptor and a recombinant G protein. The mGlu1 receptor was co-transfected with a GHQ protein, whereas the mGlu2, mGlu7a, mGlu2/7, mGlu7/2, mGlu1/2, and mGlu1/2/7 receptors were co-transfected with a chimeric G_{q19} protein. In the G_{q19} chimeric protein, the C-terminal 9 residues of the G protein α_q subunit were replaced by those of the G protein α_{i2} subunit, enabling the coupling of the receptors to phospholipase C (4, 30, 31).

Immediately before plating, cerebellar cultures were co-transfected with the mGlu2 or mGlu7a receptors or one of the chimeras, and the transfection marker green fluorescent protein (GFP)-containing plasmid, pEGFP-N1 (CLONTECH), using the transfection lipid, Transfast (32).

The wild type mGlu7a and chimeric mGlu7/2 receptors were tagged at their N terminus with a Myc epitope, as described previously (27). The presence of these Myc-tagged proteins at the cell surface was examined in living (non-permeabilized) cultured neurons. Cells were exposed for 30 min at 37 °C to a polyclonal rabbit anti-Myc (1/300; ABR, Inc.) primary antibody and then rinsed, fixed in a 4% paraformaldehyde, 0.1 M glucose-containing phosphate-buffered saline solution, and exposed for 2 h at room temperature to a goat Texas Red-conjugated anti-rabbit IgG (1/1000; Jackson ImmunoResearch). Cells were then rinsed with phosphate-buffered saline and mounted on a glass coverslip for observation on an Axiophot 2 Zeiss microscope. The intensity of Myc immunolabeling was measured on the cell body using the NIH Image program (Wayne Rasband). The ratio of total fluorescence of the cell body area over background (noise) fluorescence was determined in non-saturating conditions of the camera. The fluorescence values were then averaged and compared between neurons transfected with N-Myc-mGlu7a and -mGlu7/2 receptors.

Electrophysiology—We used the whole-cell patch clamp configuration to record Ba^{2+} current (IBa) from GFP-expressing (mGlu receptor co-transfected) cerebellar granule cells, after 9 ± 1 days *in vitro*, as described previously (32). The bathing medium contained (in mM) BaCl_2 (20), HEPES (10), tetraethylammonium acetate (10), tetrodotoxin (3×10^{-4}), glucose (10), sodium acetate (120), and MK-801 (1×10^{-3}), adjusted to pH 7.4 with NaOH and 330 mosm with sodium acetate. Drug solutions were prepared in this medium, and pH was readjusted

to 7.4. The N-methyl-D-aspartic acid receptor channel blocker, MK-801 ($1 \mu\text{M}$), was added to all solutions to avoid activation of this receptor by the D-isoform of DL-AP4.² Patch pipettes were made from borosilicate glass, coated with Sylgard, and their tip fire-polished. Pipettes had resistances of 3–5 megohms when filled with the following internal solution (in mM): cesium acetate (100), MgCl_2 (2), HEPES (10), glucose (15), CsCl (20), EGTA (20), Na_2ATP (2), and cAMP (1) adjusted to pH 7.2 with CsOH and 300 mosm with cesium acetate.

Barium currents were evoked by 500-ms voltage clamp pulses, from a holding potential of -80 mV, to a test potential of 0 mV, applied at a rate of 0.1 Hz. 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 values expressed as mean \pm S.E. of the indicated number (*n*) of experiments. The Student's *t* test ($p \leq 0.05$) was used for comparison.

Materials—DL-Aminophosphonobutyrate (DL-AP4), dihydroxyphenylglycine (DHPG), and MK-801 were purchased from Tocris Cockson (UK). Nimodipine and GF109203X were purchased from Research Biochemicals. ω -Agatoxin-IVA and ω -conotoxin-GVIA were from Alomone Labs (Israel). LY354740 was a generous gift from D. Schoepp (Lilly). An antibody raised against the G_o protein was a generous gift from V. Homburger. This antibody has been shown previously to recognize specifically the G protein α_o subunit but not the α_i subunit (33).

