Biochemical Pharmacology 99 (2016) 60–72

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Cannabinoid receptor 2 expression modulates $G\beta_1\gamma_2$ protein interaction with the activator of G protein signalling 2/dynein light chain protein Tctex-1

Marina Nagler[a,b], Lysann Palkowitsch[c], Sebastian Rading[a,b], Barbara Moepps[b], Meliha Karsak[a,b,*]

[a] Neuronal and Cellular Signal Transduction, Center for Molecular Neurobiology Hamburg (ZMNH), University Medical Center Hamburg-Eppendorf (UKE), 20246 Hamburg, Germany
[b] Institute of Pharmacology and Toxicology, Ulm University, 89081 Ulm, Germany
[c] Institute of Physiological Chemistry, Ulm University, 89081 Ulm, Germany

ARTICLE INFO

Article history:
Received 4 August 2015
Accepted 22 September 2015
Available online 26 September 2015

Chemical compounds studied in this article:
2-AG (PubChem CID: 5282280)
AM 630 (PubChem CID: 4302963)
Ammonium chloride (PubChem CID: 25517)
Bafilomycin A1 (PubChem CID: 25517)
Cycloheximide (PubChem CID: 6197)
JWH 133 (PubChem CID: 6918505)
Leupeptin hemisulfate (PubChem CID: 2733491)
MG132 (PubChem CID: 462382)

Keywords:
G protein-coupled receptor (GPCR)
Cannabinoid receptor (CB_2)
Tctex-1
G protein subunit
Protein degradation

ABSTRACT

The activator of G protein signalling AGS2 (Tctex-1) forms protein complexes with Gβγ, and controls cell proliferation by regulating cell cycle progression. A direct interaction of Tctex-1 with various G protein-coupled receptors has been reported. Since the carboxyl terminal portion of CB_2 carries a putative Tctex-1 binding motif, we investigated the potential interplay of CB_2 and Tctex-1 in the absence and presence of Gβγ.

The supposed interaction of cannabinoid receptor CB_2 with Tctex-1 and the influence of CB_2 on the formation of Tctex-1–Gβγ-complexes were studied by co- and/or immunoprecipitation experiments in transiently transfected HEK293 cells. The analysis on Tctex-1 protein was performed in the absence and presence of the ligands JWH 133, 2-AG, and AM 630, the protein biosynthesis inhibitor cycloheximide or the protein degradation blockers MG132, NH_4Cl/leupeptin or bafilomycin.

Our results show that CB_2 neither directly nor indirectly *via* Gβγ interacts with Tctex-1, but competes with Tctex-1 in binding to Gβγ. The Tctex-1–Gβγ protein interaction was disrupted by CB_2 receptor expression resulting in a release of Tctex-1 from the complex, and its degradation by the proteasome and partly by lysosomes. The decrease in Tctex-1 protein levels is induced by CB_2 expression "dose-dependently" and is independent of stimulation by agonist or blocking by an inverse agonist treatment.

The results suggest that CB_2 receptor expression independent of its activation by agonists is sufficient to competitively disrupt Gβγ–Tctex-1 complexes, and to initiate Tctex-1 degradation. These findings implicate that CB_2 receptor expression modifies the stability of intracellular protein complexes by a non-canonical pathway.

1. Introduction

The cannabinoid CB_2 receptor belongs to the family of G protein-coupled receptors (GPCR) and is activated by lipophilic substances like the phytocannabinoid delta-9-tetrahydrocannabinol (Δ^9-THC) and the dietary cannabinoid beta-caryophyllene [1]. In addition, endogenously synthesized endocannabinoids such as arachidonoyl-glycerol derivatives stimulate CB_2 receptors.

Abbreviations: AGS2, activator of G protein signalling; AM 630, 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone; 2-AG, 2-arachidonoyl-glycerol; CB_1, cannabinoid CB_1 receptor; CB_2, cannabinoid CB_2 receptor; co-IP, co-immunoprecipitation; DDM, *n*-dodecyl β-D-maltoside; DIC, dynein intermediate chain; DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethylsulfoxide; Dynlt1, dynein light chain protein 1; Erk1/2, extracellular signal-regulated kinase 1/2; FCS, fetal calf serum; Gβ, β subunit of GTP-binding protein; Gγ, γ subunit of GTP-binding protein; GEF-H1, rho guanine nucleotide exchange factor H1; GPCR, G protein-coupled receptor; GST, glutathione S-transferase; GTP, guanosine triphosphate; HEK293, human embryonic kidney cells; JWH 133, (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran; MG132, *N*-[(phenylmethoxy)carbonyl]-L-leucyl-*N*-[(1*S*)-1-formyl-3-methylbutyl]-L-leucinamide; PTH1R, parathyroid hormone 1 receptor; RhoGEF-H1, rho guanine nucleotide exchange factor H1; SD, standard deviation; Tctex-1, T-complex testis-expressed protein 1; Δ^9-THC, delta-9-tetrahydrocannabinol.

