Direct Interaction Enables Cross-talk between Ionotropic and Group I Metabotropic Glutamate Receptors*§

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Functional interplay between ionotropic and metabotropic receptors frequently involves complex intracellular signaling cascades. The group I metabotropic glutamate receptor mGlu5a co-clusters with the ionotropic N-methyl-D-aspartate (NMDA) receptor in hippocampal neurons. In this study, we report that a more direct cross-talk can exist between these types of receptors. Using bioluminescence resonance energy transfer in living HEK293 cells, we demonstrate that mGlu5a and NMDA receptor clustering reflects the existence of direct physical interactions. Consequently, the mGlu5a receptor decreased NMDA receptor current, and reciprocally, the NMDA receptor strongly reduced the ability of the mGlu5a receptor to release intracellular calcium. We show that deletion of the C terminus of the mGlu5a receptor abolished both its interaction with the NMDA receptor and reciprocal inhibition of the receptors. This direct functional interaction implies a higher degree of target-effector specificity, timing, and subcellular localization of signaling than could ever be predicted with complex signaling pathways.

Presynaptic glutamate release activates multiple receptors that are assembled onto the postsynaptic membrane by scaffolding proteins (1) and determine the neuronal response. Activation of the glutamate ionotropic receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid subtype is responsible for the fast excitatory postsynaptic currents. Concomitant activation of glutamate ionotropic receptors of the N-methyl-D-aspartate (NMDA)2 subtype mediates a slow excitatory postsynaptic current (2) that can trigger bidirectional modifications of synaptic strength: long-term potentiation (3) or long-term depression (4). The metabotropic glutamate (mGlu) receptors are subdivided into three groups based on sequence homology, preferred associated second messenger systems, and pharmacological properties (group I, mGlu1 and mGlu5; group II, mGlu2 and mGlu3; and group III, mGlu4 and mGlu6–mGlu8). mGlu1a and mGlu5a/b receptor subtypes are mainly postsynaptic and positively coupled to Gq proteins, stimulation of phospholipase C (5), protein kinase C, and mobilization of intracellular calcium via inositol 1,4,5-trisphosphate receptors. These receptors can also be synthetically activated by glutamate and participate in the modulation of postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor currents (4, 6–8). Electrophysiological experiments have shown functional interplay between mGlula/5 and NMDA receptors in various structures of the brain. Indeed, activation of mGlu5 receptors can enhance NMDA-evoked responses in the hippocampus, striatum, cortex, and spinal cord (9–18), suggesting that the functional interactions between the mGlu and NMDA receptors are of widespread significance.

Because both NMDA and mGlu receptors are important players in synaptic transmission, there has been considerable interest in identifying the mechanisms underlying the functional interactions between these two classes of glutamate receptors. In this work, using bioluminescence resonance energy transfer (BRET), we identified direct physical interaction between the mGlu5a and NMDA (NR1/NR2B) receptors, which supports mutual functional constitutive inhibition of the receptors.