RESULTS

Selective Blockade of Distinct Subtypes of Ca^{2+} Channels by the mGlu2 and mGlu7 Receptors—The effect of mGlu2 and mGlu7 receptors on Ba^{2+} currents (IBa) was studied in cultured cerebellar granule cells transfected with GFP alone (control) or in combination with either one of these receptors. In control neurons, the selective P/Q-type (ω -agatoxin-IVA; 250 nM), N-type (ω -conotoxin-GVIA; $1 \mu\text{M}$) and L-type (nimodipine; $1 \mu\text{M}$) Ca^{2+} channel blockers inhibited 41, 10, and 22% IBa, respectively. The remaining 27% IBa resulted from activation of the R-type Ca^{2+} channels. Similar data were obtained in non-transfected neurons (15) or neurons co-transfected with GFP and either the mGlu7 (Fig. 1A) or mGlu2 (Fig. 1C) receptors. These results indicated that the transfection procedure did not alter functional expression of the native Ca^{2+} channels.

As previously shown, the group III mGlu receptor agonist, DL-AP4 (500 μM), had no significant effect on IBa, in non-transfected or control (transfected with GFP alone) cerebellar granule cells (27), indicating the absence of mGlu7 receptor in the soma of these cells. After transfection, the mGlu7 receptor was expressed in the cell body, which allowed us to study the effect of this receptor on IBa (27). Under this condition, DL-AP4 selectively blocked IBa, and the inhibitory effect of this drug on IBa^{2+} was additive with those of ω -conotoxin-GVIA and nimodipine but not with that of ω -agatoxin-IVA (Fig. 1B). These results indicated that the transfected mGlu7 receptor blocked specifically P/Q-type Ca^{2+} channels.

In non-transfected (15, 34) as in mGlu2 receptor-transfected neurons (Fig. 1D), the mGlu2 receptor agonist, LY354740 ($1 \mu\text{M}$), selectively inhibited N- and L-type Ca^{2+} channels. Because cultured cerebellar granule cells express native mGlu2 receptor, LY354740 could activate both the endogenous and the transfected mGlu2 receptors. To rule out any activation of the endogenous receptor, we constructed the so-called mGlu1/2 chimeric receptor that harbored the N-terminal domain of the mGlu1 receptor and the seven transmembranes with C-terminal domains of the mGlu2 receptor (Fig. 2). This chimera was transfected in cultured cerebellar granule cells and activated with the group I mGlu receptor agonist, DHPG (400 μM). Because of the presence of native mGluR1 receptors in granule neurons, DHPG was applied in the presence of Bay36-7620 (10

²J. Perroy, G. J. Gutierrez, V. Coulon, J. Bockaert, J.-P. Pin, and L. Fagni, unpublished observations.

