Recently, evidence has emerged that seven transmembrane G protein-coupled receptors may be present as homo- and heteromers in the plasma membrane. Here we describe a new molecular and functional interaction between two functionally unrelated types of G protein-coupled receptors, namely the metabotropic glutamate type 1α (mGlu1α receptor) and the adenosine A1 receptors in cerebellum, primary cortical neurons, and heterologous transfected cells. Co-immunoprecipitation experiments showed a close and subtype-specific homology, agonist selectivity, and signal transduction pathways categorized into three subgroups on the basis of their sequence features. In homomeric and heteromeric transfected cells, and have quisqualic acid as their most potent agonist. Five splice variants of mGlu1 receptor are functionally related. Our results provide a molecular basis for adenosine/glutamate receptor interactions cross-talk and open new perspectives for the development of novel agents to treat neuropsychiatric disorders in which abnormal glutamatergic neurotransmission is involved.

Glutamate is the major excitatory neurotransmitter in the central nervous system (1), and its function through ionotropic and metabotropic (mGlu)2 glutamate receptors can be modulated by other neurotransmitters/neuromodulators (2). Eight members of the mGlu receptor family have been identified and categorized into three subgroups on the basis of their sequence homology, agonist selectivity, and signal transduction pathway. Group I contains mGlu1 and mGlu2 subtypes, which are coupled to phospholipase C in transfected cells, and have quisqualic acid as their most potent agonist. Five splice variants of mGlu1 receptor have been described, mGlu1α, mGlu1β, mGlu1γ, mGlu1δ, and mGlu1ε receptors (3, 4), all of them differing in the length of their C-terminal tail. The functional significance of the different splice variants has not yet been fully explored. It has been suggested that the C-terminal tail, which is intracellular, might play a role in the subcellular targeting of the receptor (5). Recently, we have reported that the C terminus of mGlu1α receptor interacts with tubulin (6) and that it can regulate the cell surface expression of the receptor (7) and its plasma membrane anchoring (8, 9).

Adenosine is an important neuromodulator implicated in a variety of brain activities, particularly those related to sleep and ischemic-hypoxic episodes (10). This ubiquitous nucleoside exerts its actions via specific receptors, four of which (A1, A2A, A2B, and A3) have been cloned (11). The A1R is functionally coupled to members of the pertussis toxin-sensitive family of G proteins (G i1, G i2, G i3, and G o), and its activation regulates several membrane and intracellular proteins such as adenylate cyclase, Ca2+ channels, K+ channels, and phospholipase C (11). Of the multiple neurophysiological actions of adenosine, inhibition of glutamate neurotransmission has been observed in several brain regions (12) and is probably a result of the inhibition of presynaptic calcium influx (13). Apart from this inhibitory effect, there is some evidence documenting functional interactions between adenosine and glutamate receptors in the central nervous system. Of particular interest are reports of group I mGlur receptors signaling being enhanced by group II mGlur receptors in hippocampal and cerebrogic slices (14–16) and by adenosine A1 receptors in cultured hippocampal type 1 astrocytes (17). Very recently, Toms and Roberts (18) have described that type 2 astrocytes contain group I mGlur receptors coupled to [Ca2+]i signaling and that co-activation of adenosine A1 receptors enhances group I mGlur-evoked [Ca2+]i responses in these cells via Gi/o G protein-mediated mechanism. Despite these observations, no clear molecular mechanism of this interaction between glutamate and adenosine receptors has been provided yet.

Here we report a molecular interaction between metabotropic glutamate receptor type 1α and the adenosine A1 receptor, two members of differentGPCR families. This interaction suggests that both receptors may form part of a signaling complex in vivo that could play a critical role in fine-tuning neurotransmission at glutamatergic synapses.

