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Constitutive internalization and recycling of metabotropic glutamate receptor 5 (mGluR5)

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ABSTRACT

Ligand-dependent and ligand-independent endocytic trafficking of G-protein coupled receptors (GPCRs) is critical for accurate receptor-mediated signaling and its regulation. Metabotropic glutamate receptor 5 (mGluR5) is a GPCR that plays a crucial role in circuit formation in the brain and also in various forms of synaptic plasticity including learning and memory. Outside the central nervous system this receptor also plays very important role in various other non-neuronal cells like heart cells, skin cells, hepatocytes, etc. Although the ligand-mediated endocytosis of mGluR5 has been studied in some detail, ligand-independent/constitutive endocytosis of the receptor has not been properly studied. Here, we have investigated the constitutive endocytosis of mGluR5 and also the sub-cellular fate of the receptor subsequent to internalization. We show here that mGluR5 undergoes constitutive internalization in HEK293 cells. Following endocytosis, the receptor enters the recycling compartment and no localization of the receptor was observed in the lysosome. In addition, we also report here that most of the receptors recycle to the cell surface subsequent to constitutive internalization. Thus, our data demonstrate that mGluR5 receptors internalize without the application of ligand and the internalized receptors recycle back to the cell surface following constitutive endocytosis.

1. Introduction

Metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors (GPCRs) that are classified into three groups based on their sequence identity, pharmacology and second messenger pathways [1]. The group I mGluRs are present in various regions of the brain as well as outside the brain like heart cells, skin cells, hepatocytes, etc. [1]. mGluR5, a member of the group I mGluR family, is positively coupled to the phospholipase C pathway and activation of this receptor triggers the release of Ca^{2+} from the intracellular store and activation of protein kinase C (PKC) [2]. Signaling by mGluR5 has been shown to be critical for circuitry formation during brain development and also in various forms of synaptic plasticity including learning and memory [2–5]. In addition, mGluR5 has been implicated in various neuropsychiatric disorders like fragile X syndrome [6,7]. It has been reported that the surface expression of mGluR5 plays critical role in various neuronal functions. For example, *in vivo* exposure of cocaine leads to the reduction in the surface expression of mGluR5 resulting in the loss of endocannabinoid dependent LTD [8]. The process of receptor trafficking plays a critical role in controlling the localization of the receptor. Despite this obvious significance very little is known about the trafficking of mGluR5. Furthermore, the trafficking of GPCRs has got serious attraction in the recent years because this process could serve as a mechanism for desensitization, resensitization or downregulation of the receptor depending on the type of GPCR and the system [9,10]. Due to the above reasons, it is of paramount importance to investigate the cellular and molecular mechanisms of mGluR5 trafficking but unfortunately very little is known about the trafficking of mGluR5.

Like many other GPCRs, mGluR5 also undergoes rapid internalization on exposure to the ligand and this process has been reported to be β-arrestin and dynamin dependent [11]. Although the mechanisms of ligand-dependent internalization of mGluR5 have been studied in some detail, the ligand-independent (constitutive) internalization of the receptor is not well understood. The mGluR5 has been reported to be associated with lipid rafts and constitutively internalizes via caveolar/raft pathway [12]. However, the fate of the receptor subsequent to constitutive internalization is not known. Here we have investigated the constitutive endocytosis of mGluR5 by a dual staining assay using a heterologous cell line, HEK293. HEK293 cell line is widely used as a model cell line to study the regulation of GPCRs. We find here that mGluR5 undergoes constitutive endocytosis in HEK293 cells. Subsequent to internalization the receptors enter the recycling endosome and no detectable localization of the receptor is observed

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in the lysosome. In addition, we find that almost all the constitutively endocytosed receptors recycle back to the plasma membrane after 3.5 h subsequent to internalization. These experiments suggest that mGluR5 does not get degraded and recycles back to the cell membrane after constitutive endocytosis.

2. Materials and methods

2.1. Materials

The myc tagged mGluR5 construct was a generous gift from Kathrine Roche (National Institute of Health, USA). The myc epitope was tagged at the N-terminus of the full length mGluR5 cDNA. Dulbecco's modified eagle medium (DMEM), penicillin–streptomycin solution, antibiotic–antimycotic mix, lipofectamine, OptiMEM, trypsin–EDTA, fetal bovine serum (FBS), DPBS, distilled water were obtained from Invitrogen (USA). Ampicillin, paraformaldehyde (PFA), poly-D-lysine, FluoromountTM aqueous mounting medium were purchased from Sigma (USA). Fine chemicals were obtained from Life technologies (USA), Merck limited (USA). Anti-myc mouse monoclonal antibody, anti-myc rabbit polyclonal antibody, anti-Rab11A mouse monoclonal antibody, anti-Lamp1 rabbit polyclonal antibodies were obtained from Abcam. Secondary antibodies viz., goat anti-mouse Alexa-568, goat anti-mouse Alexa-647, goat anti-mouse Alexa-488, goat anti-rabbit Alexa-568 and goat anti-rabbit Alexa-488 were purchased from Invitrogen (USA).

