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Selective regulation of recombinantly expressed mGlu7 metabotropic glutamate receptors by G protein-coupled receptor kinases and arrestins

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ABSTRACT

mGlu7 receptors are coupled to Gi/Go-proteins and activate multiple transduction pathways, including inhibition of adenylyl cyclase activity and stimulation of ERK1/2 and JNK pathways. mGlu7 receptors play an important role in cognition and emotion and are involved in stress-related disorders such as anxiety and depression and in susceptibility to convulsive seizures. In spite of these potential clinical implications, little is known on the mechanisms that regulate mGlu7-receptor signaling. Here we show that mGlu7 receptor-dependent signaling pathways were regulated in a complementary manner by different GRK subtypes, with GRK4 affecting the adenylyl cyclase and the JNK pathways, and GRK2 selectively affecting the ERK1/2 pathway. Additionally we found that the two isoforms of non-visual arrestins, i.e. β-arrestin1 and β-arrestin2, exerted opposite effects on mGlu7-receptor signaling, with β-arrestin1 positively modulating ERK1/2 and inhibiting JNK, and β-arrestin2 doing the opposite. This represents a remarkable example of "reciprocal regulation" of receptor signaling by the two isoforms of β-arrestin. Finally we found that β-arrestin1 amplified mGlu7 receptor-dependent ERK1/2 activation in response to L-AP4 (an orthosteric agonist), but not in response to AMN082 (an atypical mGlu7-receptor allosteric agonist). The different effect of β-arrestin1 on L-AP4- and AMN082-stimulated ERK1/2 phosphorylation is in line with the emerging concept of β-arrestin-biased agonists. The present study may open new perspectives in elucidating the physio-pathological roles of the mGlu7 receptor and may provide new insights for the possibility to develop specific (biased) agonists that can selectively activate different signaling pathways.

1. Introduction

mGlu7 receptors belong to group-III metabotropic glutamate receptors and share with other subtypes of the same group (e.g., mGlu4 and mGlu8 receptors) most of the functional and pharmacological properties (reviewed by Niswender and Conn, 2010; Conn and Niswender, 2006; Nicoletti et al., 2011). mGlu7 receptors are coupled to Gi/Go-proteins, and activate multiple transduction pathways, such as inhibition of adenylyl cyclase activity, and stimulation of ERK1/2 and the Jun kinase (JNK) pathways (Saugstad et al., 1994; Flor et al., 1997; Millán et al., 2002; Tian et al., 2010). Similarly to mGlu4 and mGlu8 receptors, mGlu7 receptors are localized pre-synaptically and modulate glutamate release in a context-dependent fashion. Martín et al. (2008) have found that mGlu7, $GABA_B$ and A1 adenosine receptors act in concert to reduce glutamate release mediated by N-type voltage sensitive Ca^{2+} channels in cortical synaptosomes. In contrast, repeated agonist exposure discloses a facilitatory role of mGlu7 receptors on glutamate release, which is mediated by stimulation of inositol phospholipid hydrolysis and membrane translocation of munc-13-1 (Martín et al., 2010, 2011). As opposed to mGlu4 and mGlu8 receptors, mGlu7 receptors display a very low affinity for glutamate (reviewed by Schoepp et al., 1999), and can therefore be activated by high concentrations of ambient glutamate. This suggests that

Abbreviations: GPCR, G protein-coupled receptor; mGlu, metabotropic glutamate receptor; GRK, G protein-coupled receptor kinase; FSK, forskolin; L-AP4, L-2-amino-4-phosphonobutanoate; MAPK, mytogen activated protein kinases; JNK, c-Jun N-terminal kinase; GRK2-K220R, kinase-dead GRK2 mutant; GRK2-Cter, C-terminal domain of GRK2.

