Cellular Signalling 25 (2013) 970–980

Contents lists available at SciVerse ScienceDirect

Cellular Signalling

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

Specific interactions between Epac1, β-arrestin2 and PDE4D5 regulate β-adrenergic receptor subtype differential effects on cardiac hypertrophic signaling

Magali Berthouze-Duquesnes [a,b], Alexandre Lucas [a,b], Aude Saulière [a,b], Yuan Yan Sin [c], Anne-Coline Laurent [a,b], Céline Galés [a,b], George Baillie [c], Frank Lezoualc'h [a,b,*]

[a] Inserm, UMR-1048, Institut des Maladies Métaboliques et Cardiovasculaires, 31432 Toulouse Cedex 04, France
[b] Université de Toulouse III Paul Sabatier, 31432 Toulouse Cedex 04, France
[c] Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK

ARTICLE INFO

Article history:
Received 27 November 2012
Accepted 17 December 2012
Available online 22 December 2012

Keywords:
β-Adrenergic receptor subtypes
Epac
β-Arrestin
PDE4
Cardiac hypertrophy

ABSTRACT

β1 and β2 adrenergic receptors (βARs) are highly homologous but fulfill distinct physiological and pathophysiological roles. Here we show that both βAR subtypes activate the cAMP-binding protein Epac1, but they differentially affect its signaling. The distinct effects of βARs on Epac1 downstream effectors, the small G proteins Rap1 and H-Ras, involve different modes of interaction of Epac1 with the scaffolding protein β-arrestin2 and the cAMP-specific phosphodiesterase (PDE) variant PDE4D5. We found that β-arrestin2 acts as a scaffold for Epac1 and is necessary for Epac1 coupling to H-Ras. Accordingly, knockdown of β-arrestin2 prevented Epac1-induced histone deacetylase 4 (HDAC4) nuclear export and cardiac myocyte hypertrophy upon β1AR activation. Moreover, Epac1 competed with PDE4D5 for interaction with β-arrestin2 following β2AR activation. Dissociation of the PDE4D5–β-arrestin2 complex allowed the recruitment of Epac1 to β2AR and induced a switch from β2AR non-hypertrophic signaling to a β1AR-like pro-hypertrophic signaling cascade. These findings have implications for understanding the molecular basis of cardiac myocyte remodeling and other cellular processes in which βAR subtypes exert opposing effects.

1. Introduction

Cyclic AMP (cAMP) is a universal second messenger that is produced in response to stimulation of G protein-coupled receptors (GPCRs) positively linked to adenylyl cyclase. The cAMP binding protein Epac (*ex*change *p*rotein directly *a*ctivated by *c*AMP) mediates multiple actions of cAMP in cells [1]. Epac isoforms are multidomain proteins that include an N-terminal regulatory region and a C-terminal catalytic guanine nucleotide exchange factor (GEF) region. The C-terminal catalytic region consists of a CDC25 homology domain responsible for GEF activity, a Ras exchange motif (REM), which stabilizes the CDC25 homology domain, and a Ras association (RA) domain [1]. Epac1 and Epac2 promote the exchange of GDP for GTP in the Ras-like GTPases Rap1 and Rap2 upon binding to cAMP [2]. Rap1 is the immediate effector of many biological functions of these cAMP-sensitive GEFs, such as the control of cell adhesion and cell–cell junction formation [1]. Epac proteins also represent a molecular link between different members of the Ras family. For instance, Epac1–Rap2 signaling induces cardiomyocyte hypertrophy via phospholipase C and subsequent activation of H-Ras [3,4]. Therefore, the ability of Epac to induce different cellular responses through the same second messenger suggests the existence of molecular complexes that influence Epac signaling and cellular functions [2].

Interestingly, β-adrenergic receptors (βARs) activate Epac in cardiac myocytes and other cell types [3,5–7]. Of the three βAR subtypes that have been described in mammalian heart (β1AR, β2AR and β3AR), β1AR and β2AR dominate functionally [8]. Although both are structurally related and activate the G protein stimulatory for adenylyl cyclase, the β1AR and β2AR subtypes induce different sets of signal transduction mechanisms and fulfill distinct, sometimes even opposed, physiological and pathophysiological roles [9]. For instance, β1AR, but not β2AR, stimulates protein kinase-A (PKA)-mediated phosphorylation of cardiac contractile proteins [10]. In addition, sustained activation of β1AR promotes cardiomyocyte hypertrophy and apoptosis [9,11,12]. The spatial localization and compartmentalization of β1AR and β2AR signaling may explain the functional differences between βAR subtypes [5]. Indeed, βARs are spatially and temporally regulated and exert their biological function in combination with scaffolding proteins, such as A-Kinase Anchor Proteins (AKAPs), phosphodiesterases (PDEs), which regulate the duration and intensity of cAMP signaling, and also PKA [13–15]. PDEs are represented by more than 70 isoforms