EXPERIMENTAL PROCEDURES

Cell Culture, Generation of mGlur Truncated Mutant, and Transfection—Human embryonic kidney cells, HEK-293, were grown as described (9). Rat mGlur1 receptor was truncated after amino acid position 885 (see Fig. 4). A stop codon was introduced into the coding sequence of the FLAG epitope containing mGlur1 receptor cDNA by polymerase chain reaction (8). Forward primer was MGR1-F7 (5′-GGCGCTGGGG-TGCATGTATCTC-3′), position 2833–2855 of the mGlur1 receptor cDNA...
Adenosine-Glutamate Receptor-Receptor Interactions

(Adenine–Glutamate Interactions) Cortical hemispheres from E16 rat embryos were dissected, and primary cultures of rat cortical neurons were prepared as described previously (19). The E18–21 days of age after 14–21 days in vitro. To determine the NMDA-mediated neurotoxicity, the culture-conditioned medium was collected, and the cortical neurons were washed once with serum-free Neurobasal medium (Life Technologies, Inc.) containing 50% B27-supplemented Neurobasal medium (Life Technologies, Inc.) containing 10% FBS (Life Technologies, Inc.) and then washed twice for 5 min in TBS. Fluorescein isothiocyanate-conjugated swine anti-rabbit IgG (Dako, Ely, UK) was applied in TBS at a dilution of 1:100.

Immunoprecipitation—Rat cerebellum synaptosomes (6) or transiently transfected HEK cells were collected, and the cortical neurons were washed once with serum-free B27-supplemented Neurobasal medium (Life Technologies, Inc.) containing 50% B27-supplemented Neurobasal medium (Life Technologies, Inc.) containing 10% FBS (Life Technologies, Inc.) and then washed twice for 5 min in TBS. Fluorescein isothiocyanate-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch Laboratories Inc., West Grove, PA) was applied in TBS at a dilution of 1:100.

Immunohistochemical studies showed that mGlu1a receptors are present in cerebellum, and its expression is mainly restricted to the cell body and the dendritic tree of Purkinje cells and basket cells located in the molecular layer (Fig. 1a). This is in agreement with the previously described location of these receptors in an annulus, which surrounds the post-synaptic density (27). The A1R had a more ubiquitous distribution in cerebellum being expressed in Purkinje cells and basket cells, as mGlu1 receptor, and also in granule cells (Fig. 1a). This fits with the localization of adenosine A1 receptors both presynaptically and postsynaptically (28). In addition, metabotropic glutamate receptor type 1a and adenosine A1 receptors showed a similar distribution in human cerebral cortex being expressed in large pyramidal cells located at layers V and III/IV (data not shown).

In vivo co-distribution of mGlu1a and A1 receptors in some cerebellar neurons (Fig. 1a) suggests a potential interaction between both receptors at precise brain areas. The existence of mGlu1a/A1 receptors’ hetroimers was assayed by co-immunoprecipitation experiments using a soluble extract from rat cerebellum synaptosomes that had been shown by Western blotting to contain both adenosine A1 (Fig. 1b, lane 1) and mGlu1a receptors (Fig. 1b, lane 2). When this soluble extract was immunoblotted using a specific antibody against A1 receptor (PC21-Ab), two bands of around 39 and 74 kDa of molecular size were shown (Fig. 1b, lane 3); these bands, which are glycosylated proteins (29), have been demonstrated previously to correspond to the adenosine A1 receptor monomer and dimer, respectively (22). On the other hand, a specific antibody against the mGlu1a receptor (F2-Ab), immunoblotted in the cerebellum synaptosomes extract a band with apparent molecular size of 150 kDa that corresponds to the mGlu1a receptor (Fig. 1b, lane 4), which is a glycosylated protein as it has been described previously (21, 20). From this extract, the antibody against A1R (PC11-Ab) immunoprecipitated a band of 150 kDa antibodies indicated in the figure legends and horseradish-peroxidase-conjugated swine anti-rabbit IgG (Dako, Ely, UK) as a secondary antibody. The immunoreactive bands were developed with the enhanced chemiluminescence detection kit (Pierce), as described previously (6).