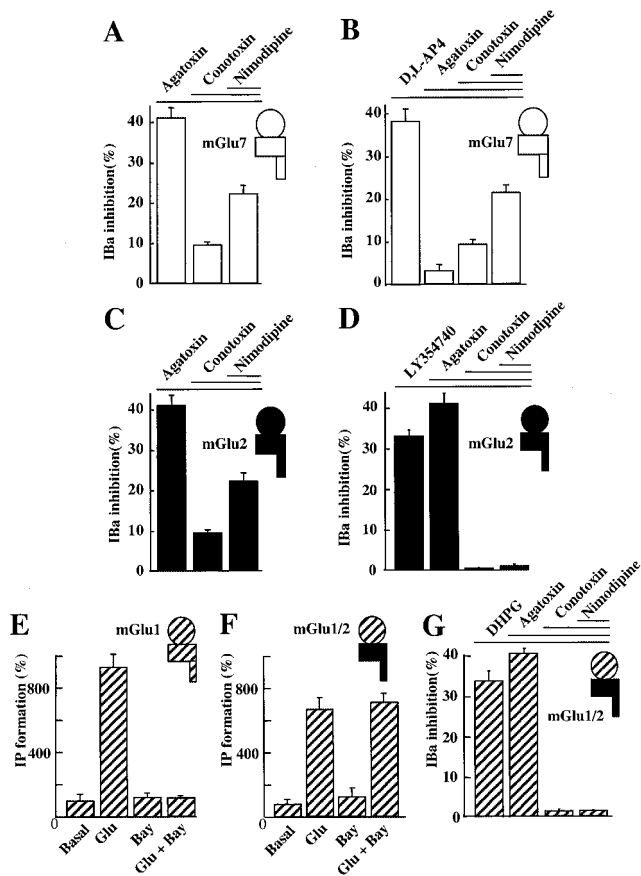


FIG. 1. Inhibitory effects of transfected mGlu2, mGlu7, or mGlu1/2 receptors on IBa. *A–D* and *G*, inhibitory effects of the indicated drugs and toxins (250 nM ω -agatoxin-IVA, 1 μ M ω -conotoxin-GVIA, 1 μ M nimodipine, 500 μ M DL-AP4, 1 μ M LY354740, and 400 μ M DHPG) obtained in cultured cerebellar granule cells co-transfected with GFP and either the mGlu7 (*A* and *B*), mGlu2 (*C* and *D*), or mGlu1/2 receptor (*G*). Each bar of histogram represents the mean (\pm S.E.) of at least eight experiments. *E* and *F*, Bay36-7620 is selective for the mGlu1 over the mGlu1/2 receptor. IP accumulation was measured in HEK-293 cells transiently expressing mGlu1 (*E*) or mGlu1/2 (*F*) receptors and incubated in the presence of 1 mM glutamate (Glu), 10 μ M Bay36-7620, or both. The data are expressed as the percentage of basal IP formation obtained in the absence of drug (*Basal*). Each bar of histogram represents the mean \pm S.E. of four independent experiments performed in triplicate.

μ M), a non-competitive and highly selective mGlu1 receptor antagonist that acts within the transmembrane domains of this receptor (30). Thus, this antagonist blocked the native (32) or recombinant mGlu1 receptor (Fig. 1*E*) but not the mGlu2 (30) or mGlu1/2 receptor (Fig. 1*F*). In neurons transfected with the mGlu1/2 receptor, DHPG induced similar IBa inhibition (Fig. 1*G*) as the mGlu2 receptor agonist (Fig. 1*D*), indicating that the transfected mGlu1/2 receptor displayed similar coupling to N- and L-type Ca^{2+} channels, as the native mGlu2 receptor, in the same cells.

In cerebellar granule cells co-transfected with the mGlu7 and mGlu1/2 receptors, the respective receptor agonists induced selective and additive inhibitions of P/Q- and N/L-type Ca^{2+} channels (Fig. 3). Thus co-transfection and overexpression of the mGlu1/2 and mGlu7 receptors did not change the selective coupling of these receptors to distinct Ca^{2+} channels in the studied neurons.

We have shown previously that in neurons transfected with the mGlu7 receptor, intracellular injection of a specific antibody raised against the G protein α_o subunit blocked the inhibitory effect of DL-AP4 on IBa, without significantly altering the current density in the absence of agonist (27). Here, the same