2.2. Methods

2.2.1. Cell culture and transfection

HEK293 cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum, antibiotic–antimycotic mix at 37 °C, 5% CO_2. Cells were transfected with myc-mGluR5 cDNA at 65–70% confluency on 35 mm coverslips coated with 50 μg/ml poly-D-lysine. Transfection was done by mixing 2 μg of the DNA with 10 μg of lipofectamine in 1 ml OptiMEM. All experiments were done 24–36 h post transfection.

2.2.2. Constitutive endocytosis assay

HEK293 cells were transfected with the myc-mGluR5 construct using lipofectamine as described before and experiments were performed 24–36 h post transfection. Cells expressing myc-mGluR5 were incubated with anti-myc mouse monoclonal antibody (1:500) for 1 h on ice. Control cells were immediately fixed (without permeabilization) with ice cold 4% PFA in PBS for 15 min on ice while other coverslips were incubated at 37 °C for 5 min, 15 min, 30 min and 60 min to allow constitutive internalization of the receptors. Subsequently, cells were fixed (without permeabilization) with ice cold 4% PFA at their respective time points for 15 min on ice. After that, cells were blocked with 2% normal goat serum (NGS) and saturating concentration of first secondary antibody viz., goat anti-mouse Alexa-568 (1:100) in NGS was applied for 1 h at 37 °C to label the surface receptors. Cells were then permeabilized with 0.1% Triton X-100 for 30 min at room temperature. After washing, cells were blocked with 2% NGS in PBS for 1 h at 37 °C followed by the application of the second secondary antibody viz., goat anti-mouse Alexa-647 (1:800) for 1 h at 37 °C to label the constitutively internalized receptors. The coverslips were then mounted on glass slides and imaged under the confocal microscope. For this and all other subsequent experiments two coverslips were used for each condition. Typically 70–100 cells were randomly chosen and imaged in any one experiment and all experiments were repeated at least three times.

In order to ensure that the Alexa-647 conjugated secondary antibody that we have used to visualize the internalized myc-mGluR5 in our experiments did not label any surface myc-mGluR5 we performed the following control experiment. Application of saturating concentration of Alexa-568 conjugated secondary antibody that we have used to visualize the surface receptors prevented any further detectable staining of the surface receptor when second secondary antibody, i.e., Alexa-647 conjugated secondary antibody was applied in non-permeabilized cells. Whereas, when cells were permeabilized with 0.1% triton X-100 for 30 min at room temperature internalized myc-mGluR5 was visible on application of Alexa-647 conjugated secondary antibody (Supplementary Fig. 1). These experiments suggest that saturating concentration of first secondary antibody (Alexa-568 conjugated secondary antibody) occupied all the surface myc-mGluR5 and the second secondary antibody (Alexa-647 conjugated secondary antibody) did not label any detectable surface myc-mGluR5. It should be noted that this particular method has been used previously by few groups to study trafficking of other neurotransmitter receptors [13,14].

2.2.3. Colocalization assay

HEK293 cells were transfected with the myc-mGluR5 DNA as described earlier. 24 h post transfection cells were incubated with either anti-myc rabbit polyclonal antibody (1:500) or anti-myc mouse monoclonal antibody (1:500) for 1 h on ice. Control cells were washed with plain DMEM and fixed with ice cold 4% PFA for 15 min. For 30 min time point, cells were incubated at 37 °C for 30 min to allow the constitutive internalization of the receptors and after that fixed in ice cold 4% PFA in PBS for 15 min on ice. Subsequently, cells were permeabilized with 0.1% Triton X-100 for 30 min at room temperature. After washing with PBS, cells were blocked with 2% NGS in PBS for 1 h at 37 °C followed by staining with either mouse monoclonal antibody against the recycling compartment marker Rab11 for 4 h at 37 °C or rabbit polyclonal antibody against the lysosomal marker LAMP1 for 1 h at 37 °C. Subsequently, appropriate secondary antibodies viz., goat anti-rabbit/goat anti-mouse Alexa-568 (1:800) and goat anti-mouse/goat anti-rabbit Alexa-488 (1:800) were applied for 1 h at 37 °C to stain the myc-mGluR5 receptors and recycling compartment or lysosomal markers. Coverslips were mounted on glass slides and imaged under the confocal microscope.