Ligand Binding Experiments—Membrane suspensions from transiently transfected HEK cells were used after transfection. The membranes were prepared from cerebellum synaptosomes extract a band with apparent molecular size of 150 kDa that corresponds to the mGlu1a receptor (Fig. 1b, lane 4), which is a glycosylated protein as it has been described previously (21, 20). From this extract, the antibody against A1R (PC11-Ab) immunoprecipitated a band of 150 kDa.
that was detected by the F2-Ab (Fig. 1b). This band was also immunoprecipitated using a different antibody against mGlu1 receptor (F1-Ab) but was not present in immunoprecipitates generated with an irrelevant antibody (Fig. 1b, lane 5). It should be noted that the efficacy of immunoprecipitation of mGlu1 receptor by the anti-adenosine receptor antibody was much less than when an anti-mGlu1 receptor antibody was used. Overall, these results indicate that there are zones in which the two receptors co-distribute and that A1 and A1 receptors do not co-distribute and that there are zones in which the two receptors co-distribute and form aggregates (heteromers).

**Interaction of mGlu1a and A1 Receptors in Transiently Transfected HEK-293 Cells**—The close association of mGlu1a and A1 receptors was subsequently studied in co-transfected HEK-293 cells by co-immunoprecipitation and double immunolabeling experiments. By confocal microscopy analysis of HEK-293 cells transiently transfected with the cDNAs encoding for mGlu1a-FLAG or mGlu1a-FLAG and A1R. After 48 h cells were washed, fixed (−Triton), and/or permeabilized (+Triton) and processed for immunostaining with anti-FLAG monoclonal antibody (Sigma, Clone M2; 10 μg/ml) and anti-A1R affinity-purified antibody (PC21-Ab, 5 μg/ml). The bound primary antibodies were detected using either fluorescein-conjugated donkey anti-mouse IgG antibody (1/50) or Texas Red-conjugated donkey anti-rabbit (1/50). Cells were analyzed by double immunofluorescence with a confocal microscopy. Superimposition of images reveals mGlu1a (green) and A1 receptor (red) co-localization in yellow. The images show a single horizontal section of representative cells. Scale bar, 10 μm.

5). This band did not appear in immunoprecipitates from cells transfected with the cDNA for either A1 receptors (Fig. 3, lane 4) or mGlu1a (Fig. 3, lane 6) or when an irrelevant antibody was used (data not shown). Conversely, when we immunoprecipitate with the FLAG antibody to pull down mGlu1a-FLAG receptor in the transiently co-transfected HEK-293 cells, and subsequently the immunoprecipitates were analyzed by SDS-PAGE and immunoblotted using anti-A1R antibodies, a band which corresponds to the A1R was observed (Fig. 3, lane 11). Interestingly, no immunoprecipitation of mGlu1a receptor, the C-terminal splice variant of mGlu1 receptor (Fig. 4a), was obtained from co-transfected cells using the antibody against A1R (PC11-Ab) (Fig. 4b, lane 7). Additionally, the construct mGlu1-M7, a deleted mutant close to the splice variant site of the mGlu1 receptor (Fig. 4a), also did not interact with A1R (Fig. 4b, lane 8). Overall, these results suggest that the C-terminal tail of the mGlu1a receptor (Fig. 4a) is implicated in the interaction of both receptors.