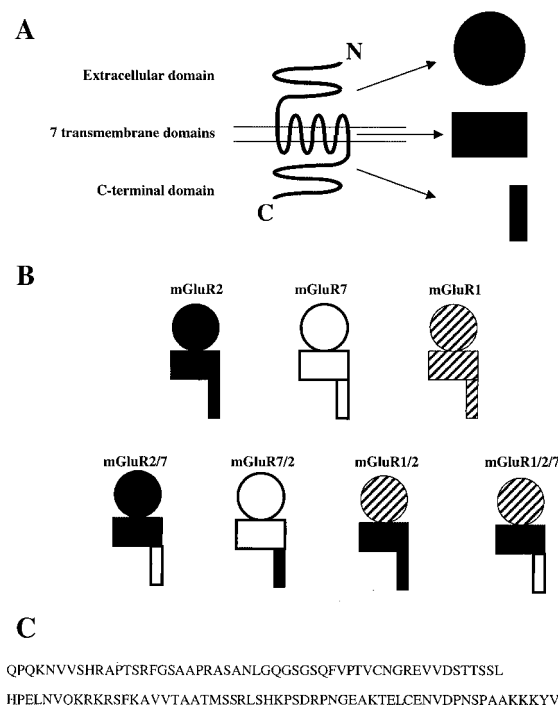


FIG. 2. Schematic representation of wild type and chimeric mGlu receptors. *A*, the circle, horizontal box, and vertical box represent the extracellular N-terminal, transmembrane, and intracellular C-terminal domains of mGlu receptors, respectively. *B*, schematic representation of the wild type and chimeric constructions. *C*, amino acid sequence of the exchanged C-terminal domains of mGlu2 and mGlu7 receptors.

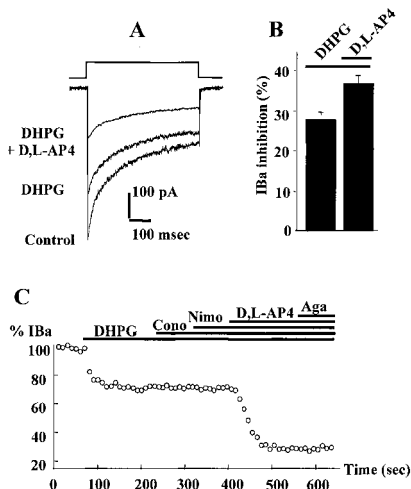


FIG. 3. Additive inhibitory effects of the mGlu1/2 and mGlu7 receptors on IBa, in mGlu1/2, and mGlu7 co-transfected cerebellar granule cells. *A*, IBa recorded in a co-transfected neuron, in the absence (*control*) or presence of the indicated drugs. *B*, inhibitory effects of the mGlu7 (DL-AP4) and mGlu1/2 (DHPG) agonists on IBa. Each bar of histogram represents the mean (\pm S.E.) of at least seven experiments. Note that the mGlu7 and mGlu1/2 agonists induced similar IBa inhibitions as in neurons transfected with mGlu7 or mGlu1/2 alone (Fig. 1, *B* and *G*). *C*, time course of the inhibitory effect of the indicated drugs and toxins.

antibody injected in mGlu2-transfected neurons antagonized the inhibitory effect of LY354740 on IBa (LY354740 induced $12 \pm 2\%$ inhibition; $n = 7$). The boiled antibody was without effect. These results showed that both mGlu2 and mGlu7 receptors acted via a G_o protein.

The C Terminus of mGlu2 and mGlu7 Receptors Determines Their Coupling Selectivity to Ca^{2+} Channels—We investigated

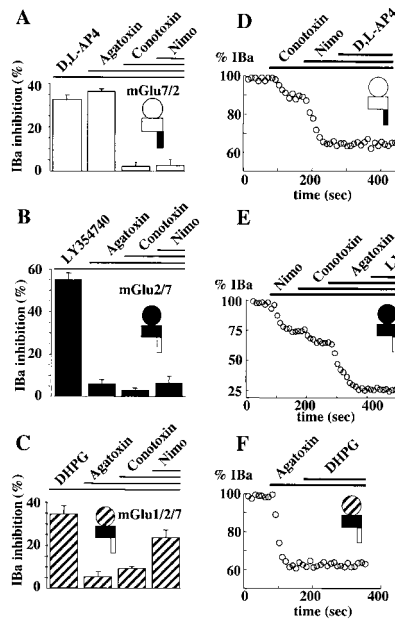


FIG. 4. Inhibitory effect of the mGlu7/2, mGlu2/7, or mGlu1/2/7 receptors on IBA in transfected cerebellar granule cells. A–C, inhibitory effects of agonist and toxins on IBA. Each bar of histogram represents the mean (\pm S.E.) of at least 10 experiments. D–F, inhibitory effect of reversed applications of the agonists and toxins on IBA. Each graph was obtained from a different single neuron and was representative of at least 5 other cells. Note the selective blockade of N- and L-type IBA by the mGlu7/2 receptor agonist (DL-AP4) in A and D, N-; L- and P/Q-type IBA by the mGlu2/7 receptor agonist (LY354740) in B and E; P/Q-type IBA by the mGlu1/2/7 receptor agonist (DHPG) in C and F.

