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Angiotensin type 1A receptor regulates β-arrestin binding of the β_2-adrenergic receptor via heterodimerization

András D. Tóth [a], Pál Gyombolai [a, b], Bence Szalai [a, b], Péter Várnai [a], Gábor Turu [a, b], László Hunyady [a, b, *]

[a] Department of Physiology, Faculty of Medicine, Semmelweis University, P. O. Box 2, H-1428 Budapest, Hungary
[b] MTA-SE Laboratory of Molecular Physiology, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

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ABSTRACT

Heterodimerization between angiotensin type 1A receptor (AT_1R) and β_2-adrenergic receptor (β_2AR) has been shown to modulate G protein-mediated effects of these receptors. Activation of G protein-coupled receptors (GPCRs) leads to β-arrestin binding, desensitization, internalization and G protein-independent signaling of GPCRs. Our aim was to study the effect of heterodimerization on β-arrestin coupling. We found that β-arrestin binding of β_2AR is affected by activation of AT_1Rs. Costimulation with angiotensin II and isoproterenol markedly enhanced the interaction between β_2AR and β-arrestins, by prolonging the lifespan of β_2AR-induced β-arrestin2 clusters at the plasma membrane. While candesartan, a conventional AT_1R antagonist, had no effect on the β-arrestin2 binding to β_2AR, TRV120023, a β-arrestin biased agonist, enhanced the interaction.

These findings reveal a new crosstalk mechanism between AT_1R and β_2AR, and suggest that enhanced β-arrestin2 binding to β_2AR can contribute to the pharmacological effects of biased AT_1R agonists.

1. Introduction

G protein-coupled receptors (GPCRs) are the largest plasma membrane receptor superfamily, and according to estimations ~40% of the marketed drugs target GPCRs (Whalen et al., 2011). Although the monomeric form of GPCRs is functional, a large number of evidence has accumulated demonstrating that they are also capable to form higher order complexes (Milligan, 2013). A very intriguing finding is that dimerization or oligomerization can greatly influence the signaling properties of GPCRs (Ferre et al., 2014). It has been reported that GPCR dimerization can result in altered ligand binding, receptor conformation or effector functions (Smith and Milligan, 2010; Szidonya et al., 2008). Heterodimerization between GPCRs widens the number of the possible physiological receptor crosstalk mechanisms, and helps fine tune receptor functions (Ferre et al., 2009; Jonas et al., 2013; Rivero-Muller et al., 2013). On the other hand, receptor dimerization can also cause unexpected drug interactions.

Angiotensin type 1A receptor (AT_1R) and β-adrenergic receptors (βAR) play crucial role in the regulation of heart function and vascular tone under physiological and pathophysiological conditions, therefore they are pivotal drug targets in cardiovascular diseases, including heart failure or hypertension (Whalen et al., 2011). Moreover, they were shown to form dimeric complexes and the blockade of either protomer with an antagonist can result in simultaneous hindering of the other protomer's G protein activation (Barki-Harrington et al., 2003).

In addition to G proteins, β-arrestin molecules are also considered to be effector proteins of GPCRs. β-arrestins govern GPCR desensitization, endocytosis and also participate in G protein-independent signaling pathways (Shenoy and Lefkowitz, 2011). β-arrestins regulate β_2AR function via several mechanisms. β-arrestin2 induces desensitization and internalization of β_2AR, and these effects have been linked to tachyphylaxis of β_2-adrenergic agonists (Deshpande et al., 2008). This phenomenon greatly limits the use of β_2-agonist drugs in the treatment of bronchial asthma. β-arrestins also mediate signaling of β_2AR. β-arrestin2 initiates the activation of MAPK cascade independently of G protein activation (Shenoy et al., 2006), and β-arrestins promote cardiomyocyte

Abbreviations: G protein-coupled receptors, GPCR; Angiotensin type 1A receptor, AT_1R; β_2-adrenergic receptors, β_2AR; serotonin 2C receptor, $5HT_{2C}R$; angiotensin II, AngII; Isoproterenol, ISO; *Renilla* luciferase, Rluc; Super *Renilla* luciferase, Sluc.