2.2.4. Recycling assay

Constitutive endocytosis of myc-mGluR5 subsequent to the live cell antibody staining (anti-myc mouse monoclonal antibody) was initiated in transfected HEK293 cells according to the method described before. Control cells were fixed with ice cold 4% PFA in PBS for 15 min on ice (without permeabilization) while other cells were incubated at 37 °C for 30 min, 2 h, 2.5 h, 3 h and 3.5 h to investigate whether the constitutively endocytosed receptors recycle back to the plasma membrane. After respective time points cells were fixed without permeabilization by ice cold 4% PFA for 15 min on ice. Surface receptors were labeled by 1 h application of saturating concentration of first secondary antibody viz., goat anti-mouse Alexa-568 (1:100) at 37 °C after blocking with 2% NGS. Next, cells were permeabilized with 0.1% Triton X-100 for 30 min at room temperature. After washing, cells were blocked with 2% NGS in PBS for 1 h at 37 °C followed by second secondary antibody viz., goat anti-mouse Alexa-647 (1:800) staining for 1 h at 37 °C to visualize the constitutively endocytosed receptors. The coverslips were mounted on glass slides and imaged under the confocal microscope.

2.2.5. Image acquisition and analysis

Coverslips were mounted in glass slides using Fluormount and imaged with a 63× oil immersion objective mounted on Zeiss LSM 580 laser scanning confocal microscope. Images from all conditions of a particular experiment were acquired using identical acquisition parameters (gain, offset, laser power, zoom, pinhole

size, etc.). The images were then analyzed in ImageJ (from NIH, USA) software using identical parameters. Control and treated cells from the same experiment were thresholded using identical values and the total thresholded area of fluorescently labeled surface and constitutively internalized myc-mGluR5 was measured using ImageJ software. The percentage of internalization for each condition was then calculated by dividing the internal fluorescence with the total labeled fluorescence (surface + internal). These values were then normalized to those of control cells from the same experiment. Each experimental treatment and analysis was done on a minimum of two coverslips and each experiment was repeated at least three times. For presentation, images were adjusted in Adobe Photoshop software using identical brightness and contrast values for each condition illustrated in the experiment. Data are presented as mean ± SEM. Group results were compared by using Student's *t*-test. $p > 0.05$ was considered not significant (n.s).

3. Results

3.1. Kinetics of mGluR5 constitutive internalization

The mGluR5 receptor is known to activate the IP_3 second messenger pathway upon ligand binding and subsequently increases the intracellular Ca^{2+} levels. In order to investigate whether the myc tagged mGluR5 undergoes constitutive internalization we transfected HEK293 cells with this construct. As expected, upon live cell antibody staining the myc-mGluR5 was seen to be localized at the cell membrane suggesting that tagging of myc at the N-terminus of the receptor did not mislocalize the receptor. Furthermore, earlier studies have also reported that this particular construct is functional and increases the level of intracellular Ca^{2+} upon activation similar to the native receptor [15]. All these results indicate that, as expected, the myc-mGluR5 fusion receptor