the role of the C terminus of mGlu2 and mGlu7 receptors in the selective coupling of these receptors to Ca^{2+} channels. To this end, we exchanged the C terminus of the mGlu2 and mGlu7 receptors to obtain the reciprocal mGlu2/7 and mGlu7/2 chimeras (Fig. 2, A and B). The amino acid sequences of the exchanged C-terminal regions are represented in Fig. 2C. In mGlu7/2-transfected neurons, DL-AP4 inhibited 32% of IBA. The remaining IBA was not significantly affected by ω -conotoxin-GVIA or nimodipine. However, the current was further depressed by ω -agatoxin-IVA (Fig. 4A) and in similar proportions as in control cells (compare with Fig. 1A). These results show that exchanging the C terminus of mGlu7 receptor with the one of mGlu2 receptor switched the selective coupling of the mGlu7 receptor to that of the mGlu2 receptor. No statistical difference was observed between cell surface immunolabeling of the mGlu7 and mGlu7/2 receptors (immunofluorescence values: 7.0 ± 0.6 , $n = 10$; 7.4 ± 0.8 , $n = 10$, respectively; $p \leq 0.05$), indicating that the difference observed in the selective coupling of these receptors to Ca^{2+} channels could not result from differential cell surface expression of the receptors.

In mGlu2/7-transfected cells, LY354740 blocked 55% of IBA. Following this inhibition, neither ω -conotoxin-GVIA, nimodipine, nor ω -agatoxin-IVA further inhibited IBA (Fig. 4B). These results were consistent with a blockade of P/Q-type as well as N- and L-type Ca^{2+} channels by the mGlu2 receptor agonist, probably through activation of the transfected mGlu2/7 and native mGlu2 receptors, respectively. To avoid any activation of the endogenous mGlu2 receptor, we transfected the mGlu1/2/7 receptor construct (N terminus of mGlu1 with the seven transmembrane domains of mGlu2 and the C terminus of mGlu7; Fig. 2) and stimulated this chimera with DHPG, in the presence of Bay36-7620. Under these conditions, DHPG inhibited 34% of IBA. Application of ω -conotoxin-IVA and nimodipine, in the presence of these drugs, inhibited 9 and 23% of total IBA, whereas ω -agatoxin-IVA had no significant additional effect

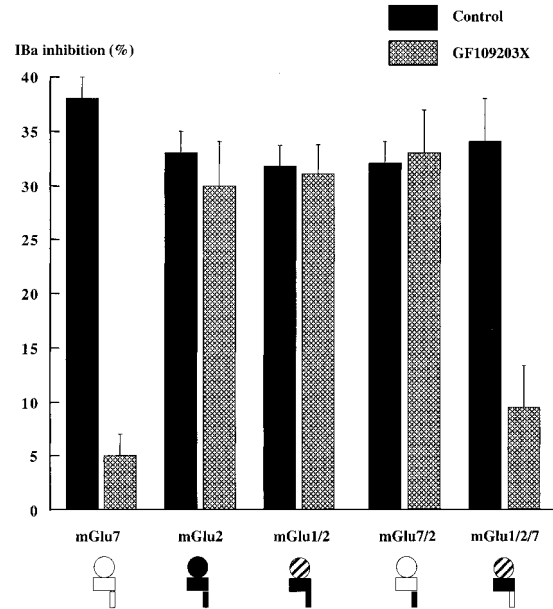


FIG. 5. Involvement of a PKC pathway in mGlu7- and mGlu1/2/7- but not in mGlu2-, mGlu1/2-, or mGlu7/2-mediated inhibitions of IBA in transfected cerebellar granule cells. Mean (\pm S.E.; $n = 5$ to 10) fractional reduction of the IBA induced by DL-AP4 (500 μ M) in mGlu7 and mGlu7/2 transfected neurons, LY354740 (1 μ M) in mGlu2 transfected neurons and DHPG (400 μ M), in mGlu1/2 and mGlu1/2/7 transfected neurons, treated or not for 30 min with the PKC inhibitor, GF109203X (10 μ M).