EXPERIMENTAL PROCEDURES

Plasmids—The plasmids encoding the wild-type mGlu5a, mGlu1a, and mGlu7a receptors tagged with a hemagglutinin (HA) or c-Myc epitope inserted immediately after the signal peptide, under the control of a cytomegalovirus promoter (pRK5-Myc-mGlu5a, pRK5-5HA-mGlu1a, and pRK5-Myc-mGlu7a), were described previously (19). The plasmids encoding the NR1A and NR2B subunits of the NMDA receptor (pRcCMVaa-NR1A and p3apA-e2-NR2B) were a generous gift from J. Neyton. For BRET experiments, a flexible linker (SGGGGS) was added in-frame to the 5′-end coding sequence of yellow fluorescent protein (YFP) and luciferase in the pcDNA3.1-YFP and phRLuc plasmids. The entire coding regions of Myc-mGlu5a (or HA-mGlu1a or Myc-mGlu7a) and NR1A were then subcloned into pcDNA3.1-YFP and phRLuc to obtain pcDNA3.1-Myc-mGlu5a-YFP and pcNR1A-hRLuc, respectively. For cell-surface immunolabeling and enzyme-
linked immunosorbent assay experiments in transfected HEK293 cells, the coding region of NR1A-hRluc was subcloned into pGFP2-C3 (PerkinElmer Life Sciences) in-frame with the 3′-end of GFP2 to obtain pGFP2-NR1A-hRluc, which codes for the NR1A-hRluc subunit tagged in its extracellular part with GFP2. The mGlut5 mutant pcDNA3.1-Myc-mGlut5a-i1YFP was generated by inserting the enzyme restriction sites MluI and NotI after the first transmembrane domain and introducing the YFP tag (amplified by PCR) in frame before the first intracellular loop. The mutant obtained, Myc-mGlut5a-i1YFP, displays the YFP tag (framed by two SGGGGS linkers) inserted between Tyr581 and Arg582. To obtain the pcDNA3.1-Myc-mGlut5a-DelCtail-YFP mutant, we amplified the Myc-mGlut5a receptor without its C-terminal tail (until the sequence GKSVT at position 861) and Arg582. To obtain the pcDNA3.1-Myc-mGlut5a-DelCtail-YFP mutant, we amplified the Myc-mGlut5a receptor without its C-terminal tail (until the sequence GKSVT at position 861) between the restriction sites SpeI and AgeI by PCR. The mGlut5a-DelCtail PCR fragment was then subcloned into pcDNA3.1-YFP.

**HEK293 Cell Culture and Transfection**—HEK293 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin and streptomycin, and 2 mM L-glutamine (all from Wisent Biocenter). For transfection experiments, cells were seeded at a density of 2 × 10⁵ cells/100-mm dish and cultured for 24 h. Transient transfections were then performed using the calcium phosphate precipitation method (20). Twenty-four hours after transfection, Dulbecco’s modified Eagle’s medium was renewed, and the cells were cultured for an additional 24 h.

**Immunolabeling in Non-permeabilized HEK293 Cells**—HEK293 cells were fixed with 3% paraformaldehyde in phosphate-buffered saline for 15 min and incubated in blocking buffer (phosphate-buffered saline containing 0.2% bovine serum albumin). HEK293 cells were incubated with rabbit IgG anti-GFP antibody (1:1000; Molecular Probes) or mouse IgG anti-Myc epitope antibody (1:200; Molecular Probes) in blocking buffer for 30 min. Cells were then washed and incubated with Alexa Fluor 594-labeled anti-rabbit secondary antibody (1:1000; Molecular Probes) and Alexa Fluor 488-labeled antimouse secondary antibody (1:1000; Molecular Probes) for 30 min in the dark. After extensive washing, the coverslips were mounted for immunolabeling analyses using a Zeiss fluorescence microscope equipped with a Plan-Apo 63× 1.40 numerical aperture oil immersion lens.

**Immunoprecipitation**—Cells transfected with Myc-mGlut5a-YFP and NR1A-Rluc + NR2B were lysed in 0.1% Triton X-100, 150 mM NaCl, 2 mM EGTA, anti-protease mixture (Roche Applied Science), and 20 μM Tris–HCl, pH 7.4 (lysis buffer), and the mixture was centrifuged. The lysate obtained from 10⁷ cells and 5 μg of goat anti-Myc antibody incubated for 1 h at room temperature were co-immunoprecipitated using solubilized Sepharose-protein A (Sigma) in lysis buffer. After extensive washing, the solid phase was incubated in Laemmli buffer at 90 °C. A control assay was performed under the same conditions but without anti-Myc antibody.

**Western Blot Analyses**—Protein samples were resolved by 7.5% PAGE, transferred to nitrocellulose, and subjected to immunoblotting using mouse anti-NR1 antibody (1:500; Zymed Laboratories Inc.). The nitrocellulose was then incubated with horseradish peroxidase-conjugated anti-mouse antibody (1:10,000; Amersham Biosciences) for 30 min and developed using the Renaissance chemiluminescence kit (PerkinElmer Life Sciences).

