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Analysis of mGluR1a constitutive internalization using a pulse–chase enzyme-linked immuno-sorbant assay (ELISA)

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Abstract

The surface expression of G protein-coupled receptors is regulated by internalization. For many receptors, a constitutive level of internalization in the absence of agonist has been reported. The constitutive internalization of metabotropic glutamate receptor 1a (mGluR1a) has been described, but in general little attention has been dedicated to this important aspect of receptor regulation. Here we describe a pulse–chase ELISA method that allows the investigation of mGluR1a constitutive internalization. When investigated by pulse–chase ELISA, the constitutive internalization of mGluR1a was inhibited by dominant negative mutant constructs of arrestin-2 or Eps-15. This observation, besides indicating the arrestin- and clathrin-dependence of mGluR1a constitutive internalization, also confirmed the physiological relevance of the method described in this article. Confocal microscopy experiments to study receptor localization further validated the pulse–chase labelling procedure. The application of the pulse–chase ELISA to mGluR1b, revealed that this splice variant undergoes marginal constitutive internalization. Two COOH-terminal deletion mutants of mGluR1a, DMI (Arg847stop) and DMII (Arg868stop), were also tested for constitutive internalization. Interestingly, only DMII underwent significant constitutive internalization, suggest-

Abbreviations: DM, deletion mutant; DNM, dominant negative mutant; ELISA, enzyme-linked immunosorbant assay; Eps15, EGFR pathway substrate clone 15; GPT, glutamate pyruvate transaminase; HEK293, human embryonic kidney cells 293; HA, Hemagglutinin; mGluR, metabotropic glutamate receptor; TBS, tris-buffered saline.

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ing that the region between Arg847 and Arg868 might play a regulatory role in mGluR1a trafficking. Taken together, the pulse–chase ELISA appears to be an efficient tool to analyze the constitutive internalization of different mGluR1 splice variants.

Keywords: Constitutive activity; Constitutive internalization; ELISA; mGluR1a; G protein-coupled receptor

1. Introduction

The internalization of membrane proteins such as G protein-coupled receptors (GPCRs) can be triggered by an agonist that activates the receptor, or alternatively can occur constitutively, that is in the absence of agonist activation. In turn, internalization is balanced by the recycling of receptor to the cell membrane from intracellular compartments (internalized vesicles, pools of ready-synthesized molecules or Golgi/ER vesicles shuttling newly synthesized protein). This cycle of internalization and recycling, despite being energetically inefficient, ensures a rapid and efficient control of membrane expression of physiologically important proteins such as GPCRs. Constitutive internalization has been observed for some wild type GPCRs, including thromboxane $A_2\beta$ [1], FP_B prostanoid [2], M2 muscarinic [3], cholecystokinin type A [4], thrombin [5] and PAR1 protease-activated [6] receptors. Interestingly, constitutive internalization of the group I metabotropic glutamate receptors (mGluRs) mGluR1a and mGluR5a has also been described [7,8], and is associated with the constitutive activity of these receptors. Group I mGluRs play a pivotal role in the modulation of synaptic activity and plasticity, and are involved in the pathogenesis of important diseases affecting the CNS. Therefore, the regulation of these receptors is of particular interest. Internalization might be an important regulatory mechanism for group I mGluRs, determining the level of surface expression of these receptors, and ultimately modulating the excitability of the glutamatergic synapse and hence neuronal activity.

The constitutive internalization of mGluR1a has been analyzed in our laboratory, leading to the identification of a direct link between constitutive activity and constitutive internalization [9]. We proposed that the constitutive activity of mGluR1a promotes the constitutive interaction of the receptor with non-visual arrestins with consequent internalization via clathrin-coated vesicles. Here, we report in detail the pulse–chase ELISA protocol used for the direct investigation of the constitutive internalization of mGluR1a. Firstly, the validation of the pulse–chase ELISA method is described. Dominant negative mutants of arrestin-2 and Eps-15 are used to confirm that this protocol enables the measurement of a physiologically relevant internalization pathway. Further confirmation that the pulse–chase labelling procedure identifies the constitutive internalization of mGluR1a from the cell membrane to lysosomes is presented using confocal immunofluorescence technology. Finally, we present data describing the use of the modified pulse–chase ELISA to investigate the differential degree of constitutive internalization between mGluR1a, mGluR1b and two receptor COOH-terminus deletion mutants, DMI (Arg847-stop) and DMII (Arg868stop).