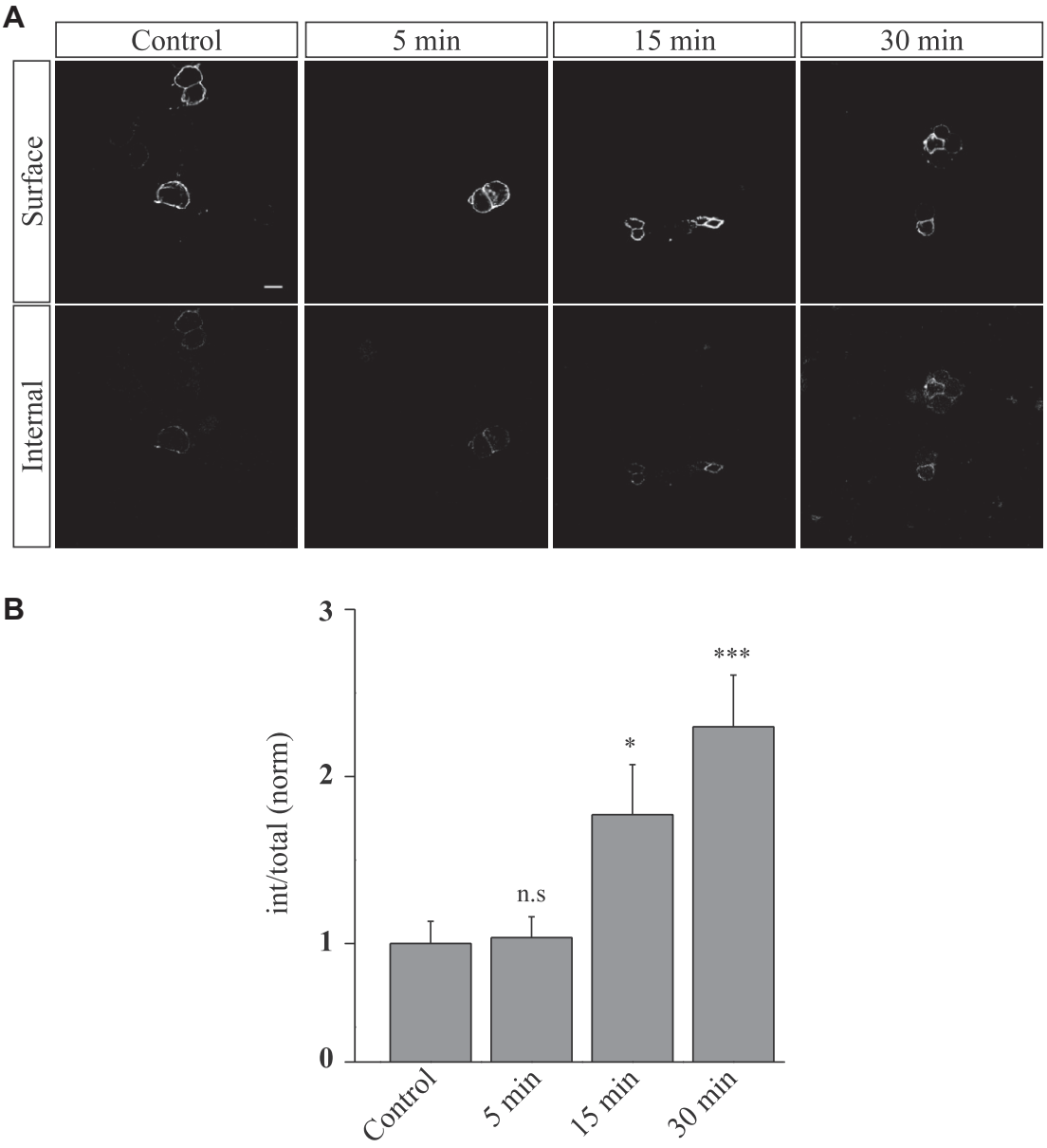

Fig. 1. Kinetics of myc-mGluR5 constitutive internalization. (A) Representative images of constitutively internalized myc-mGluR5 in HEK293 cells at various time points. Control cells showed intense surface fluorescence with very little internal fluorescence. At 5 min not much increase in the intracellular fluorescence was observed. The intracellular fluorescence increased at 15 min and at 30 min post constitutive internalization. (B) Quantitation of normalized internalization showed increase in constitutive internalization of myc-mGluR5 at 15 min and 30 min. Scale bar = 10 μm. * indicates $p < 0.05$, *** indicates $p < 0.001$ and n.s indicates $p > 0.05$.

localizes properly and this fusion receptor is functional, positively couples to the IP_3 pathway like the native receptor.

In order to investigate the constitutive endocytosis of this receptor, we studied this process using live cell dual antibody staining as described in Section 2. The advantage of this technique is it measures the proportion of the surface receptors that are internalized constitutively and thus variability in the surface expression of the receptor in different cells does not affect the endocytosis measurement. Furthermore, we have used Alexa-647 conjugated secondary antibody to visualize the internalized myc-mGluR5 since Alexa-647 is not detectable in the visible range, which enabled us for an unbiased blind sampling of cells in various conditions. Using this method we could reliably observe the constitutive endocytosis of myc-mGluR5. In control cells most of the receptors were present at the cell surface and very little internal fluorescence was observed (Fig. 1A and B) (Control = 1 ± 0.13). Subsequently, when cells were incubated at 37 °C for various times the receptors internalized in a ligand-independent manner and moved to the internal compartment. At 5 min no detectable increase in the internal fluorescence was observed (5 min = 1.03 ± 0.13). The internal fluorescence increased after cells were incubated at 37 °C for 15 min (15 min = 1.77 ± 0.3). At 30 min, further increase