**Intracellular Calcium Measurements**—After transfection, HEK293 cells were seeded in polyornithine-coated, black-walled, clear bottom 96-well plates and cultured for 24 h. Cells were then washed with freshly prepared buffer and loaded with the Ca²⁺-sensitive fluorescent dye Fluo-4-acetoxymethyl ester (1 μM; Molecular Probes) for 1 h at 37 °C. Cells were washed and incubated with 50 μl of buffer. A drug plate was prepared with the various concentrations of agonist to be tested, and 50 μl of 2× drug solution was added to each well after 20 s of control recording. Fluorescence signals (excitation at 485 nm and emission at 525 nm) were measured using a FlexStation fluorescence microplate reader (Molecular Devices) at sampling intervals of 1.5 s for 60 s.

**Electrophysiology**—HEK293 cells cotransfected with the transfection marker GFP and the plasmids of interest were selected based on their fluorescence for whole-cell patch-clamp recording at room temperature. The recording pipettes had resistances of 3–5 megohms when filled with the following medium: 140 mM CsCl, 0.5 mM CaCl₂, 20 mM EGTA, 10 mM HEPES, and 10 mM D-glucose, pH 7.2 (osmolarity of 300 mosM). Cells were continuously perfused with the following external medium: 140 mM NaCl, 2 mM CaCl₂, 3 mM KCl, 10 mM HEPES, 10 mM D-glucose, 0.01 mM glycine, and 0.0003 mM tetrodotoxin, pH 7.4 (osmolarity of 330 mosM). The NMDA receptor currents were recorded through an Axopatch 200B amplifier (Molecular Devices) and filtered at 1 kHz. The data were then digitized at 3 kHz, and maximal current amplitude was measured using pCLAMP 9 software (Molecular Devices). Agonists were applied using a fast gravity perfusion system.

**BRET**—Cells were counted by measuring protein concentration (DC protein assay kit, Bio-Rad). For total fluorescence and luminescence measurements, cells were dispersed in 96-well plates with clear bottoms (Costar) at a density of 100,000 cells/well. We measured the total fluorescence of the cells in a Mithras LB 940 instrument (Berthold Technologies) using an excitation filter at 485 nm and an emission filter at 530 nm. The light emitted at 530 nm upon light excitation at 485 nm (fluorescence) is indicative of the amount of YFP-tagged proteins. We then incubated cells with DeepBlue C coelenterazine (5 μM) to measure the total luminescence using the Mithras instrument. The light emitted at 400 nm in the presence of DeepBlue C (luminescence) is indicative of the amount of Rluc-tagged proteins. For BRET measurements, cells coexpressing NR1A-Rluc + NR2B and mGlut5a-YFP constructs were distributed in white 96-well microplates (Corning) at a density of 100,000 cells/well. We initiated BRET by adding 5 μM coelenterazine H and measured the ratio of the light emitted by YFP (510–550 nm) to the light emitted by Rluc (460–500 nm) using the Mithras instrument. Values were corrected by subtracting the background ratio detected when the Rluc construct was expressed alone.

**Quantification of Cell-surface Receptor Expression Using Enzyme-linked Immunosorbent Assay**—Cells expressing Myc-mGlut5a and/or GFP-NR1A + NR2B were fixed with 4% paraformaldehyde in phosphate-buffered saline and then
blocked with phosphate-buffered saline plus 5% fetal bovine serum. After a 30-min reaction with one of the primary antibodies (rabbit IgG anti-GFP antibody (1:1000) or mouse IgG anti-Myc antibody (1:200; Molecular Probes)) in the same buffer, goat anti-rabbit (or anti-mouse) antibody coupled to horseradish peroxidase (1:500; Jackson Immunoresearch Laboratories, Inc., West Grove, PA) was applied for 30 min. Secondary antibody was detected and instantaneously quantified by chemiluminescence (SuperSignal West Femto, Pierce) using a Wallac VICTOR2 luminescence counter (PerkinElmer Life Sciences).

Statistical Analyses—Statistical significance of the mean value differences was determined using Student’s t test analysis, with a p value of 0.05.