(Fig. 4C). These results indicated that DHPG blocked selectively P/Q-type Ca^{2+} channels. In a separate series of experiments, an initial application of a given channel blocker was immediately followed by application of the transfected receptor agonist. Thus, in mGlu7/2 receptor-transfected cells, after blockade of N- and L-type Ca^{2+} channels with ω -conotoxin-GVIA and nimodipine, respectively, the resistant IBA was not affected by subsequent application of DL-AP4 (Fig. 4D). In mGlu2/7-transfected cells, after blockade of N-, L-, and P/Q-type Ca^{2+} channels with ω -conotoxin-GVIA, nimodipine, and ω -agatoxin-IVA, the drug/toxin-insensitive IBA was not further depressed by application of LY354740 (Fig. 4E). Finally, in mGlu1/2/7 receptor-transfected cells, after blockade of P/Q-type Ca^{2+} channels with ω -agatoxin-IVA, DHPG did not further inhibit the remaining IBA (Fig. 4F). Altogether these results indicated that exchanging the C terminus of mGlu2 with the one of mGlu7 receptor switched the selective coupling of mGlu2 to the one of mGlu7 receptor.









We verified that the switch in the coupling to Ca^{2+} channels by exchanging mGlu receptor C termini resulted from a switch in the receptor signaling pathway. The PKC inhibitor, GF109203X (10 μ M), strongly reduced the amount of mGlu7 or mGlu1/2/7 receptor-induced IBA inhibition without affecting mGlu2, or mGlu1/2, or mGlu7/2 receptor-induced inhibition (Fig. 5). These results indicated that the C terminus of mGlu2 and mGlu7 receptors specified the receptor signaling pathway, and thus the selectivity of the receptor coupling to Ca^{2+} channels, in neurons.

DISCUSSION

As reviewed in Table I, our results show for the first time that the C terminus of mGlu2 and -7 receptors is a molecular determinant involved in the selectivity of coupling to specific effectors. To characterize this molecular feature, we used chimeric receptors transfected in neuronal cultures. This type of preparation provided a more physiological environment than classical heterologous expression systems, notably for studying

TABLE I
Results summary

Receptors harboring the mGlu7 C terminus select a PKC pathway and specifically inhibit P/Q-type Ca^{2+} channels, whereas receptors harboring the mGlu2 C terminus do not activate the PKC pathway and selectively inhibit N- and L-type Ca^{2+} channels. The C terminus of these receptors is necessary and sufficient to select a specific signaling pathway.

Activated receptors		Inhibited Ca^{2+} channel type		Involvement of PKC pathway
Transfected	Native	P/Q	N,L	
mGlu7 		*		YES
mGlu2 	mGlu2 		*	NO
mGlu1/2 			*	NO
mGlu7/2 			*	NO
mGlu2/7 	mGlu2 	*	*	NOT TESTED
mGlu1/2/7 		*		YES

receptor transduction signaling, because the natural transduction pathway and effectors were expected to be present in our model. Moreover, we verified that the transfection and overexpression of the studied wild type receptors did not modify their signaling pathway and coupling to Ca^{2+} channels as compared with the endogenous receptors. We also verified that the differences observed between functional responses of the wild type and corresponding chimeric receptors did not result from differential cell surface expression of these receptors.