To test the functional significance of an mGlu1a/A1 receptors interaction, measurements of calcium mobilization in co-transfected HEK-293 cells were performed. In HEK cells transiently expressing A1R or A1R plus mGlu1a-FLAG R-PIA mobilized intracellular calcium in a concentration-dependent manner, with an EC50 value of 46.8 ± 4.4 nM for the single expressing A1R cells or 45.7 ± 5.1 nM for the doubly A1R plus mGlu1a-FLAG expressing cells. On the other hand, in these cells quisqualicacid also mobilized intracellular calcium in a concentration-dependent manner, with an EC50 value of 4.1 ± 1.1 μM for the single expressing mGlu1a-FLAG cells or 5.6 ± 1.1 μM for the doubly A1R plus mGlu1a-FLAG expressing cells. The densities of the transiently expressed receptors were controlled by
FIG. 3. Interaction of mGlu₁₆ and A1 receptors in transiently transfected HEK-293 cells. Cells transiently expressing AIR alone (lanes 1 and 7), AIR plus mGlu₁₆-FLAG (lanes 2 and 8), or mGlu₁₆-FLAG alone (lanes 3 and 9) were washed and solubilized in ice-cold lysis buffer and processed for immunoprecipitation using anti-AIR antibodies (PC11-Ab; 10 μg/ml; lanes 4–6) and anti-FLAG monoclonal antibody (Sigma, Clone M2; 10 μg/ml; lanes 10–12). Solubilized membranes (Crude, lanes 1–3 and 7–9) and immunoprecipitates (IP, lanes 4–6 and 10–12) were analyzed by SDS-PAGE and immunoblotted (IB) using anti-mGlu₁₆ receptor antibodies (F2-Ab; 5 μg/ml; lanes 1–3) and anti-FLAG monoclonal antibody (FLAG; 10 μg/ml; lanes 4–6). Immunoreactive bands were detected as before. IgG indicates the position of the immunoglobulins used in the immunoprecipitation.

FIG. 4. Specificity of the interaction between mGlu₁₆ and A1 receptors in transiently transfected HEK-293 cells. a, schematic representation of the primary structure of rat mGlu₁₆ and mGlu₁₆ receptors. The gray arrowhead indicates the position of the stop codon introduced to generate the deleted mutant mGlu₁₆-M7. b, HEK-293 cells transiently expressing AIR alone (lane 1), AIR plus mGlu₁₆-FLAG (lane 2), AIR plus mGlu₁₆-FLAG (lane 3), and AIR plus mGlu₁₆-M7-FLAG (lane 4) were processed for immunoprecipitation using anti-AIR antibodies (PC11-Ab; 10 μg/ml). Solubilized membranes (Crude, lanes 1–4) and immunoprecipitates (IP, lanes 5–8) were analyzed by SDS-PAGE and immunoblotted (IB) using anti-mGlu₁₆ antibodies (F1-Ab; 5 μg/ml). Immunoreactive bands were detected as before. IgG indicates the position of the immunoglobulins used in the immunoprecipitation.

means of ligand binding experiments performed in these cells (see “Experimental Procedures”). Cells transfected with the cDNA for AIR alone express 3.4 ± 0.4 pmol of AIR/mg protein as detected by [³H]R-PIA binding with a Kᵦ of 27 ± 3 nM. In these cells the AIR agonist R-PIA leads to a calcium peak (Fig. 5). On the other hand, cells transfected only with the cDNA for mGlu₁₆ receptor express 3.3 ± 0.9 pmol of mGlu₁₆ receptor/mg protein as detected by [³H]quisqualic binding with a Kᵦ of 177 ± 46 nM, and a weak calcium peak was detected when treated with the agonist for the metabotropic glutamate receptor, quisqualic acid (Fig. 5). Interestingly, in HEK-293 cells co-transfected with both receptors (3.3 ± 0.2 pmol of AIR/mg of protein and 2.8 ± 1.5 pmol of mGlu₁₆/mg of protein), preincubation with quisqualic acid markedly potentiated the calcium peak obtained in response to AIR activation (140 ± 10%; n = 3). Conversely, preincubation of co-transfected cells with the agonist for AIR led to a marked enhancement of the signal provided by quisqualic acid (180 ± 20%; n = 3) (Fig. 5). Quisqualic acid or R-PIA failed to bind or to provide any signal in nontransfected HEK-293 cells. These results clearly show a heterologous sensitization or synergistic effect upon mGlu₁₆ and A1 receptors activation.

Interaction of mGlu₁₆ and A1 Receptors in Primary Rat Cortical Neurons—To assess the physiological relevance of the mGlu₁₆/A1 receptors interaction, we analyzed the distribution of both receptors in primary rat cortical neurons. Both receptors showed a similar punctate distribution throughout the proximal and distal dendrites, and the degree of co-localization at these locations was very high (Fig. 6, a–c). In fact, some of the mGlu₁₆ receptor or AIR-containing puncta co-distributed with the synaptic marker protein synaptophysin (Fig. 6, d–i), suggesting that they could be localized to synapses. Thus, synapses are one of the specific cellular sites where mGlu₁₆ and A1 receptors interact.