RESULTS

Direct Interaction between NMDA and Group I mGlu Receptors—In an attempt to detect direct interaction between the mGlu5a and NMDA receptors, human NR1A and mGlu5a cDNAs were fused at their C termini to the energy donor Rluc and acceptor YFP, respectively (GFP2-NR1A-Rluc and Myc-mGlu5a-YFP) for BRET measurements. We verified the correct cell-surface expression of the tagged proteins (Myc-mGlu5a-YFP and GFP2-NR1A-Rluc) using antibodies raised against their extracellular tags (anti-Myc and anti-GFP2, respectively). Immunolabeling of non-permeabilized HEK293 cells transfected with Myc-mGlu5a-YFP or GFP2-NR1A-Rluc and NR2B revealed that both fusion proteins were present at the cell surface (supplemental Fig. S1A). Moreover, both constructs were functional when transfected in HEK293 cells. NMDA-induced currents were not significantly different in HEK293 cells cotransfected with wild-type NR1A and NR2B compared with cells coexpressing NR1A-Rluc and NR2B (supplemental Fig. S1B), and stimulation of recombinant mGlu5a receptor or mGlu5a-YFP induced similar intracellular Ca²⁺ release (supplemental Fig. S1C).

Having these tagged receptors functionally expressed, we studied the energy transfer between Rluc and YFP in cells coexpressing NR1A-Rluc, NR2B, and Myc-mGlu5a-YFP receptors using the membrane-permeable luciferase substrate coelenterazine H. Hydrolysis of coelenterazine H leads to the emission of light with a spectrum overlapping the excitation spectrum of YFP, thus allowing BRET. At a constant level of NR1A-Rluc + NR2B expression, the BRET signal increased hyperbolically as a function of the mGlu5a-YFP expression level (Fig. 1A), indicating a specific interaction between the two proteins (21). Furthermore, at a constant donor/acceptor ratio (fluorescence/luminescence = 0.104 ± 0.015), the BRET signal remained constant whatever the total level of receptor expression (Fig. 1B). This demonstrated that the interaction between the NMDA and mGlu5 receptors was not just a phenomenon of highly overexpressed proteins. To confirm that the signal specifically reflects the interaction process, experiments were performed in the absence of the NR2B subunit, which precluded membrane targeting of the NR1A-Rluc subunit (22). This led to dramatically weaker energy transfer with mGlu5a-YFP. Moreover, the BRET signal increased linearly (rather than hyperbolically) with mGlu5-YFP expression, which most likely reflected random collision (bystander BRET) (Fig. 1A). To confirm the interaction between the mGlu5a and NMDA receptors, we coimmunoprecipitated Myc-mGlu5a-YFP with the NR1A-Rluc receptor subunit in HEK293 cells cotransfected with these proteins and NR2B (Fig. 1C). Taken together, the results demonstrated that the measured energy transfer between NR1A-Rluc + NR2B and Myc-mGlu5a-YFP resulted from close interaction, rather than from a nonrandom clustering of these two proteins.

To further investigate the specificity of the receptor interactions, we monitored the energy transfer between the NMDA
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**FIGURE 2.** Detection of agonist modulation of the interaction between the NR1A-Rluc and Myc-mGlu5a-YFP constructs. A, HEK293 cells cotransfected with a constant concentration of NR1A-Rluc + NR2B and increasing concentrations of plasmid encoding Myc-mGlu5a-YFP were incubated in 5 μM coelenterazine H. Cells were then stimulated or not with DHPG (100 μM) or NMDA (100 μM) for 10 s before BRET measurement. The data shown are representative of five independent experiments. B, shown is the real-time measurement of receptor interaction upon agonist stimulation. Cells cotransfected with NR1A-Rluc + NR2B and Myc-mGlu5a-YFP (fluorescence/luminescence (Fluo/Lumi) ratio of 0.09 ± 0.005) constructs were incubated in the presence of coelenterazine H before stimulation with DHPG (100 μM) or NMDA (100 μM). BRET measurements were then immediately performed for 100 s (every 2.7 s) in the continued presence of the agonist. The real-time agonist-promoted increase in BRET is expressed as the difference in the absolute BRET value obtained in the presence and absence of DHPG or NMDA.

