Metabotropic glutamate receptors: Beyond the regulation of synaptic transmission

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Summary
Metabotropic glutamate (mGlu) receptors are G-protein coupled receptors activated by glutamate, the major excitatory neurotransmitter of the CNS. A growing body of evidence suggests that the function of mGlu receptors is not restricted to the regulation of synaptic transmission. mGlu receptors are expressed in a variety of peripheral cells, including inter alia hepatocytes, pancreatic cells, osteoblasts and immune cells. Within the immunological synapses, mGlu receptors expressed by T cells might contribute to the vast array of signals generated by the antigen-presenting cells. mGlu receptors are also found in embryonic and neural stem cells. This suggests their involvement in the pathophysiology of brain tumors, which likely originates from cancer stem cells similar to neural stem cells. Ligands of mGlu3 and mGlu4 receptors are potential candidates for the experimental treatment of malignant gliomas and medulloblastomas, respectively.

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1. Introduction
Metabotropic glutamate (mGlu) receptors are members of class-C G-protein coupled receptors (GCPRs), which also includes the GABA_B receptor, the Ca^{2+}-sensing receptor, taste receptors, and pheromone receptors. Structurally,
mGlu receptors are characterized by (i) a long N-terminus domain, which contains a glutamate binding region termed Venus fly trap; (ii) an heptahelical domain, which is common to all GCPRs; and (iii) an intracellular C-terminus domain of various length depending on the specific mGlu receptor subtype. mGlu receptors function as dimers, with two molecules of glutamate being required for a full receptor activation. Positive and negative allosteric modulators bind to the heptahelical domain (Kunishima et al., 2000; Pin et al., 2004). There are eight mGlu receptor subtypes divided into three groups on the basis of their amino acid sequence, pharmacological profile, and transduction mechanisms. Group I includes mGlu1 and mGlu5 receptors (splice variants: mGlu1a-e; mGlu5a,b), which are coupled to Gq. Their activation stimulates the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P2) generating the second messengers inositol-1,4,5-trisphosphate (Ins-1,4,5-P3) and diacylglycerol (DAG). Ins-1,4,5-P3 releases Ca2+ from intracellular stores, whereas DAG facilitates the activation of protein kinase C (PKC). Both mGlu1 and mGlu5 receptors are activated by 3,5-dihydroxyphénylglycine (DHPG). Compounds Ro-016128 and CPCCOEt are examples of positive and negative allosteric modulators of mGlu1 receptors, respectively. Compounds CDPBP, CPHPA, and ADX47273 behave as positive allosteric modulators of mGlu5 receptors, whereas MEP, SIB1893, and the anxyolitic drug, fenobam, negatively modulate mGlu5 receptors. Group II includes mGlu2 and mGlu3 receptors, which are both coupled to Gi. Their activation produces pleiotropic effects including inhibition of adenyl cyclase, inhibition of voltage-sensitive Ca2+ channels, and activation of K+ channels. Both receptor subtypes are potently activated by a series of conformationally constrained glutamate analogues, which include LY354740 and LY379268. Compound LY341495 acts as an orthosteric mGlu2/3 receptor antagonist at nanomolar concentrations. A growing number of positive allosteric modulators including compounds 3-MPPTS and yPPTS selectively amplify responses mediated by mGlu2 receptors. Group III includes mGlu4, mGlu6, mGlu7, and mGlu8 receptors. All these receptors are coupled to Gi/Go proteins and produce effects similar to those produced by mGlu2/3 receptors. All these receptor subtypes are activated by L-2-amino-4-phosphonobutanoate (L-AP4) and by the endogenous compound, L-serine-O-phosphate. PHCCC behaves as a selective positive allosteric modulator of mGlu4 receptors (reviewed by Nakanishi, 1992; Pin and Duvoisin, 1995; Schoepf et al., 1999; De Blasi et al., 2001). Since the time of their discovery (Sladeczek et al., 1985; Nicoletti et al., 1986), mGlu receptors have been considered as classical synaptic receptors. mGlu1 and mGlu5 receptors are preferentially localized on postsynaptic cells and suggest that these receptors are key regulators of basic processes of cell biology, such as cell proliferation, differentiation, and survival. This moves from three curious findings related to mGlu receptors. First, the ability of glutamate to stimulate PtdIns-4,5,-P2 hydrolysis in brain slices is prominent in the first 10 days of postnatal life (up to 20–30 fold increase in inositolphosphate formation) but disappears in slices from adult animals, where mGlu1 and mGlu5 receptors are presumed to regulate synaptic transmission (Nicoletti et al., 1986). Second, mGlu1a and mGlu5 receptors show constitutive activity, i.e., they are also active in the absence of synaptic glutamate (reviewed by Pin and Duvoisin, 1995). Third, functional mGlu1 and mGlu5 receptors have been found in the cell nuclear membrane, and their activation generates Ca2+ transients inside the nucleus (O’Malley et al., 2003; Jong et al., 2005). What activates nuclear mGlu receptors and whether these receptors are involved in the regulation of the cell cycle or other nuclear processes is unclear, as yet.