The existence of a large number of GPCR-mediated signaling pathways, with respect to the limited number of functional G protein subunits, raises the question of how a particular receptor is selectively coupled to a given effector. We provide insight into this question by showing that the C terminus of two G_o protein-coupled mGlu receptors is a key molecular determinant for selecting a specific signaling pathway. This part of the mGlu receptors has not been reported to be involved in the coupling specificity to G protein α subunit (3, 4). This was consistent with the idea that activation of a specific class of G protein by a given GPCR does not seem to be sufficient to determine the specificity of the receptor transduction pathway. For instance in sympathetic neurons, various neurotransmitter receptors have been shown to inhibit N-type Ca^{2+} channels whatever the nature of the α and $\beta\gamma$ subunits of the activated recombinant G protein (21).

Consistent with our findings, a growing body of evidence suggests that in addition to the G protein activation, the C terminus of mGlu receptors plays an essential role in the selection of a specific signaling pathway. Because the C terminus of the receptor is the target of a number of kinases and intracellular proteins (35), these factors may support the selectivity of the receptor coupling. The two following examples are in favor of this hypothesis. A first example is the differential coupling of the mGlu5 receptor to either combined IP_3 and cAMP or single IP_3 pathways, depending on the PKC-mediated phosphorylation of two C-terminal serine residues at positions 881 and 890 (35–37). A second example is the PKC-mediated phosphorylation of a C-terminal threonine residue, at position 840 of the mGlu5 receptor. This phosphorylation is responsible for generation of Ca^{2+} oscillations in response to agonist. By contrast, the mGlu1a receptor displays an aspartate residue in

this corresponding position and triggers a single peak Ca^{2+} response (38). Therefore, because of differential phosphorylation of their C terminus, mGlu1 and mGlu5 receptors can display different signaling cascades, although both receptors activate G_q proteins. The same might apply to mGlu2 and mGlu7 receptors, but no such functional specific differential phosphorylation sites have been reported so far for these receptors. Therefore, such a hypothesis would need further specific examination.

Proteins that interact differentially with the C terminus of mGlu2 and mGlu7 receptors are also good candidates for selective activation of different transduction pathways. To our knowledge, no protein has been shown to interact directly with the C terminus of the mGlu2 receptor. By contrast, the mGlu7 receptor C terminus displays binding sites for a number of intracellular proteins (39). Indeed, both calmodulin and $\text{G}\beta\gamma$ subunits can bind, in a mutually exclusive manner, to a subdomain in the C terminus of the mGlu7 receptor. Thus, dissociation of the $\beta\gamma$ subunits from the activated G protein interacts with the mGlu7 receptor C terminus. However, the binding of calmodulin to the receptor will promote dissociation of the G protein $\beta\gamma$ subunits from the receptor, thus making these subunits available for inhibiting P/Q-type Ca^{2+} channels (40). Therefore, it appears that inhibition of Ca^{2+} channels by the mGlu7 receptor may not solely depend on activation of a G protein but also on protein-protein interaction in the C terminus of the receptor. A second candidate is the PDZ domain-containing protein, protein interacting with C kinase 1 (PICK1). This protein interacts with the very last 3 C-terminal amino acids of the mGlu7a receptor (41–43). The PDZ domain of PICK1 also interacts with $\text{PKC}\alpha$ (44) thus creating a functional complex comprising the mGlu7 receptor, a PICK1 dimer and $\text{PKC}\alpha$ (42). These associations may be functionally important in the PKC-dependent coupling of the mGlu7 receptor to P/Q-type Ca^{2+} channels.

Our results may be of particular interest in understanding the physiological significance of alternative splicing of GPCRs. Strikingly, the variable part in GPCR splicing, including the class of mGlu receptors (12), is their C-terminal domain. For example, the natural mGlu7a receptor splice variant, mGluR7b, is generated by an out-of-frame insertion in the C terminus that results in the replacement of the last 16 amino acids of the mGlu7a receptor by 23 different amino acids (45, 46). According to our finding that the C terminus of mGlu receptors plays a key role in the receptor coupling to specific effectors, different mGlu receptor splice variants could lead to different signaling pathways, depending on the splice site choice.

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