A mGlu1a receptor was tagged with HA in its extracellular domain and with YFP in the C-terminal intracellular part (see “Experimental Procedures”). At a constant level of NR1A-Rluc + NR2B expression, the BRET signal increased hyperbolically as a function of HA-mGlu1a-YFP expression, indicating a specific interaction between the NMDA and mGlu1a receptors. Thus, both group I mGlu receptors (mGlu5 and mGlu1a) could interact with the NMDA receptor. In contrast, the weak and linear BRET signal observed between NR1A-Rluc + NR2B and increasing concentrations of the group III Myc-mGlu7a-YFP receptor did not reflect specific interaction between the tagged partners, but rather random collisions (Fig. 1D). This observation is indicative of the specificity of the interaction between the NMDA receptor and group I over group III mGlu receptors.

**FIGURE 3.** Reciprocal modulation of the NMDA and mGlu5a receptors. A, NMDA whole-cell currents were recorded in HEK293 cells transfected with the NMDA receptor NR1 and NR2B subunits in the presence or absence of the mGlu5a receptor at −65 mV. In the last experimental condition, DHPG (100 μM) was co-applied with NMDA (100 μM). Each bar of the histogram is the mean ± S.E. of 5–10 cells. B, intracellular Ca2+ release was induced by the mGlu5 receptor agonist DHPG in HEK293 cells transfected with the mGlu5a receptor in the presence or absence of the NMDA receptor subunits. In the last experimental condition, NMDA was co-applied with DHPG. Each bar of the histogram is the mean ± S.E. of five experiments. C, left panel, the total amount of receptors was estimated by the emission of GFP2 at 535 nm when excited at 480 nm (Fluo) and the light emitted at 400 nm in the presence of DeepBlue C (Lumi). Right panel, enzyme-linked immunosorbent assay was performed to quantitate cell-surface receptor expression upon single transfection or cotransfection. Results are expressed as a percentage of the N-terminally tagged Myc-mGlu5a receptor (left bars) or GFP2-NR1A (right bars). Data represent the mean ± S.E. of three independent experiments. *p < 0.05.

Reciprocal Receptor Cross-talk—The spontaneous interaction between the NMDA and mGlu5a receptors and its regulation by agonists suggested the existence of a relevant functional interaction between the receptors. As shown in Fig. 3A, HEK293 cells cotransfected with the NR1A and NR2B subunits displayed NMDA-induced current. Interestingly, coexpression of the mGlu5a receptor strongly reduced this current in the absence of DHPG. These results indicate an agonist-independent/constitutive inhibition of the NMDA receptor by the mGlu5a receptor. Moreover, no additional effect was obtained by co-stimulation of the mGlu5a receptor with DHPG (Fig. 3A).

Reciprocally, when expressed alone in HEK293 cells, the stimulated mGlu5a receptor (100 μM DHPG) induced a marked intracellular Ca2+ release, which was significantly reduced when the NMDA receptor (NR1A + NR2B) was coexpressed (Fig. 3B). Again, concomitant application of NMDA did not significantly affect the DHPG response. Thus, the NMDA receptor constitutively inhibited the mGlu5a receptor. Notably, the total amount of receptors (estimated by the fluorescence emitted by GFP2-NR1A and the luminescence of Myc-mGlu5a-Rluc) was not significantly different when those receptors were expressed alone or together. We subsequently verified using enzyme-linked immunosorbent assay measurements that the cell-surface expression of the receptors was not affected by their cotransfection (Fig. 3C). The results indicate a
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Loss of Physical Interaction Results in Loss of Reciprocal Modulation between the mGlu5a and NMDA Receptors—We further studied whether the direct physical interaction between the receptors could underlie their reciprocal modulation. We generated a mGlu5a-YFP mutant deleted of its C-terminal tail. This mutant was no longer able to physically interact with the functional NMDA receptor (NR1A-Rluc + NR2B) as assessed by loss of the BRET signal in cotransfected HEK293 cells (Fig. 5A) and the absence of co-immunoprecipitation (supplemental Fig. S2). It was also unable to spontaneously inhibit NMDA currents (Fig. 5B) and was not constitutively modulated by the NMDA receptor (Fig. 5C), although it was still expressed at the cell surface data (not shown) and correctly coupled to intracellular Ca\(^{2+}\) stores (Fig. 5C). These results demonstrate that the reciprocal and constitutive inhibition of the mGlu5a and NMDA receptors is entirely dependent on their physical interaction. This further supports the hypothesis of a mutual steric effect between the co-assembled mGlu5a and NMDA receptors. In addition, the C-terminal tail of the group I mGlu receptor fused to the lymphocyte transmembrane protein CD4 was sufficient for co-immunoprecipitation with the NMDA receptor (supplemental Fig. S3). More precisely, progressive deletions of the CD4-mGlu1 C-terminal tail indicated the distal 108 amino acids to be necessary for interaction with the NMDA receptor (supplemental Fig. S3).

