European Journal of Pharmacology 668 (2011) 107–114

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and Cellular Pharmacology

Bradykinin B_2 receptor-mediated transport into intact cells: Anti-receptor antibody-based cargoes

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ARTICLE INFO

Article history:
Received 9 February 2011
Received in revised form 9 June 2011
Accepted 23 June 2011
Available online 7 July 2011

Keywords:
Bradykinin
B_2 receptor
β-Arrestin
Receptor endocytosis

ABSTRACT

Endocytosis of the bradykinin-stimulated B_2 receptors is parallel to the transport and subsequent degradation of the ligand. To implement biotechnological applications based on receptor-mediated transport, one strategy is to conjugate the agonist ligand to a cargo. Alternatively, we studied whether the B_2 receptor can transport large antibody-based cargoes into intact cells and characterized the ensuing endosomal routing. Myc-tagged B_2 receptors (coded by the vector myc-B_2R) and a truncated construction devoid of the Ser–Thr phosphorylation domain (myc-B_2R_{trunc} vector) were coupled to anti-myc monoclonal antibodies that did not impair bradykinin binding or elicit calcium signaling in intact cells. Anti-myc antibodies, conjugated or not with secondary antibodies optionally coupled to Qdot nanomaterials, were transported into early endosome autoantigen 1-, and β-arrestin-positive vesicles in bradykinin-stimulated intact cells expressing receptors encoded by myc-B_2R. Antibody-conjugated cargoes progressed into late-endosomes-lysosomes within 3 h without evidence of autophagy. Receptors encoded by myc-B_2R_{trunc} did not support the ligand-controlled endocytosis of anti-myc antibodies. Aside from small ligand-conjugated cargoes, very large antibody-based cargoes can be transported by agonist-stimulated B_2 receptors into intact cells. The latter type of cargo requires a receptor competent for interaction with β-arrestins, enters the degradation pathway separately from the receptor as a function of time and has the potential to confer a qualitatively novel function to a receptor.

1. Introduction

Following agonist stimulation, a specific Ser–Thr-rich C-terminal domain of the bradykinin B_2 receptor is phosphorylated, leading to the recruitment of β-arrestin and adaptor proteins that mediate agonist-induced endocytosis (Leeb-Lundberg et al., 2005). The agonist-stimulated B_2 receptors actually transport and translocate bradykinin into the endosomal compartment, where it is degraded (Munoz and Leeb-Lundberg, 1992). We have recently illustrated B_2 receptor-mediated transport into intact cells using a bradykinin analog conjugated with a carboxyfluorescein cargo (Gera et al., 2011); this agent modeled the endocytic transport, trafficking and intracellular degradation of the peptide ligand.

In the present study, we addressed a novel strategy to exploit receptor-mediated transport for cargoes much heavier than the agonist and showed that receptors encoded by the myc-B_2R vector mediate the endocytosis of anti-myc monoclonal antibodies in HEK 293a cells stimulated with bradykinin, the N-terminal myc epitope merely representing a model for a receptor extracellular epitope that can be recognized by an antibody present in the extracellular space. The routing of both the fluorescent agonist or antibody-based cargoes theoretically hinges on the function of β-arrestins, which was verified using a truncated version of myc-tagged B_2 receptors devoid of the domain substrate for the G protein coupled receptor kinases. Further, the trafficking of receptor-transported cargoes to early endosome autoantigen 1- or Rab7-positive endosomes and lysosomal or autophagic structures was also addressed. This opens the way to exploiting the particular distribution of a given G protein coupled receptor to confer new types of response to cells.

2. Materials and methods

2.1. Drugs and reagents

Cell culture reagents were purchased from Invitrogen. The bradykinin B_2 receptor antagonist LF 16-0687 was a gift from Laboratoires Fournier (Daix, France). Preassembled quantum dot nanocrystals with an average emission wavelength of 705 nm (Qdot 705 goat F(ab′)$_2$ anti-mouse IgG conjugate) and BSA-Alexa Fluor 594 were purchased from

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doi:10.1016/j.ejphar.2011.06.041

Invitrogen (Carlsbad, CA, USA). All other drugs and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell culture and transfection

A subclone of HEK 293 cells, called HEK 293a, originally obtained from Sigma-Aldrich was used in all experiments. This cell type was grown in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 0.1% L-glutamine and 0.1% penicillin–streptomycin. HEK 293a cells were grown until they reached 70% confluence and were then transfected with one of the myc-B_2R-coding vectors optionally co-transfected with vectors coding for fusion proteins: β-arrestin$_1$-cherry fluorescent protein (CherryFP), β-arrestin$_2$-green fluorescent protein (GFP), or early endosome autoantigen 1 (EEA1)-FYVE-GFP using the EX-Gen 500 transfection reagent (MBI Fermentas Inc., Flamborough, ON, Canada) as recommended by the manufacturer. Vectors coding for the myc-tagged B_2 receptors (myc-B_2R) and its truncated version (myc-B_2R_{trunc} vector) deprived of the substrate domain of the G protein coupled receptor kinase but pharmacologically intact, are described elsewhere (Bawolak et al., 2007; Gera et al., 2011). Briefly, truncation of the C-terminus in the myc-B_2R_{trunc} vector was obtained by mutating Glu^{340} in the myc-B_2R sequence to the stop codon, thus eliminating the 28 C-terminal residues, a sequence that comprises the conserved and phosphorylable Ser–Thr-rich domain (Leeb-Lundberg et al., 2005). The receptor encoded by myc-B_2R_{trunc} binds [^{3}H]bradykinin with an affinity identical to that of the myc-tagged B_2 receptor, but its surface expression (B_{max}) is slightly inferior to that of the non-truncated construction (Gera et al., 2011). Several plasmids were gifts; the β-arrestin$_2$-GFP fusion protein in pcDNA3 was a kind gift from Dr. M. Bouvier (Université de Montréal, Canada; Bernier et al., 2004). GFP-Rab7 was graciously given by Dr. M. J. Tremblay (Université Laval, Canada) and the vector coding for EEA1-FYVE-GFP, by Dr. Tamas Balla (NICHD, NIH, Bethesda, MD, USA). GFP-LC3 labels autophagosomes in mammalian cells (Kabeya et al., 2000); the pEGFP-LC3 expression vector for this chimerical protein was a generous gift from Dr. T. Yoshimori (Osaka University, Japan).

