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Current Perspective

β-Arrestin-Mediated Signaling Improves the Efficacy of Therapeutics

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Abstract. *β*-Arrestins (*β*-arrestin-1 and *β*-arrestin-2) were first identified as proteins that have the ability to desensitize G protein-coupled receptors (GPCRs). However, it has recently been found that *β*-arrestins can activate signaling pathways independent of G protein activation. The diversity of these signaling pathways has also been recognized. This leads to an appreciation of *β*-arrestin-biased agonists, which is a new class of drugs that selectively activate *β*-arrestin-mediated signaling without G protein activation. In this review, we will discuss the recent advance of *β*-arrestin-mediated signaling pathways, including a brief account of different biased agonists, their pharmacological applications, and novel *β*-arrestin research.

Keywords: G protein-coupled receptor, biased agonist, *β*-arrestin, G protein

1. Introduction

β-Arrestins are known as non-visual arrestins and consist of two proteins: *β*-arrestin-1 and *β*-arrestin-2. These are also referred to as arrestin-2 and arrestin-3, respectively (1). As their names imply, *β*-arrestins were identified as proteins that "arrest" the agonist-stimulated β_2-adrenergic receptor signaling, analogous to the role of arrestin in the turn-off mechanism of rhodopsin activation (1). However, it has been well recognized that *β*-arrestins have their own signaling pathways (2). These signaling pathways are independent of G protein activation. This review will focus on the new signaling pathways of *β*-arrestins and the clinical benefits of targeting them.

2. Roles of *β*-arrestins in signaling pathways

2.1. Receptor endocytosis

The first recognized function of *β*-arrestins is to promote desensitization and internalization of G protein-coupled receptors (GPCRs) (1). Agonist activation of GPCRs is usually followed by receptor phosphorylation by G protein-coupled receptor kinases (GRKs). This phosphorylation promotes recruitment of *β*-arrestins to the receptor (1). When *β*-arrestins bind to the activated GPCRs, *β*-arrestins undergo a conformational change and act as scaffolding proteins that form a complex with heterotetrameric clathrin adaptor protein (AP-2) and clathrin. Clathrin is recruited to the plasma membrane by receptor-bound *β*-arrestins, and the membrane buds are formed inwardly. Two other proteins, amphiphysin and dynamin, act on the pit and pinch off the plasma membrane to form an endocytic vesicle. The internalized receptors will be either recycled back to the plasma membrane or targeted to lysosomes for degradation. In addition to the roles of *β*-arrestins in internalization of GPCRs, they are also involved in the internalization of the membrane proteins that do not belong to the GPCR family, such as transforming growth factor-*β* receptor-2 (3), insulin like growth factor receptors (4), nicotinic acetylcholine receptor (5), and transient receptor potential ion channel (TRPV4) (6).

2.2. Extracellular signal-regulated kinase (ERK) activation

It is well established that *β*-arrestins can activate ERK1 and ERK2 (ERK1/2) through their own pathways without G protein activation. *β*-Arrestin-mediated ERK activation requires GRK5 and GRK6 but not GRK2 activation

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(7). The role of β-arrestin-dependent ERK activation differs from that of G protein-dependent ERK activation. β-Arrestin-dependent ERK1/2 activation is generally associated with decreased apoptosis and increased activation of survival signaling in cardiomyocytes and HEK293 cells. In contrast to β-arrestin-mediated ERK1/2 activation, G protein-dependent ERK1/2 activation is often associated with increased apoptosis in cardiomyocytes and HEK293 cells. This suggests that β-arrestin-dependent signaling is a promising target for controlling cardiovascular diseases, especially heart failure. It has been recently reported that acute mechanical stress of the heart induces β-arrestin-2-mediated ERK1/2 activation through angiotensin II type 1 receptor (AT1R). Essentially, the same pathway is involved in the activation of ERK in response to acute mechanical stress and β-arrestin-biased agonists (8). Although chronic mechanical stress induces heart failure, acute mechanical stress through β-arrestin-dependent signaling inhibits cardiomyocyte apoptosis and may protect against heart failure (9). Localization of signaling proteins is one of the important factors for proper cellular responses. Localization of ERK1/2 activated by a β-arrestin-mediated pathway is different from that of ERK1/2 activated by a G protein-dependent pathway. ERK1/2 activated by a β-arrestin-mediated pathway is more localized in the cytoplasm than in the nucleus, while ERK1/2 activated by a G protein-dependent pathway is widely distributed in the cytoplasm and the nucleus. The mechanism of β-arrestin-dependent ERK1/2 localization in the cytoplasm of the heart and its role in cardiomyocyte apoptosis is still unknown and under investigation. It has been reported that the roles of β-arrestin-1 and β-arrestin-2 in ERK activation differ from each other. AT1R-stimulated activation of ERK1/2 in HEK293 cells increases when the cellular level of β-arrestin-1 is strongly inhibited (10). This suggests that β-arrestin-1 and β-arrestin-2 may each antagonize ERK activation.