This mutual regulation probably resulted from a direct steric blockade of the co-assembled receptors. Indeed, the effect was observed in the absence of agonist, and the presence of agonist did not potentiate the effect. Moreover, the mGlu5a receptor did not spontaneously induce intracellular Ca\(^{2+}\) release (compare the calcium release obtained in the absence of agonist in untransfected (mock) and Myc-mGlu5a-transfected cells) (Fig. 3B), although it constitutively blocked NMDA receptor currents (Fig. 3A). To further test the hypothesis of a G-protein-independent blockade between the mGlu5a and NMDA receptors, we generated a mGlu5a receptor tagged with YFP within the first intracellular loop. The so-called Myc-mGlu5a-i1YFP receptor was successfully expressed at the cell surface (Fig. 4A). However, this construct was unable to activate G-protein (Fig. 4B). Despite the lack of G-protein activation, the BRET signal increased hyperbolically as a function of the mGlu5a-i1YFP expression level at a constant level of NR1A-Rluc + NR2B expression (Fig. 4C). This reflected a specific interaction between the two proteins. Furthermore, the mGlu5a-i1YFP construct was still able to inhibit the NMDA receptor currents in the absence of agonist (Fig. 4D). It is therefore likely that the constitutive regulation of the NMDA receptor by the mGlu5a receptor is independent of the conventional mGlu5 receptor activity and intracellular signaling pathway.
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**DISCUSSION**

In this study, we have reported that the mGlu5a and NMDA receptors can physically interact together and undergo a reciprocal constitutive inhibition. To study the occurrence and dynamics of this protein-protein interaction, we used the BRET technology in living cells. In this assay, the efficacy of the energy transfer depends on the close proximity (<10 nm) and orientation of the donor and acceptor entities (23, 24). With the average radius of proteins being 50 Å, the occurrence of resonance energy transfer is interpreted as a strong indication that the proteins attached to the energy donors and acceptors, respectively, are indeed in direct contact. Our data thus provide evidence of a direct interaction between the mGlu5a and NMDA receptors. Although BRET measurements were performed between the mGlu5a and NR1A subunits, our data do not allow us to conclude whether the interaction occurred between mGlu5a and the NR1A or NR2B subunit of functional NMDA receptors.

Our study also clearly demonstrates that the interaction allowed reciprocal modulation of the mGlu5a and NMDA receptors. Concomitant expression of both receptors induced a basal mutual functional inhibition without affecting cell-surface receptor expression. Combined with the lack of agonist-promoted functional regulations, these results suggest that the mutual regulation is an agonist-independent/constitutive regulation.

This reciprocal cross-talk between the mGlu5a and NMDA receptors indicates a non-canonical mechanism of action. The mGlu5a receptor did not display constitutive activity with respect to its intracellular calcium signaling, but this lack of classical G-protein pathway activation did not prevent the constitutive inhibition of NMDA receptor currents. Moreover, the mGlu5a receptor mutant mGlu5a-i1YFP, which was defective in G-protein activation and displayed perfect interaction with the NMDA receptor, still inhibited NMDA currents in a constitutive manner. This definitively excluded a possible involvement of the conventional G-protein pathway in this effect. Furthermore, a truncated mGlu5a receptor construct lacking the entire C-terminal domain (mGlu5a-DelCtail-YFP) efficiently coupled to G-protein activation but was unable to promote the BRET signal with the NMDA receptor and totally lost its ability to inhibit NMDA currents. The loss of BRET was certainly attributable to the suppression of a direct physical interaction between the two receptors, rather than an increase in donor and acceptor tag distance, because the YFP tag in the mGlu5a-DelCtail-YFP mutant was positioned under the membrane cell surface, as was the case for the mGlu5a-i1YFP construct, which still displayed a BRET signal with the interacting NR1A-RLuc subunit. Taken together, these results strongly suggest that the physical interaction between the NMDA and mGlu5a receptors was responsible for their mutual functional regulation.