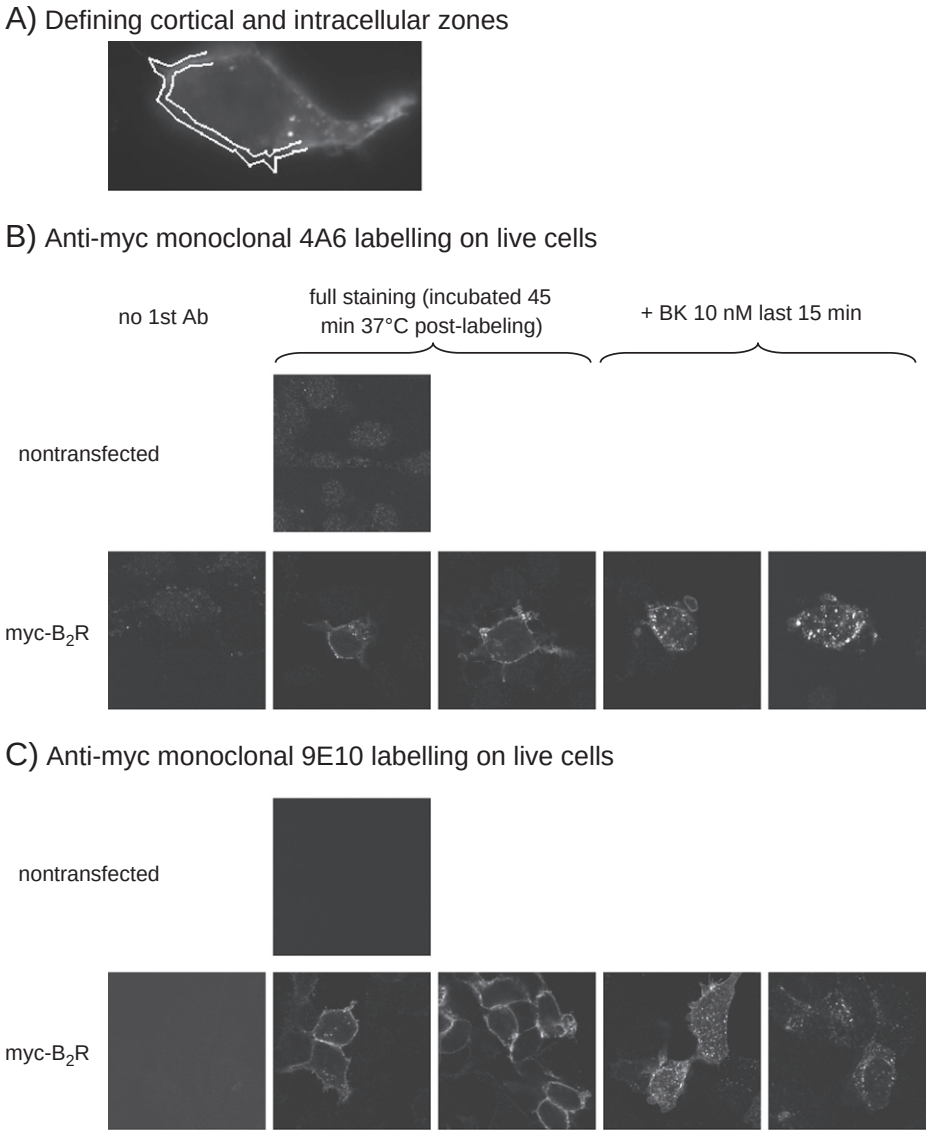

Fig. 1. A. Microscopic analysis of cortical and intracellular fluorescence in a microphotography of an intact HEK 293a cell. Epifluorescence, original magnification 1000×. See Materials and methods for the description. B. and C. Endocytosis assays of a monoclonal antibody cargo mediated by receptors encoded by the myc-B_2R vector in live HEK 293 cells. Labeling with either monoclonal antibody 4A6 or 9E10 (1:1000 dilution for either; the latter antibody purchased from Covance) was performed at room temperature, the cells were washed and then incubated at 37 °C in serum-free culture medium. Optionally, cells were further stimulated with the agonist bradykinin (BK; 10 nM) for the last 15 min of the incubation period. Thereafter, the cells were fixed, permeabilized and stained with the secondary Alexa Fluor 488-labeled antibody. Confocal microscopy (BioRad 1024 apparatus; sides of square fields = 60 μm).