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Invited review

Molecular mechanisms that desensitize metabotropic glutamate receptor signaling: An overview

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ABSTRACT

The purpose of the present article is to review our actual knowledge on the desensitization of metabotropic glutamate receptors based on the literature available so far, with the attempt to emphasize all converging data and to give a possible explanation to those evidences that still remain controversial. 1. We review our knowledge on the regulation of mGlu receptors based on the available literature 2. We report converging data and we comment on issues that still remain controversial.

This article is part of a Special Issue entitled 'Metabotropic Glutamate Receptors'.

1. Introduction

Receptor responsiveness is regulated at the level of the G-protein coupled receptors (GPCR) by a process termed desensitization. This involves a combination of events including receptor phosphorylation by different kinases, internalization and interaction with specific regulatory proteins. Receptor homologous desensitization is a "feedback" mechanism that protects against both acute and chronic receptor over-stimulation and occurs shortly after exposure of GPCR to agonists (Liggett, 2011). In the process of homologous desensitization, G-coupled receptor kinases (GRKs) were initially identified as serine/threonine kinases that, acting in concert with their functional partner arrestins, regulate the activity of most GPCR (recently reviewed by Penela et al., 2010). The GRKs, a family of seven members in mammals, phosphorylate the agonist-occupied receptor. Arrestin then binds to the phosphorylated receptor, which in turn uncouples from heterotrimeric G-proteins and becomes desensitized (Penela et al., 2003; Premont and Gainetdinov, 2007). Phosphorylated receptors are then targeted to clathrin-coated vesicles, where they are resensitized and recycled back to plasma membranes (Reiter and Lefkowitz, 2006; Moore et al., 2007). Besides this "classical paradigm", emerging evidence indicates that both GRKs and arrestins actively participate to signal propagation. They can interact with proteins involved in signal transduction, driving the signal and finely modulating the cellular responses to GPCR activation. The potential for GRKs to interact with different proteins is predicted by their molecular structure, which clearly indicates that these are multi-domain proteins. All GRKs share a highly conserved catalytic domain; in the βARK subfamily (which includes GRK2 and GRK3), this catalytic domain is flanked by an N-terminal domain and by a C-terminal domain. The N-terminal domain is important for receptor recognition, for intracellular membrane anchoring, and also contains an RH domain (regulator of G protein signaling (RGS) homology domain) which enables GRK2 and GRK3 to specifically interact with Gαq family members, thus blocking their interaction with their effector, phospholipase C beta (PLCβ). In the C-terminal region, GRK2 and GRK3 contain a pleckstrin homology domain (PH) that allows the interaction with the phosphatidylinositol 4,5-bisphosphate and free Gβγ subunits (Penela et al., 2010). It has been documented in several studies that GRK2 has a wide pattern of interacting proteins, ranging from GPCR, tyrosine kinase receptors such as PDGF-Rβ (Hildreth et al., 2004), to non-receptor substrates such as tubulin, synucleins, phosducin, ribosomal protein P2, the ERM family protein ezrin, the calcium-binding protein DREAM, IκBα or the p38 MAPK (Peregrin et al., 2006; Patial et al., 2009). Different studies have documented that β-arrestins act as scaffold proteins bringing to the receptors signaling

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molecules such as src, the phosphodiesterase PDE4, components of the MAPK cascade, components of the NFκB cascade and many others (reviewed in Reiter and Lefkowitz, 2006; Premont and Gainetdinov, 2007; DeWire et al., 2007).

Metabotropic glutamate (mGlu) receptors belong to class C of the GPCR superfamily. Similar to all GPCRs, mGlu receptors contain a heptahelical domain in the membrane region and they share with other members of class C GPCRs an extended extracellular N-terminal domain in which the binding region for glutamate is located. The 8 subtypes identified are classified into 3 groups based on amino-acid sequence, transduction mechanisms and pharmacological profile. Group I includes mGlu1 and mGlu5 receptors, which are coupled to Gq and activate phospholipase C-β (PLCβ). mGlu1 and mGlu5 receptors are mainly found in the peripheral portions of postsynaptic elements, where they modulate excitatory synaptic transmission and synaptic plasticity. Group II (mGlu2 and mGlu3) and Group III (mGlu4, mGlu6 mGlu7 and mGlu8) mGlu receptors are coupled to Gi/Go and negatively regulate adenylyl cyclase activity. All these receptor subtypes, with the exception of mGlu6, are preferentially localized on presynaptic terminals, where they inhibit neurotransmitter release (see Nicoletti et al., 2011 for a recent review). As the mGlu receptors have a significant similarity in overall structure, it sounds interesting to study the desensitization of these receptors, in order to establish the molecular determinants that drive their signal transduction towards specific cellular responses. The characterization of these mechanisms and their regulation might be important for the pathophysiology of the diseases in which mGlu receptors are known to be involved.

The purpose of the present article is to review our knowledge on the desensitization of mGlu receptors based on the literature available so far, with the attempt to emphasize all converging data and to comment on issues that still remain controversial.