Abbreviations: βARs, β-adrenergic receptors; β-arr, β-arrestin; ANF, Atrial Natriuretic Factor; HDAC4, histone deacetylase 4; Epac, exchange protein directly activated by cAMP; Iso, isoproterenol; RA, Ras association domain.

* Corresponding author at: Inserm, UMR-1048, Institut des Maladies Métaboliques et Cardiovasculaires, BP-84225, 31432 Toulouse Cedex 04, France. Tel.: +33 5 61 32 56 46; fax: +33 5 61 32 56 22.
E-mail address: Frank.Lezoualch@inserm.fr (F. Lezoualc'h).

0898-6568/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cellsig.2012.12.007

and the PDE3 and PDE4 families are predominantly expressed in the heart [14,16]. Besides their spatial regulation by AKAPs, PDEs interact differently with β1AR and β2AR thereby contributing to the specificity of βAR subtype signaling [17,18]. Particularly, β-arrestin (β-arr), which is classically associated with the termination of GPCR signaling and receptor desensitization, also acts as a dynamic scaffold allowing the recruitment of specific GPCR partners, including βAR subtypes and thus enforcing the specificity of various cell signaling pathways.

Epac represents a novel mechanism for governing cAMP signaling specificity and given its involvement in the manifestation of diseases, such as cardiac myocyte hypertrophy, it is important to determine the role of this cAMP sensor in GPCR signaling. We thus focused our study on βARs as they are canonical GPCR and key regulators of cardiac function [9]. We show that the β1AR and β2AR subtypes differentially interact with the cAMP-sensor Epac1 to induce distinct signaling pathways. Indeed, although both βAR subtypes activate the Epac downstream effector Rap1, only β1AR was able to regulate Epac1-induced H-Ras activation. We then explored the mechanistic basis of the differential effects of βAR subtypes on Epac signaling. We found that Epac1 recruitment to β1AR via its interaction with β-arrestin2 (β-arr2) plays an essential and coordinating role in the specific activation of the β1AR/Ras cardiac hypertrophic signaling. Interestingly, knock-down of β-arr2 prevents β1AR/Epac1-induced histone deacetylase 4 (HDAC4) nuclear export and the expression of the Atrial Natriuretic Factor (ANF), a marker of cardiac myocyte hypertrophy. In addition, we showed that Epac1 and the PDE variant PDE4D5 competed for the binding to β-arr2 upon β2AR activation. Comparison of the interacting domains between Epac1 and β-arr2 with those previously reported between PDE4D5 and β-arr2 [19] revealed a β-arr2 sequence (58-Pept) that was specific for the interaction of PDE4D5 with β-arr2. Disrupting the PDE4D5–β-arr2 complex by incubation with 58-Pept allowed the recruitment of Epac1 to activated β2AR and switched β2AR signaling to a β1AR-like pro-hypertrophic signaling.

2. Materials and methods

2.1. Reagents

Anti-Flag M2 affinity gel, anti-HA affinity gel, Phenylephrine (PE) and isoproterenol (Iso) were obtained from Sigma-Aldrich. Coelenterazine *h* and Deep Blue C were from PerkinElmer Life and Analytical Sciences. Collagen (rat tail) was from Roche Applied Science. Antibodies and their suppliers were; anti-Flag M2 (Sigma); anti-β1AR, anti-β-2AR H20, anti-phospho-HDAC4, anti-PARP, anti-GAPDH (Santa Cruz); anti-β-Arrestin2, anti-Rap2B, anti-Rap1A (BD Biosciences); anti-β-Actin; horseradish peroxidase-conjugated secondary antibodies (Cell Signaling); and anti-Ras (Clone Ras10, Millipore). Alexa 594 and 488 were from Invitrogen Life Technologies.

2.2. Cell culture

HEK293 cells that stably express β1AR or β2AR were generous gifts of Dr. HA Rockman and Dr. SK Shenoy (Duke University), respectively. HEK293 cells were maintained in MEM with 10% FBS (fetal bovine serum) and 1% penicillin–streptomycin. Neonatal rat cardiac myocytes were isolated as previously described [20]. All procedures for cardiac myocyte isolation were performed in accordance with the guide for the care and use of laboratory animals and the veterinary committee was informed about the myocyte isolation protocol used.