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mGlu7 receptors regulate glutamate release during high synaptic activity. Accordingly, mice lacking mGlu7 receptors show an increased susceptibility to convulsive seizures (Sansig et al., 2001), and disruption of the interaction between mGlu7 receptors and PICK1 (protein interacting with protein kinase C-1) causes absence seizures (Bertaso et al., 2008). mGlu7 receptors play an important role in cognition and emotion and are involved in stress-related disorders such as anxiety and depression (O'Connor et al., 2010). Genetic deletion of mGlu7 receptors reduces anxiety and depression in several behavioral tests (Cryan et al., 2003; Callaerts-Vegh et al., 2006), and causes changes that are consistent with an antidepressant phenotype, e.g. increased BDNF levels in the hippocampus, and an enhanced sensitivity to the suppressive action of dexamethasone on the hypothalamic–pituitary–adrenal axis (Barden, 2004; Mitsukawa et al., 2006).

mGlu7 receptors are also found outside of the CNS, e.g. in hair cells and spiral ganglion cells of the inner hair, and in the colon mucosa (reviewed by Nicoletti et al., 2011). The evidence that common single nucleotide polymorphisms of GRM7 (the gene encoding the mGlu7 receptor) are strongly associated with the risk of developing age-related hearing impairment (presbycusis) links mGlu7 receptors to human pathology (Friedman et al., 2009). Activation of mGlu7 receptors in the colon mucosa increases fecal water content in a stress-induced defecation paradigm that models the irritable bowel syndrome (Julio-Pieper et al., 2010).

In spite of these potential clinical implications, little is known on the mechanisms that regulate mGlu7-receptor signaling. Homologous desensitization is a mechanism of G protein-coupled receptor (GCPR) regulation that rapidly occurs in response to prolonged agonist exposure. This process is mediated by two classes of proteins: G protein-coupled receptor kinases (GRKs) and arrestins. There are three subfamilies of GRKs: (i) the rhodopsin kinase (RK) subfamily, which includes RK (GRK1) and GRK7; (ii) the βARK subfamily, which includes GRK2 and GRK3; and (iii) the GRK4 subfamily, which includes GRK4, GRK5, and GRK7. The arrestin family includes four subtypes: the visual arrestin1 and arrestin4 are localized in the retina, where they regulate phototransduction while β-arrestin1 and β-arrestin2 are ubiquitous and modulate the signaling of the majority of GPCR. The GRKs phosphorylate the intracellular domains of agonist-activated GPCRs, thereby allowing arrestins to bind to phosphorylated GPCRs and uncouple GPCRs from heterotrimeric G proteins (Kohout and Lefkowitz, 2003). More recently it was documented that non-visual arrestins (β-arrestin1 and 2) can also behave as scaffolding proteins, linking receptors to downstream signaling pathways, such as the MAPK pathway (Luttrell and Lefkowitz, 2002; DeWire et al., 2007; Reiter et al., 2012).

In this study we investigate the mechanisms that regulate mGlu7-receptor signaling pathways and we address this issue on mGlu7 receptors transiently expressed in HEK293 cells.

2. Materials and methods

2.1. Materials

Monoclonal anti-Ha (used to probe mGlu7 receptor) and anti-JNK antibodies were purchased from Millipore (Temecula, CA); Monoclonal anti-GRK2/3 (clone C5/1) and monoclonal anti-GRK4–6 (used to probe GRK4) antibodies were purchased from Upstate Biotechnology (Lake Placid, NY); polyclonal anti-ERK1/2 and polyclonal anti-GRK6 (C-20) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA); monoclonal anti-phospho-ERK1/2 and polyclonal anti-phospho-JNK antibodies were from Cell Signaling Technology (Beverly, MA); monoclonal anti-β-arrestin1 antibody was from BD Transduction Laboratories (Lexington, KY); pertussis toxin (PTX) and forskolin (FSK) were purchased from Calbiochem (San Diego, CA). L-2-Amino-4-phosphonobutanoate (L-AP4), N,N′-bis(diphenylmethyl)-1,2-ethanediamine dihydrochloride (AMN082) and 6-(4-Methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo [4,5-*c*]pyridin-4(5*H*)-one hydrochloride (MMPIP) were purchased from Tocris Cookson (Bristol, UK). All other drugs were purchased from Sigma–Aldrich (Milan, Italy).