2. Group I mGlu receptors

Group I mGlu receptors, mGlu1 and mGlu5 are by far the most extensively investigated among mGlu receptors. As expected, several mechanisms of receptor desensitization were identified, involving signaling-dependent kinases, GRK/arrestins, RGS proteins and also phosphorylation-independent GRK effects. It is conceivable that the relative contribution of these different mechanisms drives the final receptor-mediated response and this interplay likely depends on the relative expression of these proteins in different cell types.

2.1. mGlu1 receptors

2.1.1. Receptor signaling

After prolonged or repeated stimulation, mGlu1 receptors are profoundly desensitized. PKC is clearly involved in this process. Francesconi and Duvoisin (2000) have shown that mGlu1a receptor desensitization is mediated by PKC-induced phosphorylation at the critical theronine residue (T695) in the G protein-coupling domain localized to intracellular loop 2 of the receptor. The mutation of this residue to alanine retards mGlu1a receptor desensitization, whereas mutation to glutamic acid residue to mimic phosphorylation tends to uncouple the receptor from Gαq. The activated α subunit of the Gq (Gαq) can in turn be inhibited by RGS proteins (Saugstad et al., 1998). These RGS proteins work by interacting with Gα and by increasing the intrinsic GTPase activity of Gα, acting as GTPase-activating proteins (Berman and Gilman, 1998; Hepler, 1999). A PKC-independent component of mGlu1 receptor desensitization had been already identified in early studies (Catania et al., 1991). Accordingly, it was shown that GRKs are directly involved in the phosphorylation and homologous desensitization of mGlu1a receptors. A number of GRK family members (GRK2, GRK4, GRK5, and GRK6) phosphorylate mGlu1a receptor and contribute to the desensitization of this splice variant (Dale et al., 2000; Sallese et al., 2000). Overexpression of a catalytically inactive GRK2 mutant (GRK2-K220R) prevents the agonist-stimulated phosphorylation of mGlu1a receptors. This process, rather than facilitating mGlu1a receptor signaling, significantly attenuates agonist-stimulated inositol phosphates (InsP) formation, suggesting that GRK2-mediated mGlu1a receptor desensitization involves a phosphorylation-independent mechanism (Dale et al., 2000). Consistent with this observation, another study showed that in HEK293 cells GRK2 and GRK4 regulate mGlu1 receptor signaling by different mechanisms (Iacovelli et al., 2003). GRK4-dependent desensitization was fully phosphorylation-mediated, whereas GRK2 regulated receptor-stimulated InsP production by a phosphorylation-independent mechanism, which involves the functional RH domain present within the GRK2 N-terminus. The difference between GRK4 and GRK2 likely reflects the different ability of their N-terminal domains to interact with the activated Gαq and supports the idea that only GRK2 and GRK3 (members of the βARK subfamily) possess a functional RH domain, whereas the members of the GRK4 subfamily (namely GRK4, GRK5, and GRK6) do not interact with the G protein to regulate receptor signaling (Iacovelli et al., 2003). Accordingly, GRK2 mutants impaired in Gαq/11 binding (R106A, D110A, and M114A), interacted with mGlu1 receptors, but did not mediate mGlu1 receptor desensitization (Dhami et al., 2004; Sterne-Marr et al., 2004).

Sallese et al. (2000) showed that GRK4 is expressed in cerebellar Purkinje cells, where it regulates mGlu1 receptors. It was shown that an antisense treatment of cultured Purkinje cells, which significantly reduced the levels of GRK4 expression, induced a marked modification of mGlu1-receptor mediated responses, which was indicative of an impaired receptor desensitization. This suggests an important role for GRK4 in the physiology of Purkinje cells and the regulation of motor learning. β-Arrestins were not required for the homologous desensitization of mGlu1a (or mGlu1α) receptor signaling (Dale et al., 2001). Expression of the β-arrestin dominant negative mutant, βarrV53D, inhibited mGlu1 receptor-agonist stimulated MAPK activation, suggesting that βarrV53D prevents the interaction of endogenous β-arrestin with protein(s) involved in the formation of a signaling complex that mediates MAPK activation. The involvement of β-arrestin in agonist-dependent MAP kinases activation was confirmed in cerebellar Purkinje cells using an adenovirus vector to express βarrV53D (Iacovelli et al., 2003).

2.1.2. Receptor internalization

Several studies have shown that agonist-independent (constitutive) internalization represents an important feature of group I mGlu receptors in neurons and recombinant cells. In these cells, constitutive internalization was insensitive to receptor antagonists or to strategies that lower the amount of endogenous glutamate released into the culture medium (Dale et al., 2001). Constitutive internalization of mGlu1a receptors was independent of GRK-mediated phosphorylation, and relied on a constitutive receptor activity because it was suppressed by treating the receptor with inverse agonists (Pula et al., 2004). Controversial data exist regarding the role of β-arrestins in agonist-independent internalization of mGlu1a receptors. Dale et al. (2001) showed that the constitutive internalization of mGlu1a receptors was β-arrestin- and dynamin-independent, while Pula et al. (2004) demonstrated that it was both β-arrestin- and clathrin-dependent. As proposed by Pula et al. (2004) a possible explanation for this discrepancy is the difference in the experimental conditions employed, principally