2. Expression and function of mGlu receptors in non-neuronal cells that reside outside the CNS

The strongest suggestion that mGlu receptors are not exclusively synaptic receptors derives from numerous studies that demonstrate the presence of functional mGlu receptors in a number of peripheral non-neuronal cells, many of which do not even originate from the neural crest. Gu and Publicover (2000) found the presence of mGlu1b in rat femoral osteoblasts, where mGlu1 receptor agonists behave similarly to parathyroid hormone in inhibiting responses mediated by NMDA receptors. As a follow-up of these findings, Yoneda’s group found that functional mGlu4 and mGlu8 receptors are present in cultured osteoblasts (Hinoi et al., 2001), and that activation of these receptors with L-AP4 inhibits chondral mineralization in the rodent cartilage (Wang et al., 2005). Liver cells instead express mGlu5 receptors, the activation of which stimulates PtdIns-4,5-P2 hydrolysis, and is protective against cell damage produced by hypoxia or acetaminophen (Sureda et al., 1997; Storto et al., 2000, 2003, 2004). mGlu5 receptors have been implicated in the regulation of pancreatic hormone secretion, although their exact role is unclear (Tong et al., 2002; Uehara et al., 2004; Storto et al., 2006). An intriguing finding is the presence of mGlu5 receptors in insulin-containing vesicles purified from clonal pancreatic beta-cells, which also express vesicular glutamate transporters.
mGlu receptors and cell-to-cell communication

(Storto et al., 2006). Perhaps the metabolic glutamate that is synthesized in response to beta-cell activation and is transported inside the insulin-containing granules transmits signals across the granule membrane by activating mGlu5 receptors (Storto et al., 2006). Expression of mGlu1 and mGlu5 receptors is also found in rat and human testis, with mGlu5 receptors being present and functionally active in mature human sperm (Storto et al., 2001). Finally, an exciting set of data move mGlu receptors from the classical neurosyapsis to the “immunological synapsis” between the antigen-presenting cell (APC) and the T lymphocyte. Storto et al. (2000) first showed the expression of group I and group II mGlu receptors in isolated thymocytes and thymic stromal cell lines. Interestingly, mGlu1 receptors were found in immature CD4+/CD8+ thymocytes, whereas expression of mGlu5 receptors was restricted to the more mature CD4+/CD8+ and CD4+/CD8- thymocytes (Storto et al., 2000). The existence of mGlu receptors in the thymus has been confirmed by Rezzani et al. (2003), who have found that (i) mGlu2/3, mGlu4, and mGlu5 receptors are widely expressed in thymic cells; (ii) mGlu5 receptors are preferentially expressed in thymic medullary cells; and (iii) a 2-day treatment with the immunosuppressant, cyclosporin-A, inhibits the expression of all mGlu receptor subtypes in the thymus. A role for mGlu receptors in the activation and function of lymphocytes is emerging from a series of studies that focus on group I and group III mGlu receptors. Both mGlu1 and mGlu5 receptors are expressed by human Jurkat T-cell lines, whereas only the mGlu5 receptor is expressed by the human B-cell line, SKW6.4. In blood lymphocytes, the mGlu5 receptor is expressed constitutively and is positively coupled to adenyl cyclase, whereas the mGlu1 receptor is expressed in response to T-cell receptor activation, and is coupled to the activation of the MAPK pathway (Pacheco et al., 2004). Group III mGlu receptors are also expressed by rodent lymphocytes, and their pharmacological activation generates intracellular radical oxygen species and causes cell death (Bolldrey et al., 2004, 2005). In contrast, activation of group I mGlu receptors protects human T lymphocytes against activation-induced cell death (Miglio et al., 2005; Chiocchetti et al., 2006). Of great interest is that glutamate is now considered as a putative non-peptidic mediator of the immunological synapsis. APC in contact with T cells release substantial amount of glutamate through the membrane cystine/glutamate co-transporter. “Immunosynaptic” glutamate appears to activate group I mGlu receptors, thus contributing to regulate the initiation of T-cell-mediated immune responses (Pacheco et al., 2006).