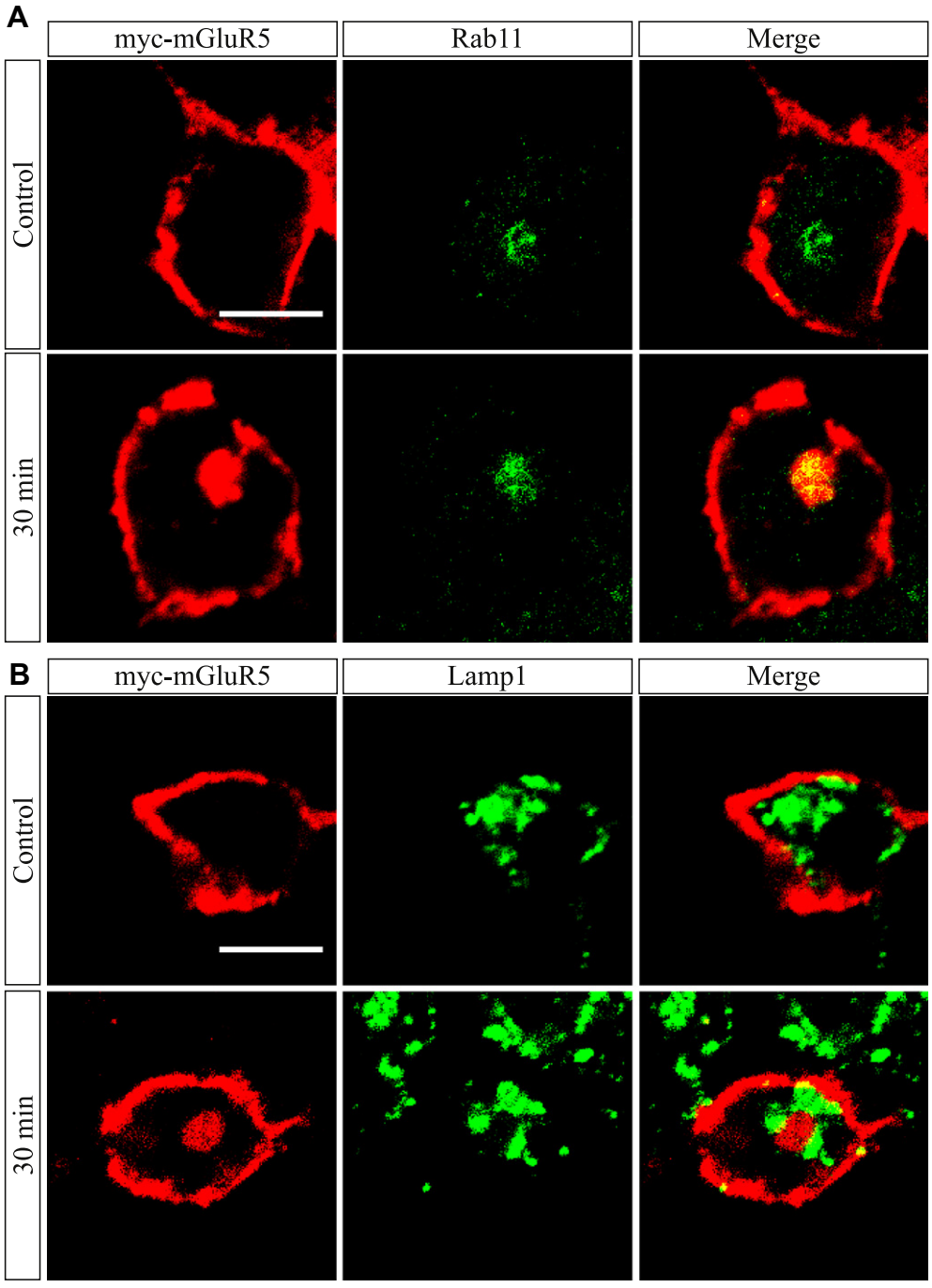

Fig. 2. myc-mGluR5 enters the recycling compartment after constitutive endocytosis. (A) Control cells showed presence of most of the myc-mGluR5 at the cell surface and not much internal fluorescence was observed in these cells. At 30 min of constitutive internalization the receptors were seen to be localized at the recycling compartment as observed by the colocalization with Rab11, a recycling compartment marker. (B) The constitutively endocytosed myc-mGluR5 did not colocalize with the lysosomal marker Lamp1 at 30 min time point. Scale bar = 5 μm.

in the internal fluorescence was observed (30 min = 2.3 ± 0.31). We also measured the internalization of the receptor at 1 h and no significant difference between the internalization at 30 min and at 1 h were observed (data not shown).