* Corresponding author.
E-mail address: Hunyady@eok.sote.hu (L. Hunyady).

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contraction (Carr et al., 2016). Chronic activation of β_2-adrenergic receptor by catecholamines leads to DNA damage via β-arrestin1 (Hara et al., 2011). β-arrestin1 facilitates the MDM2 promoted ubiquitination and degradation of p53. In the absence of β-arrestin1 this effect of β_2AR is greatly abrogated. These examples show the central role of β-arrestins in the function of β_2AR.

Activation of G proteins by AT_1R is considered to evoke deleterious effects in several pathophysiological conditions. However, stimulation of the G protein-independent, β-arrestin-mediated signaling pathways through AT_1R has been shown to have beneficial outcomes (Hunyady and Catt, 2006; Whalen et al., 2011). The clinically used conventional AT_1R antagonist drugs antagonize both pathways, so the desired β-arrestin-mediated favorable effects are also blocked. Thus, it is proposed, that ligands which are able to antagonize the G protein activation of a GPCR, but still able to induce the β-arrestin dependent signaling, could be prosperous drugs in many pathological circumstances (Whalen et al., 2011). Such β-arrestin biased agonist ligands have been already discovered for AT_1R. The first such ligand was [Sar^1,Ile^4,Ile^8]-AngII, however its clinical use was seriously hindered because of its poor receptor affinity (Wei et al., 2003). Since then, new peptides with higher affinity, like TRV120023 or TRV120027, have been developed, which offered the possibility of the clinical application (Rajagopal et al., 2011; Szakadati et al., 2015; Violin et al., 2014).

In this study, we investigated the consequences of angiotensin type 1A receptor-β2-adrenergic receptor (β_2AR) heterodimerization on β-arrestin binding using a bioluminescence resonance energy transfer (BRET)-based approach. We found that dimerization alters the β-arrestin binding of β_2AR. The physiological AT_1R agonist angiotensin II or the β-arrestin biased AT_1R ligand TRV120023, but not the unbiased AT_1R inverse agonist candesartan could potentiate β-arrestin coupling to the β_2AR. These findings reveal a possible new physiological crosstalk mechanism between AT_1R and β_2AR.

2. Materials and methods

2.1. Materials

The AT_1R, AT_1R-DRY/AAY (Gaborik et al., 2003), AT_1R-Δ319 (Hunyady et al., 1994), β_2AR, β_2AR-Sluc, untagged $5HT_{2C}R$-VGV (I156V, N158G, I160V), $5HT_{2C}R$-VGV-Sluc, PM-mRFP (mRFP fused to plasma membrane target sequence of Lyn) (Toth et al., 2012), AT_1R-Rluc (Szakadati et al., 2015), AT_1R-Venus, β-arrestin1-Venus, β-arrestin2-Venus (Gyombolai et al., 2013), β-arrestin2-Rluc, β_2AR-Venus (Turu et al., 2006), Cameleon D3 Ca^{2+}-BRET sensor (Gulyas et al., 2015), EPAC cAMP-BRET sensor (Erdelyi et al., 2014) and L10-Venus (Venus fused to plasma membrane target sequence of Lck) (Toth et al., 2016) constructs were previously described. $5HT_{2C}R$-Venus was generated by replacing the Super *Renilla* luciferase (Sluc) tag to monomeric Venus (Venus) in the $5HT_{2C}R$-Sluc construct. To create the Cerulean tagged β_2AR construct, the Sluc tag of β_2AR-Sluc was replaced with Cerulean. To generate the YFP-β-arrestin2 construct the cDNA of rat β-arrestin2 was cloned into pEYFP-N1 vector between *Age*I and *Kpn*I restriction sites. The plasmids encoding HA epitope-tagged wild type and K44A mutant dynamin-2A were kindly provided by Dr. K. Nakayama (Tsukuba Science City, Ibaraki, Japan).