* Corresponding author at: Neuronal and Cellular Signal Transduction, Center for Molecular Neurobiology Hamburg (ZMNH), University Medical Center Hamburg-Eppendorf (UKE), Martinistr. 52, 20246 Hamburg, Germany.
E-mail address: meliha.karsak@zmnh.uni-hamburg.de (M. Karsak).

http://dx.doi.org/10.1016/j.bcp.2015.09.017
0006-2952/

Increased CB_2 receptor expression is associated with a wide range of human diseases and it has been shown to contribute to various pathological states in animal models of liver dysfunction, bone degeneration, atherosclerosis, and neurodegenerative diseases [2]. For example, whereas CB_2 receptors are absent in healthy brain microglia [3], microglial expression levels of CB_2 were found to be increased in Alzheimer's brain tissue, amyotrophic lateral sclerosis, multiple sclerosis, as well as in models of neuropathic pain [4]. The increase in CB_2 expression and/or activity possibly contributes to the observed increase in microglia activity in the course of the diseases [4]. Of note, a role of CB_2 receptors in regulating neuronal progenitor proliferation and differentiation has been reported [5]. Higher CB_2 expression is also observed in a number of tumours and cancer cells and the inhibitory effect of CB_2 receptor activation on cell cycle progression and proliferation in different cancer types has been shown in a considerable number of studies [6]. In regard to these findings, modulation of CB_2 receptor expression and/or activity has been proposed as an attractive potential therapeutic approach [2].

Due to the importance of CB_2 receptors in pathological conditions, there is great interest in the biochemical characterization of CB_2 receptor signalling and its regulation by *e.g.* intracellularly interacting proteins. Lack of specificity of commercially available CB_2 antibodies [7] limits biochemical analysis in cells endogenously expressing CB_2. Of note, studies raising concerns against specificity of antibodies increased generally for GPCRs [8–13]. Thus, we set out to identify and characterize receptor interacting proteins and/or receptor function regulating proteins in cultured model cells reconstituted with an epitope-tagged CB_2 receptor.

As an activator of G protein signalling (AGS), Tctex-1 has been reported to interact with different GPCRs, like rhodopsin [14] parathyroid hormone 1 receptor (PTH1R) [15] and orexin 1 receptor [16]. Tctex-1 was identified as part of the cytoplasmic dynein motor complex, linking dynein cargos and adaptor proteins to dynein [17]. However, Tctex-1 also acts independently from the dynein complex by *e.g.* interacting with the β-subunit of GTP-binding protein (Gβ) [18]. Accordingly, a Tctex-1–Gβγ-complex was reported to be involved in the regulation of initial neurite sprouting, axonal specification and elongation of cultured neurons [19], and to be of relevance for cellular proliferation of neural progenitors [20].

For the dynein intermediate chain (DIC) and several Gβ proteins an interaction with Tctex-1 *via* a putative binding domain K/R-K/R-X-X-K/R has been described [19,21]. Of interest, the CB_2 receptor carries a putative Tctex-1 binding motif comprising the amino acid sequence K-K-C-V-R in its carboxyl terminal portion (Fig. 1A). Due to this fact, we analysed the putative interaction of Tctex-1 and CB_2 receptor in detail. To this end, we investigated the potential interaction of both proteins by immunoprecipitation from lysates of HEK293 cells exogenously expressing Tctex-1 and CB_2. We identified a correlation of the CB_2 receptor expression level and the formation of Tctex-1-containing intracellular protein complexes, and studied if cannabinoids induce changes in these effects. Furthermore, results obtained by using protein degradation inhibitors indicate an induction of specific proteolytic pathways by a non-canonical CB_2 receptor function. Our findings open future perspectives of CB_2 receptor functions putatively relevant on various pathophysiological conditions where the receptor is up-regulated.