Glutamate/adenosine receptors interaction may be important for modulating the role of mGlu₁₆ receptor in neurodegeneration/neuroprotection, an issue that is still controversial. When examining this role a number of factors, including the...
heteromeric composition of NMDA receptors, the time of exposure to drugs or to ambient glutamate, and the function of astrocytes clearing extracellular glutamate and producing neurotoxic or neuroprotective factors must be taken into account (20). On the other hand, glutamate could also modulate the well known function of adenosine as neuroprotective factor (12). It is thus likely that the interaction of mGlu1/A1 receptors could be beneficial in situations of enhanced neuronal activity, in which potentiation of postsynaptic adenosine A1 receptor limits evoked depolarization and results in decreased activation of voltage-dependent Ca\(^{2+}\) channels and NMDA receptor ion channels, through which Ca\(^{2+}\) enters cell bodies (30). We have therefore examined the effect of activating of both mGlu1 and A1 adenosine receptors on NMDA-mediated neurotoxicity in primary neuronal cultures. In agreement with previously described data (20), submaximal concentrations of NMDA induced neuronal death, which was enhanced by the presence of quisqualic acid during the NMDA treatment (Fig. 7). In contrast, when the adenosine A1 receptor agonist, R-PIA, was present during the NMDA treatment, the induced neurotoxicity was reduced by nearly 50%. When added simultaneously, R-PIA reduced the enhancement of the neurotoxicity induced by quisqualic acid (Fig. 7). On the other hand, pre-exposure of neurons to R-PIA or to quisqualic acid also reduced the NMDA-induced neurotoxicity. This reduction was more marked if both quisqualic acid and R-PIA were present during the pre-exposure, showing that the simultaneous activation of both receptors appears to increase the protection of the neurons against the NMDA treatment compared with the effect evoked by either quisqualate or R-PIA applied separately. These results show the relevance of the interaction of mGlu1/A1 receptors and support the concept of specificity and complexity of this interaction, being the spatiotemporal segregation profile of adenosine/glutamate during synaptic activity of special importance to achieve a neuroprotective or a neurotoxic effect.

**FIG. 6. Co-localization of mGluR1α and A1R in primary cultures of rat cortical neurons.** Neurons (days in vitro 14–21) were fixed, permeabilized, and processed for immunostaining using rhodamine-conjugated anti-A1R antibody (PC21-Ab; 15 μg/ml) and fluorescein-conjugated anti-mGlu1α receptor antibody (F2-Ab; 15 μg/ml) (a–c), anti-A1R antibody (PC21-Ab, 5 μg/ml) and anti-synaptophysin monoclonal antibody (1/20) (d–f), or anti-mGlu1α receptor antibody (F2-Ab; 5 μg/ml) and anti-synaptophysin monoclonal antibody (1/20) (g–i). Primary antibodies in d–i were detected using fluorescein-conjugated donkey anti-mouse IgG antibody (1/50) or Texas Red-conjugated donkey anti-rabbit (1/50) antibody (1/20) or Texas Red-conjugated donkey anti-mouse IgG antibody (1/50) or Texas Red-conjugated donkey anti-rabbit (1/50) (a–c). Cells were analyzed by double immunofluorescence with a confocal microscopy. Images show A1R receptor (a) in red mGlu1α receptor (b) in green, A1R (d) and mGlu1α (g) receptors in red, and synaptophysin (h) in green. Superimposition of images reveals co-localization in yellow (c, f, and i). Scale bar, 10 μm.