2.3. RhoA/ROCK pathway

New lines of evidence indicate that β-arrestin-dependent signaling is involved in RhoA/ROCK pathway activation. This pathway is dependent upon β-arrestin-1 but not β-arrestin-2 (11). Although the exact mechanisms of β-arrestin-1-mediated RhoA/ROCK signaling are still under investigation, it has been reported that β-arrestin-1 enhances angiotensin II-stimulated RhoA/ROCK signaling by inhibiting ArhGAP (12). The function of ArhGAP is to deactivate RhoA. In many cases, β-arrestin-mediated signaling is protective against cellular stresses. However, there are many reports indicating that activation of RhoA causes the development of many diseases including heart failure (13). The role of RhoA activation by β-arrestin-biased agonists in cardiomyocyte apoptosis remains to be determined. Like the inhibitory effect of β-arrestin-1 on β-arrestin-2-dependent ERK1/2 activation, it has been found that β-arrestin-2 reduces β-arrestin-1-dependent RhoA activation (11). These results suggest that β-arrestin-1 and β-arrestin-2 may regulate each other's effects to adjust the intracellular signaling intensity.

2.4. Other signaling pathways

β-Arrestin-2 can mediate negative regulation of interleukin (IL)-1β, IL-12, and IL-6 production; natural killer cell–mediated toxicity; NF-κB transcription factor activity; and tumor necrosis factor production (14, 15). β-Arrestin-2 can also mediate positive regulation of the Akt (also known as protein kinase B) signaling cascade (16). β-Arrestin-1 is reported to be involved in the positive regulation of the activity of a small GTPase such as RhoA and histone acetylation (11, 17).

Signaling pathways through GPCR are summarized in Fig. 1. In this scheme, β-arrestins participate in two signaling pathways: GPCR desensitization and G protein-independent signaling.

3. β-Arrestin-biased agonists with promising therapeutic potential

3.1. Carvedilol

Carvedilol is a non-selective β-blocker / α_1-blocker, and it is widely used for the treatment of mild to moderate congestive heart failure (18). Recent studies on carvedilol indicate that it activates cardioprotective signaling through β-adrenergic receptors, β-arrestin, and ERK1/2 activation. It has also been shown that carvedilol activates a survival signal in the heart through a β-arrestin–Src–EGF receptor signaling pathway (19, 20). As carvedilol inhibits excess stimulation of catecholamine by blocking the binding to the β_1-adrenergic receptor, activation of a survival signal through a β-arrestin-dependent pathway is more effective for controlling chronic heart failure.

3.2. SII-AngII and TRV120027

Angiotensin II is an eight-amino-acid peptide (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) and regulates many important cardiovascular functions, including constriction of blood vessels and stimulation of aldosterone production. Angiotensin II binds AT1R and activates G_q and G_{12} family proteins, as well as β-arrestins. SII-AngII ([Sar^1, Ile^4, Ile^8]angiotensin II) and TRV120027 (Sar-Arg-Val-Tyr-Ile-His-Pro-D-Ala-OH) are β-arrestin-biased agonists of AT1R (21, 22). Both agonists can decrease blood pressure, increase cardiomyocyte contractility, increase cardiomyocyte performance, and de-