We found that the other group I mGlu receptor, mGlu1a-YFP, could also interact with the NMDA receptor, whereas mGlu7a-YFP (group III) could not. This specificity of interaction with the NMDA receptor was in harmony with the endogenous subcellular location of these receptors. Indeed, group I mGlu receptors as postsynaptic entities are neighbors of the NMDA receptor, whereas mGlu7a receptors are confined to the presynaptic compartment. Previous reports have already emphasized functional modulations between group I mGlu and NMDA receptors. However, this work highlights mutual antagonism, whereas synergism is often observed in neurons of various brain regions (9–13, 17, 18). Our report describing mutual inhibition between the mGlu5a and NMDA receptors therefore exacerbates the controversy regarding the role of mGlu receptors in the modulation of NMDA receptor functions (14, 25, 26). Group I mGlu receptors have been reported to both potentiate and paradoxically depress excitatory synaptic transmission in the CA1 region of the hippocampus (26). Furthermore, co-activation of the NMDA and mGlu5 receptors results in a profound enhancement of NMDA receptor currents and miniature excitatory postsynaptic currents. If, however, the mGlu5 receptor is activated in the absence of NMDA receptor stimulation, the resulting activity of the NMDA receptor and the miniature excitatory postsynaptic current display a modest depression (14). This apparent discrepancy may rely on the physical association of NMDA receptors with mGlu receptors probably via the Homer-Shank complex (27). The direct association and mutual antagonism of the two receptors that we describe here might be precluded in neurons by the binding of scaffolding proteins to the C termini of the receptors (Homer-Shank on the mGlu5 receptor and guanylate kinase-associated protein-PSD95 on the NMDA receptor). Nevertheless, such multiprotein receptor complexes are certainly not immutable, and the receptor-protein interactions may rather undergo regulated association/dissociation features depending on the physiological conditions. For example, Homer1a is a short splice variant of Homer1b/c that lacks the ability to link mGlu1a/5 receptors to other postsynaptic proteins, including Shank, and thus disassembles the synaptic glutamate receptor signaling complex (28–33). Interestingly, Homer1a expression is regulated by neuronal activity. When the multiprotein postsynaptic receptor complex is disrupted by Homer1a, the mGlu1a/5 and NMDA receptors may become free to directly interact in neurons and undergo reciprocal constitutive inhibition.

Such an activity-dependent regulation of mGlu1a/5a and NMDA receptor function may explain the dual role of group I mGlu receptors in modulation of NMDA receptors that has been described in the literature. A recent report emphasizes the importance of the C-terminal tail of group I mGlu receptors in attenuation of NMDA-induced neurotoxicity (34). This neuroprotective effect is independent of G-protein activation and is removed following NMDA receptor activation through a calpain-mediated truncation of the mGlu receptor C-terminal tail. Our findings may contribute to a better understanding of this effect.

It is generally accepted that activation of metabotropic receptors engages one or more intracellular signaling cascades that control the function and/or localization of a cohort of ligand-gated ion channels. Conversely, stimulating ionotropic receptors can indirectly affect metabotropic signaling cascades. Our results show that metabotropic and ionotropic glutamate receptors can reciprocally modulate each other via more direct interaction, thus contrasting with this consensual paradigm.
Such an emerging theme of direct interaction between structurally unrelated membrane receptors is indeed reinforced by the discoveries of other receptor couples: D5-GABA\(_A\) (35), D1-NMDA (36), and GABAB-GABA\(_A\) (37). Together, these studies illuminate a previously unappreciated richness in the functional interactions between transmembrane receptor proteins. This type of direct interaction could impart a much higher degree of target-effector specificity, timing, and subcellular signaling than could ever be envisaged with the mass effects expected from activating second messenger cascades.

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