3. mGlu receptors in stem/progenitor cells

That the action of glutamate extends beyond the classical niche of synaptic regulation is further suggested by the presence of mGlu receptors in stem cells of different origin. Interestingly, the mGlu5 receptor is the only mGlu receptor subtype expressed by undifferentiated embryonic stem cells, which are pluripotent cells isolated from the inner mass of the blastocyst. In these cells, activation of mGlu5 receptor cooperates with the cytokine, leukemia inhibitory factor in supporting self-renewal (Cappuccio et al., 2005). Pharmacological blockade of mGlu5 receptors with MPEP or receptor knockdown inhibits self-renewal and promotes differentiation of cultured embryonic stem cells by enhancing ubiquitination and degradation of c-Myc (Spinsanti et al., 2006). Differentiation of embryonic stem cells into “embryoid bodies” (floating aggregates of cells differentiating into cells of the three main germ layers) is associated with the induction of mGlu4 receptors, the activation of which drives cell differentiation toward the endoderm and mesoderm lineages (Cappuccio et al., 2006). At least mGlu3 and mGlu5 receptors are also expressed by neural stem cells, i.e., by stem/progenitor cells resident in the CNS, which give rise to neurons, astrocytes, and oligodendrocytes (Luyt et al., 2003, 2004). Neural stem cells can be isolated either from the embryonic brain or from two regions of persistent neurogenesis of the adult brain: the subventricular zone (SVZ) lining the lateral ventricles, and the subgranular zone (SGZ) of the hippocampal dentate gyrus. In neural stem cells isolated from the developing brain, activation of mGlu3 and mGlu5 receptors supports cell proliferation and survival, and knockout mice lacking mGlu5 receptors show a lower number of proliferating neuroprogenitors in the SVZ and SGZ (Di Giorgi-Gerevini et al., 2005). In cultured neural stem cells isolated from the mouse SVZ, pharmacological activation of mGlu3 receptors inhibits cell differentiation into GFAP+ astrocyte-like cells, an effect that may follow the inhibition of cAMP formation (Ciceroni et al., 2006). Functional mGlu3 and mGlu5 receptors are also found in progenitor cells of the astrocytic/oligodendrocytic lineage (Luyt et al., 2003, 2004). How mGlu receptors function in these cells is unknown, although activation of mGlu5 receptors has been found to protect cultured oligodendrocyte progenitors against excitotoxic death (Kelland and Toms, 2001).

4. mGlu receptors and cancer cells

The increasing awareness that cancer stem cells are the putative tumor-initiating cells in leukemias and a variety of solid tumors provides a link for the study of mGlu receptors in oncology. Putative stem cells of malignant gliomas (the most frequent malignant brain tumor of the adult age) bear similarities with type-B cells of the SVZ, including the generation of neurospheres in culture, the expression of immature markers, the response to the same mitogens and differentiating agents (Doetsch et al., 1999; Gritti et al., 1999; Reya et al., 2001; Sanai et al., 2005; Piccirillo et al., 2006; Schuurbiers, 2006; Vescovi et al., 2006). If activation of mGlu3 receptors stimulates proliferation and inhibits differentiation of neural stem cells, then one expects that receptor activation produces similar effects in glioma stem cells. Although this has not been examined, as yet, a number of studies indicate that mGlu3 receptor blockade inhibits proliferation of glioma cells. This has been shown using cultured cells prepared from human grade IV astrocytomas (glioblastoma multiforme) and glioma cell lines (D’Onofrio et al., 2003), and has been replicated in in vivo studies. Systemic administration of the mGlu2/3 receptor antagonist, LY341495, inhibits the growth of glioma cells implanted either under the skin or in the brain parenchima of nude mice (Arcella et al., 2005). This is particularly promising because malignant gliomas are highly resistant to chemo-

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and radiotherapy, and almost invariably recur following surgery. Activation of mGlu4 receptors can instead reduce the growth of medulloblastomas. These tumors, which are most frequent in children, originate from the transformed neuroprecursors of cerebellar granule cells. Initial studies have shown that granule cell neuroprecursors grown in culture respond to the mGlu4 receptor enhancer, PHCCC, with a reduced proliferation and an increased differentiation into mature neurons (Canudas et al., 2004). The same drug is also able to reduce proliferation of medulloblastoma cells in culture, and to limit the growth of medulloblastomas in mice lacking one allele of the Sonic Hedgehog receptor, Patch-1 (Iacovelli et al., 2006). What is even more interesting is that mGlu4 receptors are detected in biopptic samples of human medulloblastomas, and their expression is inversely related to the severity of medulloblastomas (Iacovelli et al., 2006). Finally, mGlu receptors may have a role in tumor growth even outside the CNS. Suzy Chen and collaborators have found by serendipity that the insertional mutant mouse TG3 develops spontaneous melanomas. Interestingly, the transgene is inserted into the mGlu1 receptor gene, and causes an ectopic expression of mGlu1 receptors in melanocytes (Pollock et al., 2003). Transgenic mice expressing mGlu1 receptors in melanocytes also form malignant melanomas (Pollock et al., 2003). It is intuitive that mGlu1 receptor antagonists/negative modulators may have a future in the experimental treatment of malignant melanomas.

5. Final comment

The emerging function of mGlu receptors in non-neuronal cells rises the question of how these receptors can be activated in the absence of synaptic glutamate. One should take into account that glutamate has a large metabolic pool and can be synthesized from â-oxo-glutarate, a by-product of the Krebs cycle. Metabolic glutamate that is not cleared intracelullarly can be transported outside the cell where it activates paracrine mechanisms of cell-to-cell communication and autocrine mechanisms of self-regulation. Both membrane mGlu receptors and intracellular mGlu receptors (e.g., nuclear receptors) might sense the state of metabolic activation of the cell. Finally, as discussed above, some mGlu receptors are constitutively active, and, therefore, their function critically depends on the expression levels. The new findings we have highlighted pave the way to a more integrated approach to the study of mGlu receptors, which may increase our knowledge of the real function of mGlu receptors and may gain new insights into basic mechanisms regulating cell biology and cell-to-cell communication.

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Conflict of interest

None declared.

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