3.2. mGluR5 enters the recycling compartment subsequent to constitutive internalization

After internalization, GPCRs can have multiple sub-cellular fates. Many GPCRs enter the recycling compartment subsequent to internalization and recycle back to the cell surface. This process has been shown to be important for the resensitization of many receptors. For some GPCRs, after internalization, they enter the lysosome for degradation and this process is believed to be important for the downregulation of the receptor. Various factors like type of GPCR, ligand and cell type determine the fate of the receptor. Our earlier results suggest that mGluR5 endocytosed constitutively, even without the application of ligand. But the fate of this receptor after the constitutive internalization is not known. In order to investigate the sub-cellular fate of this receptor subsequent to constitutive internalization, we performed co-localization studies with recycling endosome marker Rab11 and lysosomal marker Lamp1. In our earlier studies we observed that constitutive endocytosis of myc-mGluR5 was maximal at 30 min. So, we chose 30 min time point for our colocalization studies. HEK293 cells were transfected with myc-mGluR5. 24 h after transfection, myc-mGluR5 receptors were labeled with anti-myc mouse monoclonal antibody or anti-myc rabbit polyclonal antibody. Subsequently, constitutive internalization was allowed for 30 min at 37 °C followed by fixation and permeabilization of cells. Respective secondary antibodies tagged with Alexa-568 was applied to visualize the constitutively endocytosed receptors. For staining of Rab11 and Lamp1, mouse monoclonal and rabbit polyclonal primary antibodies were used respectively. Subsequently, appropriate secondary antibodies tagged with Alexa-488 were used to visualize these markers (as described in Section 2.2). As expected, control cells did not show observable internalized receptors in both the colocalization experiments (Fig. 2A and B). Importantly, after 30 min of constitutive internalization myc-mGluR5 was observed to be co-localized with Rab11 (Fig. 2A), whereas not much colocalization was observed with Lamp1 (Fig. 2B). Together these results suggest that constitutively endocytosed mGluR5 goes to the recycling