**FIG. 7. Excitotoxicity in rat cortical neurons.** Cortical neurons were exposed for 10 min to 30 μM of NMDA. Quisqualic acid (Quis, 100 μM) and/or R-PIA (100 nM) were transiently applied (for 1 min) 5 min prior to the addition of NMDA (Pre-exposure) and/or added together with NMDA for 10 min (Treatment). Quisqualic acid treatments were always made in the presence of 50 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Sigma) to block non-NMDA receptors. Death neurons were computed by assessing the propidium iodide staining in photomicrographs of 10 representative fields from each monolayer of treated neurons and expressed as percent of NMDA toxicity. Asterisks denote differences from the control (*, p < 0.05; **, p < 0.01, two-tail t test).

**DISCUSSION**

Here we describe a novel interaction between two unrelated G protein-coupled receptors (GPCR), namely the metabotropic glutamate type 1α and the adenosine A1 receptors. Although the cooperativity of the agonist binding to GPCR suggested the possibility of oligomerization of G proteins and their associated receptors (31), the existence and the precise function of GPCR homo- or heteromerization has not been fully elucidated.

Metabotropic glutamate type 1α and adenosine A1 receptors show a high degree of co-localization in co-transfected cells and in more physiological systems as neurons from rat cerebellum and from rat cortex. Both receptors co-immunoprecipitate from co-transfected cells and from rat cerebellum synaptosomes,
suggesting that mGlu_{1a} and A1 receptors interact and that this interaction is physiologically relevant. From a functional point of view, heteromerization often results in facilitatory responses or synergistic effects. Thus, GABABR1 and GABABR2 receptors need to be co-expressed and assembled into heteromers to reach the cell surface (32–35). On the other hand, two functional opioid receptors, κ and δ, can heteromerize, which changes the pharmacology of the individual receptors and potentiates signal transduction (36). Also, it has been described for heterodimerizations for the CC chemokine receptor 2 (CCR2) and the CX chemokine receptor 4 (CXC4) and/or CC chemokine receptor 5 (CCR5) (37). Recently, it has been demonstrated that κ and δ opioid receptors can form heteromers with β_{2}-adrenergic receptors affecting their trafficking properties without any significant alteration in the ligand binding or coupling properties of the receptors (38). There are also some papers reporting similar interactions betweenGPCR and nonGPCR proteins, for example the receptor activity-modifying protein, a small protein containing only one putative transmembrane domain and a short cytosolic tail, acting as a chaperone protein that facilitates the cell surface targeting and modulator function of the calcitonin-receptor-like receptor (39).

Interestingly, recent data (40) indicated that a GPCR, the D5-dopamine receptor, physically interacts with the γ-aminobutyric acid A (GABAa) receptor, a GABA-operated Cl⁻ channel. This physical interaction was dependent on the presence of agonist for both receptors and necessary for the functional cross-talk between the D5 and GABAa receptors (40).

In the case of cells co-expressing mGlu_{1a} and A1 receptors, a glutamate/adenosine synergism was found at the level of calcium mobilization (Fig. 5). Furthermore, in experiments of neuroprotection performed in neuronal cultures, preincubation with quisqualic acid plus adenosine was much more effective than pretreatment with any of the compounds. These results suggest that activation of mGlu_{1a} and A1 receptors in the same neuron results in synergism. Heteromerization, however, does not always lead to facilitation or synergistic events. Thus, in basal ganglia, there is an adenosine-mediated antagonism of dopaminergic neurotransmission. This antagonism is in part due to cross-talk at the level of second messengers but is also mediated by formation of adenosine/dopamine receptor heteromers (41). Although it is difficult to ascertain to what extent the antagonism is mediated by heteromerization or to interference in signaling, there is evidence indicating that both events operate and are closely interrelated. Thus, in cells where A1R and D1-dopamine receptors are present, as in nigrostriatal GABAergic neurons, adenosine leads to both the disappearance of the high affinity site of D1 receptors, probably via conformational changes in A1R/D1R heteromers, and a reduction in dopamine-induced cAMP increases, an effect due to cross-talk at the adenylate cyclase level (41).

There is experimental and molecular modeling evidence that intramembrane domains are involved in the formation of homodimers of G protein-coupled receptors. Gouldson et al. (42) have hypothesized that domain swapping with involvement of transmembrane regions 5 and 6 is responsible for homo- and heteromerization of G protein-coupled receptors. In contrast, heterodimerization of GABAR1 and GABAB2 receptors is mediated by the coiled-coil interaction of the C-terminal cytoplasmic tails (35).