Cell culture reagents were from Invitrogen (Carlsbad, CA). Cell culture dishes and white 96-well plates for BRET measurements were obtained from Greiner (Kremsmunster, Austria). TRV120023 (Sar-Arg-Val-Tyr-Lys-His-Pro-Ala-OH) was synthetized by Proteogenix (Schiltigheim, France). Coelenterazine h was purchased from Regis Technologies (Morton Grove, IL). Unless otherwise stated, all other chemicals and reagents were from Sigma (St. Louis, MO).

2.2. Cell culture and transfections

HEK 293T and COS-7 cells were cultured in DMEM supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% fetal bovine serum in 5% CO_2 at 37 °C. For the BRET-experiments, the transfection was performed on cell suspension using Lipofectamine 2000 in OptiMEM according to the manufacturer's instructions, thereafter the cells were plated on polylysine covered white 96-well plates. The measurements were performed 24 or 48 h after transfection of HEK 293T and COS-7 cells, respectively.

CHO cells were cultured in Ham's F12 supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% FBS. The day before transfection the cells were plated on 6-well plates, the transfection was achieved using Lipofectamine 2000 according to manufacturer's protocol.

For confocal microscopy experiments, HEK 293T cells were grown on glass coverslips in 6-well plates the day before transfection, and were transfected with plasmids encoding β_2AR-Cerulean, AT_1R-Δ319 and β-arrestin2-Venus (1 μg, 4 μg, and 0.5 μg pro well, respectively) using Lipofectamine 2000. The experiments were performed the day after transfection.

2.3. Bioluminescence resonance energy transfer (BRET) measurements

After a washing step, the medium of HEK 293T or COS-7 cells was changed to modified Kreb's-Ringer medium containing 120 mM NaCl, 4.7 mM KCl, 1.2 mM $CaCl_2$, 0.7 mM $MgSO_4$, 10 mM glucose 10 mM, pH 7.4 Na-HEPES, unless otherwise stated. 5 μM coelenterazine h, as *Renilla* luciferase substrate, was added to cells, thereafter luminescence was measured at 480 nm and 530 nm wavelengths by a Thermoscientific Varioskan Flash Reader (Perkin Elmer). BRET ratio was calculated by dividing the emission collected at 530 nm with the emission measured at 480 nm.

BRET signal of CHO cells was measured in cell suspension using Mithras LB 940 multilabel reader (Berthold Technologies), as earlier described (Gyombolai et al., 2014).

For the statistical analysis Two-Way-ANOVA tests were performed. An effect was considered statistically significant, when the p value of the interaction between the two treatments was less than 0,05.

2.4. BRET-titration experiments

Increasing amount of donor (Sluc containing) and acceptor (Venus containing) proteins were expressed in HEK 293T cells. Similarly to the conventional BRET experiments, before measurement the medium was changed to modified Kreb's-Ringer medium. Before addition of 5 μM coelenterazine h, Venus fluorescence was measured by excitation at 510 nm and emission collected at 535 nm. After coelenterazine h treatment, luminescence was measured using 480 nm and 530 nm filters and total luminescence was determined without filter. The data analysis in details was earlier described (Szalai et al., 2014). Briefly, measured points were grouped into high/low luminescence group by the median luminescence value for β_2AR-Sluc and AT_1R-Venus expressing cells. The effect of luminescence on the measured BRET ratio was evaluated by covariance analysis, forcing the regression line through the origin.

2.5. Confocal laser-scanning microscopy and image analysis

The media of the cells were changed to modified Kreb's-Ringer medium. Time-series images were taken every 10 s for 190 s from the bottom of the cells with a Zeiss LSM 710 confocal laser-scanning