2.3. Plasmid construct and transfection

The plasmid constructs were generously provided by Dr K. Knowlton (the rat ANF promoter fused to the luciferase reporter gene (ANF–Luc)), Dr J.L Bos (wild type Epac1 and Epac1$^{\Delta 1-148}$ plasmid constructs), Dr J. Backs (HDAC4 construct), and Dr R. Jockers (β1AR and β2AR). The β-arrestin2-HA construct was from Addgene (Dr R.J Lefkowitz); the β1–GFP$_{10}$ [21], β-arr2–YFP [2] and the RLuc–β-arr2 plasmids [22] have been previously described. Epac–YFP was generated after subcloning the coding sequence of Epac1 in peYFP–C1 (BD-Biosciences Clontech), while Epac1–Rluc was provided by Dr C. Galés. Human *β-arr2* siRNA (5′GGACCGCAAAGUGUUUGUGTT-3′), rat *β-arr2* siRNA (5′ GGUACAGUUUGCUCCUGAGTT-3′), human *Rap2B* SiRNA (5′GUACG ACCCGACCAUCGAATT-3′) and control siRNA (5′GUGGACCCUGUAGA UGGCGTT-3′) were purchased from Ambion. Transient transfection experiments with HEK293 cells and neonatal cardiac myocytes were performed with the X-tremeGENE 9 reagent (Roche Applied Science) and Lipofectamine 2000 (Invitrogen Life Technologies), respectively, in the presence of various quantities of plasmid constructs and according to the manufacturer's instructions. SiRNA transfections were carried out using Lipofectamine 2000 according to the manufacturer's instructions.

2.4. Subcellular fractionation

Cells were harvested in ice cold PBS. After centrifugation at 5000 rpm for 2 min, cells were lysed on ice (15 min) in hypotonic buffer (20 mM Hepes pH 7.5, 10 mM NaCl, protease and phosphatase inhibitor cocktail) and supplemented with 0.5% Nonidet P40. After centrifugation (2500 rpm, 4 °C, 2 min), supernatants containing the cytosolic fraction were collected and pellets were resuspended in hypertonic buffer (20 mM Hepes pH 7.5, 400 mM NaCl), and left on ice for 15 min. Buffers were supplemented with a cocktail of protease and phosphatase inhibitors. After centrifugation (10,000 rpm, 4 °C, 10 min), supernatants containing the nucleic fraction were collected. The protein concentration of both fractions was determined with a BCA protein assay kit. 15 μg of total proteins was boiled for 10 min in reducing Laemmli sample buffer and resolved by SDS-PAGE.

2.5. Immunoprecipitation and western blotting

HEK293 cells were serum-starved for 4 h and then stimulated with Iso for 10 min. Cells were solubilized in RIPA buffer (150 mM NaCl, 50 mM Tris; pH 8, 0.5 mM EDTA, 1% Nonidet P-40 and 0.5% deoxycholate) supplemented with a cocktail of protease and phosphatase inhibitors (Roche Applied Science). After centrifugation, protein concentration was determined with a BCA protein assay kit (iNtRON Biotechnology) and soluble extracts were incubated with anti-Flag M2, anti-HA or anti-Myc agarose beads on a rotating wheel at 4 °C overnight. Non-specific binding was avoided by repeated washes with RIPA buffer and bound proteins were eluted with sample buffer containing SDS. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Hybond-P; GE Healthcare) for western blotting. Immunoblots were revealed by using enhanced chemiluminescence (ECL$^+$, GE Healthcare). Signals were quantified by densitometry using the Image Lab Biorad software.

2.6. Pull-down assays

Ras and Rap pull-down experiments were performed using a GST fusion protein containing respectively the Ras binding domain of Raf1–RBD and the Rap1 binding domain of Ral–GDS as previously described [3].

2.7. Immunostaining and confocal imaging

HEK293 cells that stably express β1AR or β2AR were transiently transfected with HA-Epac1 and/or β-arr. 24 h post-transfection, they were plated on collagen-coated 35-mm plates and, 24 h later, starved in serum-free medium for 1 h. Cells were then fixed in 4% paraformaldehyde for 30 min and permeabilized with 0.1% Triton X-100 in PBS containing 3% BSA for 1 h. Incubation with the appropriate primary