The plasmids encoding for GRK2, GRK4, GRK6, β-arrestin1 (βarr1), β-arrestin2 (βarr2) and βarrV53D, the dominant negative of βarr1 (βarrDN) cDNAs were previously described (Calabrese et al., 1994; Iacovelli et al., 2003). The plasmids encoding for the kinase-dead mutant of GRK2 (GRK2-K220R) and the C-terminal domain of GRK2 (Gly^{495}-Leu^{689}) (GRK2-Cter) were kindly provided by C. Scorer (GlaxoSmithKline, Uxbridge, Middlesex, UK); DynK44A cDNA was kindly provided by J. Benovic; Ha-tagged mGlu7 receptor and human EAAC1 cDNA were kindly provided by J. P. Pin (CNRS, Montpellier, France).

2.2. Cell culture and transfection

Human embryonic kidney (HEK) 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin). Cells were transfected in 10-mm Falcon dishes using 8 μl of LipofectAMINE2000 in OptiMEM medium (Invitrogen, Carlsbad, CA), and 15 μg of cDNA. The cells used for determination of cAMP were co-transfected with 2.5 μg/dish of adenylyl cyclase type V cDNA (Aramori et al., 1997). 3 μg/dish of excitatory amino acid carrier 1 (EAAC1) cDNA were co-transfected in each experiment. After 4 h, the transfection medium was removed and the cells were seeded into 6-well plates for MAPK and JNK assay or in 48-well plates for cAMP assay, previously coated with poly(L-lysine) (0.01%). The experiments were performed 72 h after transfection and the cells were serum starved 16–18 h before.

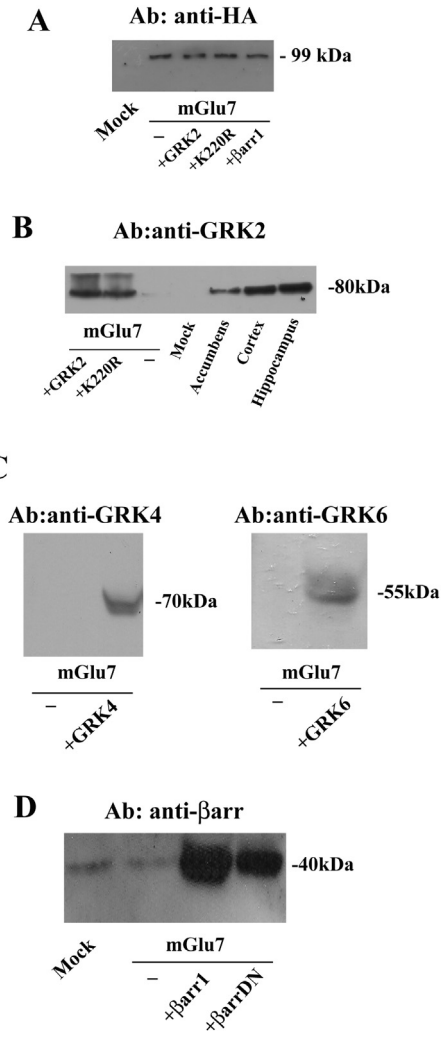

Fig. 1. (A) Immunoblots of mGlu7 receptor: the protein levels are similar when the mGlu7 receptor was expressed alone or co-expressed with GRK2, with GRK2 kinase-dead mutant, GRK2-K220R (K220R) or with β-arrestin1 (βarr1). The mGlu7 receptor was HA-tagged and its expression was detected using an anti-HA antibody; (B) immunoblots of GRK2 and GRK2 kinase-dead mutant, GRK2-K220R (K220R). The GRK2 protein levels in transiently transfected HEK293 cells are compared with the levels in rat brain regions (nucleus accumbens (Accumbens), cortex and hippocampus); (C) immunoblots of GRK4 and GRK6; (D) immunoblots of β-arrestin1 (βarr1) and β-arrestin1 dominant negative mutant (βarrDN) in HEK293 cells transfected with the respective cDNAs. Mock indicates HEK293 cells transfected with empty vector. Each lane was loaded with 80 μg of proteins from individual culture dishes.