2. Methods

2.1. Chemicals

The chemicals, used in this work, were purchased from following companies. DMEM (Dulbecco's Modified Eagle Medium), Penicillin-Streptomycin (10,000 U/ml), Lipofectamine™ 2000 and Opti-MEM® for cell culture were obtained from Life Technologies, Germany. FCS (fetal calf serum) with low endocannabinoid levels (#F7524, Lot: 030M3396, about 2 ng/ml anandamide and about 50 ng/ml 2-arachidonoyl-glycerol measured by the laboratory of Jürg Gertsch) was ordered from Sigma–Aldrich, Germany. PBS (phosphate buffered saline), DDM (*n*-dodecyl β-D-maltoside), EDTA (ethylenediaminotetraacetic acid), glycerol, sodium fluoride, bafilomycin A1, cycloheximide, GTP[γS] and anti-Flag® M2 Affinity Gel were purchased from Sigma–Aldrich, Germany. Leupeptin hemisulfate, SDS pellets (sodium dodecylsulfate) and IPTG (isopropyl-β-D-1-thiogalactopyranoside) were obtained from Roth, Germany. Ammonium chloride, sodium chloride, magnesium chloride, HEPES (4-(2-hydroxyethyl)-1-piperazine-1-ethanesulfonic acid), PMSF (phenylmethanesulfonyl fluoride), β-glycerophosphate, Tris, DTT (2,3-dihydroxybutane-1,4-dithiol), skimmed milk powder, Tween® 20, Triton X-100 and aprotinin were purchased from AppliChem, Germany. Complete Mini protease inhibitor cocktail tablets and protein A agarose were obtained from Roche Applied Science, Germany. CB_2 ligands JWH 133, AM 630, 2-AG as well as proteasome inhibitor MG132 were from Tocris, UK. Bromophenol blue was used from Merck, Germany. Glutathione sepharose beads were purchased from GE Healthcare, UK.

2.2. Primary and secondary antibodies

Commercial antibodies were obtained from the following companies, rabbit anti-Flag (#F7425, dilution 1:750), mouse anti-Flag M2 (#F1804, dilution 1:750) and mouse anti-α-tubulin antibody (#T5168, dilution 1:1000) from Sigma–Aldrich, Germany; rabbit anti-Gβ antibody (T-20, #sc-378, dilution 1:1000) from Santa Cruz Biotechnology, Inc., Germany; mouse anti-Myc (#2276, dilution 1:1000), mouse anti-Erk1/2 (#9107, dilution 1:2000), rabbit anti-phospho-Erk1/2 (#4370, dilution 1:1000) antibody from Cell Signaling Technology, USA; mouse anti-GST antibody (#34860, dilution 1:750) from Qiagen, Germany and rabbit anti-Tctex-1 antibody (#11954-1-AP, dilution 1:1000) from Proteintech, UK. Secondary anti-mouse and anti-rabbit horseradish peroxidase-conjugated antibodies (#sc-2030 and #sc-2005, dilution 1:5000) were purchased from Santa Cruz Biotechnology, Inc., Germany. Also anti-mouse and anti-rabbit IRDye® 680LT-conjugated secondary antibodies (#926-68022 and #926-68023, dilution 1:5000) or anti-mouse and anti-rabbit IRDye® 800CW-conjugated secondary antibodies (#926-32212 and #926-32213, dilution 1:5000) from LI-COR, Germany were used. Information about used pairs of primary and secondary antibodies for detection is given for each experiment in the corresponding figure legend.

2.3. Cell lines

Human embryonic kidney cells HEK293 were purchased from CLS Cell Lines Service GmbH (Germany). The cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FCS (fetal calf serum) and 1% Penicillin-Streptomycin (10,000 U/ml).

2.4. DNA constructs

The DNAs encoding for human proteins were cloned into pcDNA™ 3.1(+) expression vector (#V790-20, Life Technologies, Germany). Open reading frames of the human CB_2 and PTH1R were cloned using primers attaching a DNA sequence encoding a Flag-tag to the 5′ ends of the coding regions. For cloning of CB_2 receptor variant Q_{63}–H_{316} human genomic DNA was used. The cDNA clone for human PTH1R was obtained from the ORFeome Collaboration and the Center for Cancer Systems Biology *via* Harvard PlasmID