In the case of mGlu_{1a}/A1 receptors’ heteromers the interaction depends on the C terminus of mGlu_{1a} receptor as its splice variant, mGlu_{1d} receptor, which has a short and different C terminus, does not interact with A1R. Also a deleted mutant of mGlu_{1a} receptor, close to the splice variant site and missing nearly all the C terminus of the receptor, does not interact with A1R. The co-immunoprecipitation of mGlu_{1a} and A1 receptors might be due to a physical association between them, but it also could be the case that both receptors are recruited into a specific signaling complex at specific synapses via common interactions with other proteins.

One possible mechanism for this mGlu_{1a}/A1 receptor coupling is that it is directed by interactions of the cytoplasmic C terminus with specific targeting proteins. This type of targeting mechanism appears to operate for the synaptic localization of the ionotropic glutamate receptors and a number of different proteins, containing PDZ domains, which interact with specific C-terminal sequences of these receptors (43–47). Also, the EVH1-like domain (ENA/VASP homology domain) containing protein, which binds specifically to the C-terminal residues of mGlu1α receptor, has been described (48). This protein, termed Homer-1A, was isolated as a synaptic plasticity-regulated gene from rat hippocampus (48, 49). Additional proteins related to Homer-1A have also been described, namely Homer-1B, Homer-1C, Homer-2A, Homer-2B, Homer-2C, Homer-2D, Homer-3A, Homer-3B, Homer-3C, and Homer-3D (50–53).

The ability of Homer to link mGlu_{1a} receptor to Shank, a scaffolding multimeric signaling protein, may contribute to anchoring the mGlu_{1a} receptor to specific sites at the plasma membrane (8, 9).

Our efforts are directed to find a network of protein interactions that are shared by both receptors and that likely play a key role in the signaling mechanisms of both mGlu_{1a} and A1 receptors. The nature of the signaling complexes formed would depend on the type of receptors present in a given neuron, and the effect will be determined by the balance between concentrations of agonists in the synaptic cleft and timing of receptor activation. It has been proposed that the mechanism of the biphasic effects of quisqualic acid to increase NMDA toxicity if added together with NMDA or to reduce it if pretreated before NMDA is in part due to the functional switch described for the mGlu_{1a} receptor (54, 55, 20).

In mixed cortical or pure hippocampal neuronal cultures a first application of 3,5-dihydroxyphenylglycine, an agonist of the group I mGlur receptors, potentiated toxicity induced by submaximal concentrations of NMDA, whereas the same drug applied shortly after a brief pre-exposure, protected against neuronal death (20). Interestingly, the switch in the regulation of excitotoxic neuronal death was sensitive to protein kinase C inhibitors (20). This mechanism may explain the opposite results obtained with group I mGlur receptors, assuming that the influence of group I mGlur receptors on excitotoxic neuronal death will depend on the “functional status” of group I mGlur receptors (naive versus experienced or unphosphorylated versus phosphorylated receptors) (20).

In Fig. 7 we show that the timing in mGlu_{1a} and A1 receptors activation is very important to achieve a maximum effect in adenosine- and glutamate-mediated neuroprotection/ neurodegeneration. In fact, although adenosine added together with glutamate was protective for cultured neurons, this protection was nearly total by preincubation with the metabotropic glutamate receptor agonist.

Our data provide biochemical and functional evidence for mGlu_{1a} and A1 receptor-receptor interaction. The way A1R is involved in interactions with receptors for other neurotransmitters, using different receptor system to synchronize synaptic transmission, opens new perspectives to understand the actual role of this autacoid. Since mGlu_{1a} receptors seem to be involved in the pathophysiology of neuropsychiatric diseases such as Alzheimer’s and related disorders, this molecular interaction offers a new basis for the design of novel strategies to study the genesis and evolution of these diseases and of novel agents to treat them.

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