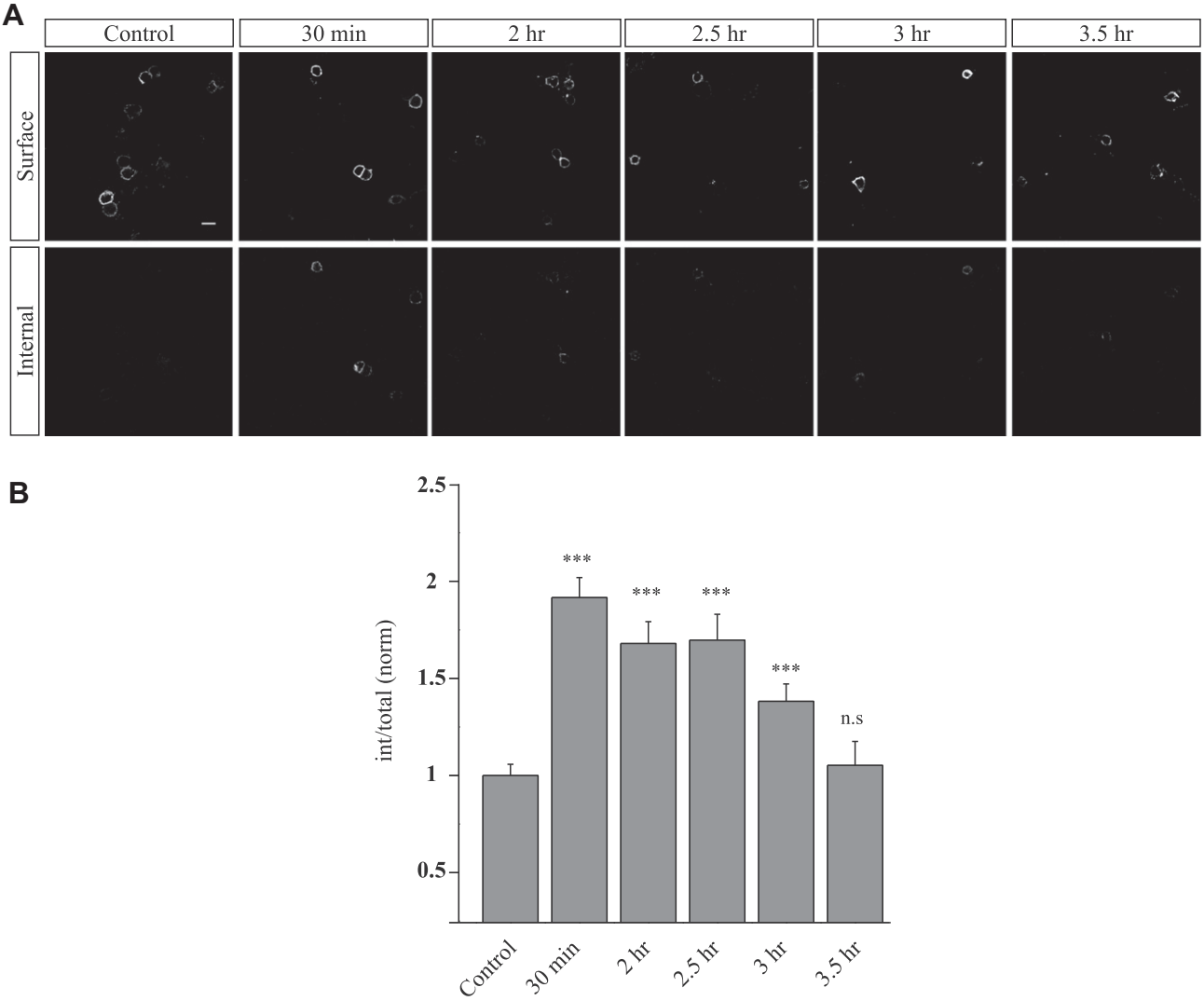

Fig. 3. myc-mGluR5 recycles to the cell membrane after constitutive internalization. (A) Control cells showed presence of the myc-mGluR5 at the cell surface and not much intracellular fluorescence was observed in these cells. The receptors internalized without the application of ligand as seen by the increase in intracellular fluorescence at 30 min. When cells were chased for longer time i.e., 2 h, 2.5 h, 3 h and 3.5 h almost all the receptors recycled back to the cell surface. (B) Quantitation showed the constitutive internalization of myc-mGluR5 receptors at 30 min followed by recycling of the receptor to the cell surface at 3.5 h. Scale bar = 10 μm. *** indicates $p < 0.001$ and n.s indicates $p > 0.05$.

compartment subsequent to internalization while very little or no receptors enter the lysosomal compartment.

3.3. mGluR5 recycles back to the cell surface after constitutive internalization

Our earlier experiments suggest that constitutively endocytosed myc-mGluR5 enters the recycling endosome and does not go to the lysosome for degradation. To determine whether myc-mGluR5 recycles back to the surface after constitutive internalization, cells were incubated for longer time periods at 37 °C subsequent to constitutive internalization. As expected, receptors were internalized constitutively at 30 min (Control = 1 ± 0.06, 30 min = 1.92 ± 0.1) (Fig. 3A and B). When receptors were chased for longer time, they recycled back to the surface. A decrease in the normalized internalization was observed with time and after 3.5 h most of the receptors recycled back to the cell surface with very little fluorescence was observed in the internal compartment of the cells (2 h = 1.68 ± 0.11, 2.5 h = 1.7 ± 0.13, 3 h = 1.38 ± 0.09, 3.5 h = 1.05 ± 0.12) (Fig. 3A and B). This result suggests that the mGluR5 that were internalized constitutively recycled back to the surface.

4. Discussion

Endocytosis of G-protein coupled receptors is a major mechanism to desensitize and downregulate the surface receptors. Desensitization and downregulation of surface receptors are necessary mechanisms to protect the receptors from chronic over-stimulation. Till date, the desensitization, endocytosis, recycling and downregulation mechanisms of various GPCRs have been studied. It has become apparent from these studies that due to the diversity in the GPCR regulation, each GPCR has its own unique characteristics and an extensively studied GPCR such as the β2-adrenergic receptor may not provide all the details about other GPCRs. Whereas the cellular and molecular mechanisms of trafficking of some GPCRs have been studied extensively in the last few years, till now the mechanisms of metabotropic glutamate receptor trafficking has not been studied in detail. Furthermore, most of the GPCRs could undergo internalization in both ligand-dependent and ligand-independent manner. Although some amount of work has been done on the ligand-mediated endocytosis of mGluR5, nothing is known about the constitutive (ligand-independent) internalization of mGluR5.

Here we have studied the constitutive trafficking of mGluR5 in HEK293 cells, which is a widely used cell line to study regulation of GPCRs. Most of the previous work that investigated the trafficking of GPCRs employed various techniques like surface biotinylation assays, surface fluorescence measurement and internal fluorescence measurement assays. All these methods do not take into account the variability in the receptor expression among the cells. To overcome this, we have used dual labeling assay in our endocytosis experiments that enables us to normalize the expression of the receptor within the cell and allows us to compare the expression between the cells without any perturbation. Using this method we show here that like many other GPCRs, mGluR5 also undergoes constitutive endocytosis. Subsequent to constitutive endocytosis the receptors were seen to be localized in the recycling compartment and not much presence of the receptor was observed in the lysosome. Furthermore, almost all endocytosed receptors recycled back to the cell surface in 3.5 h. These experiments suggest that the existing receptors are probably reused again after a cycle of endocytosis and majority of them are not replaced by newly synthesized receptors. It would be important to study the structural determinant of the receptor as well as the molecular players that are involved in this recycling process of mGluR5. It would also be important to perform a comparative analysis of constitutive vs. ligand-dependent trafficking of metabotropic glutamate receptor 5.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.09.040.

References

[1] G.K. Dhami, S.S. Ferguson, Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis, Pharmacol. Ther. 111 (2006) 260–271.
[2] C.H. Kim, J. Lee, J.Y. Lee, K.W. Roche, Metabotropic glutamate receptors: phosphorylation and receptor signaling, J. Neurosci. Res. 86 (2008) 1–10.
[3] M. Tsanov, D. Manahan-Vaughan, Synaptic plasticity in the adult visual cortex is regulated by the metabotropic glutamate receptor, mGluR5, Exp. Brain Res. 199 (2009) 391–399.
[4] B.A. Grueter, Z.A. McElligott, A.J. Robison, G.C. Mathews, D.G. Winder, In vivo metabotropic glutamate receptor 5 (mGluR5) antagonism prevents cocaine-induced disruption of postsynaptically maintained mGluR5-dependent long-term depression, J. Neurosci. 28 (2008) 9261–9270.
[5] S. Neyman, D. Manahan-Vaughan, Metabotropic glutamate receptor 1 (mGluR1) and 5 (mGluR5) regulate late phases of LTP and LTD in the hippocampal CA1 region in vitro, Eur. J. Neurosci. 27 (2008) 1345–1352.
[6] G. Dolen, E. Osterweil, B.S. Rao, G.B. Smith, B.D. Auerbach, S. Chattarji, M.F. Bear, Correction of fragile X syndrome in mice, Neuron 56 (2007) 955–962.
[7] A. Michalon, M. Sidorov, T.M. Ballard, L. Ozmen, W. Spooren, J.G. Wettstein, G. Jaeschke, M.F. Bear, L. Lindemann, Chronic pharmacological mGlu5 inhibition corrects fragile X in adult mice, Neuron 74 (2012) 49–56.
[8] L. Fourgeaud, S. Mato, D. Bouchet, A. Hemar, P.F. Worley, O.J. Manzoni, A single in vivo exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens, J. Neurosci. 24 (2004) 6939–6945.
[9] A.C. Magalhaes, H. Dunn, S.S. Ferguson, Regulation of GPCR activity, trafficking and localization by GPCR-interacting proteins, Br. J. Pharmacol. 165 (2012) 1717–1736.
[10] E. Kelly, C.P. Bailey, G. Henderson, Agonist-selective mechanisms of GPCR desensitization, Br. J. Pharmacol. 153 (Suppl. 1) (2008) S379–388.
[11] L. Fourgeaud, A.S. Bessis, F. Rossignol, J.P. Pin, J.C. Olivo-Marin, A. Hemar, The metabotropic glutamate receptor mGluR5 is endocytosed by a clathrin-independent pathway, J. Biol. Chem. 278 (2003) 12222–12230.
[12] A. Francesconi, R. Kumari, R.S. Zukin, Regulation of group I metabotropic glutamate receptor trafficking and signaling by the caveolar/lipid raft pathway, J. Neurosci. 29 (2009) 3590–3602.
[13] S. Bhattacharyya, V. Biou, W. Xu, O. Schluter, R.C. Malenka, A critical role for PSD-95/AKAP interactions in endocytosis of synaptic AMPA receptors, Nat. Neurosci. 12 (2009) 172–181.
[14] V. Biou, S. Bhattacharyya, R.C. Malenka, Endocytosis and recycling of AMPA receptors lacking GluR2/3, Proc. Natl. Acad. Sci. USA 105 (2008) 1038–1043.
[15] K.Y. Choi, S. Chung, K.W. Roche, Differential binding of calmodulin to group I metabotropic glutamate receptors regulates receptor trafficking and signaling, J. Neurosci. 31 (2011) 5921–5930.