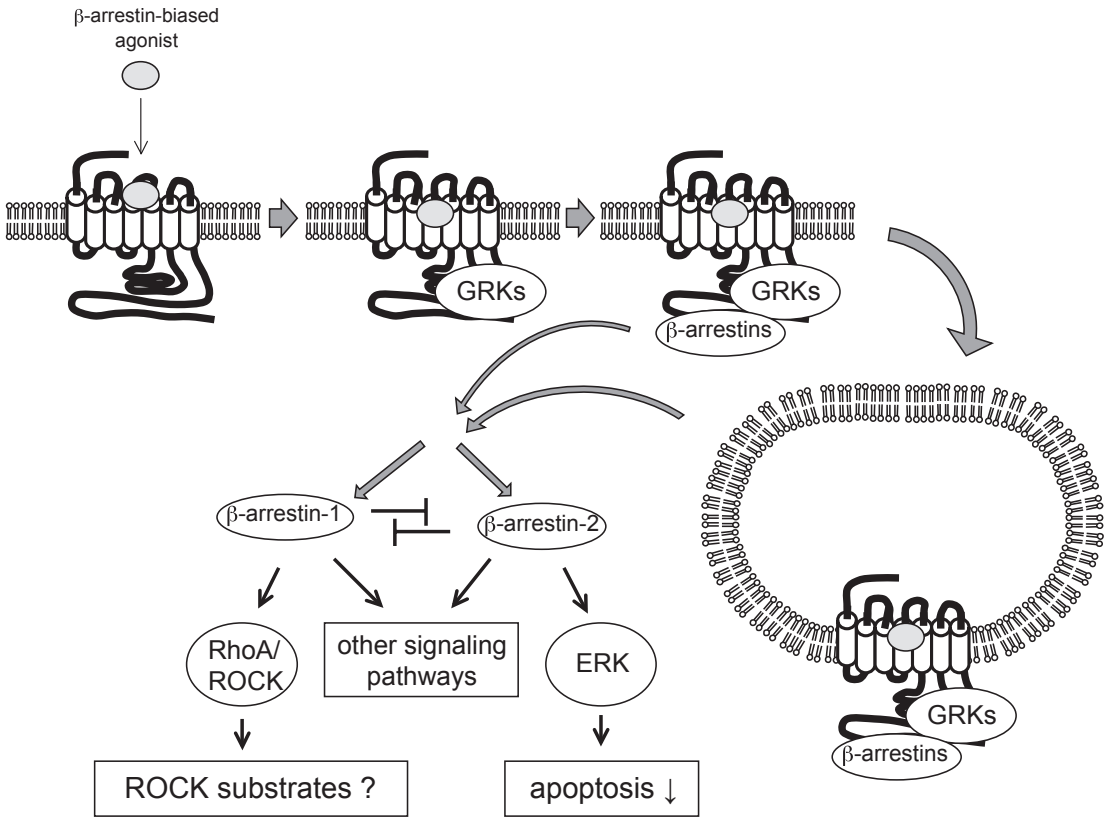

Fig. 1. GPCR-mediated signaling pathways. *β*-Arrestins participate in two signaling pathways: one is desensitization/internalization, and the other is G protein-independent signaling. The internalized GPCRs can activate signaling cascades like GPCRs expressed on the plasma membrane.

crease cardiac fibrosis (22, 23). TRV120027 is also the first biased agonist designed to treat acute heart failure patients and is under phase 2a trial.

3.3. PTH-*β*arr

Parathyroid hormone (PTH) is an 84-amino-acid polypeptide, and plays an important role in the homeostasis of Ca^{2+} and phosphate. PTH binds to a PTH receptor and activates the G_q and G_s family proteins, as well as *β*-arrestins. PTH(1-34) is approved as a drug for the treatment of osteoporosis. PTH(1-34) activates not only G protein signaling but also *β*-arrestin-mediated signaling. G protein-mediated signaling activates both bone resorption and formation. In contrast to G protein-mediated signaling, a *β*-arrestin-biased agonist selectively activates bone formation. PTH-*β*arr (D-Trp^{12}, Tyr^{34}-*β*PTH(7-34)) is found to be a PTH receptor–biased agonist that activates *β*-arrestin-2 but not classic G protein signaling (24). PTH-*β*arr stimulated anabolic bone formation, and this effect was greatly decreased in *β*-arrestin-2-knockout mice (25). This suggests that *β*-arrestin-2-mediated signaling selectively activates bone formation without bone resorption. Thus, a *β*-arrestin-biased agonist could be better than classical drugs depending on the purpose of treatment.

3.4. JNJ7777120

Histamine receptors are divided into four subtypes: H_1 to H_4. H_1 mediates inflammatory responses and H_2 plays an important role in acid secretion from gastric parietal cells. H_3 works as the receptor that regulates neurotransmitter release, such as histamine and GABA. H_4 participates in the migration of neutrophils, mast cells, and other inflammatory cells. JNJ7777120 is an indole derivative {1-[(5-chloro-1*H*-indol-2-yl)carbonyl]-4-methylpiperazine} and has been described as a selective antagonist at the H_4 histamine receptor (26). JNJ7777120 is widely used to characterize the physiological role of the H_4 receptor. It has been recently found that JNJ7777120 is a biased/inverse agonist that activates *β*-arrestin signaling and inhibits G protein signaling. As the H_4 histamine receptor is highly expressed in hemopoietic cells, it is suggested that JNJ7777120 is a promising new compound for the treatment of chronic inflammatory diseases (26, 27).

3.5. M_3-muscarinic receptor–biased agonists

M_3-muscarinic receptor is a member of the muscarinic receptor family. A recent study showed that M_3-muscarinic receptor activation in the brain regulates learning and memory in a β-arrestin-dependent manner (28). The M_3-muscarinic receptor is expressed in the central nervous system, including the hippocampus, and is proposed to be involved in learning and memory induced by fear conditioning. Knock-in mice of GRK-catalyzed phosphorylation-deficient M_3-muscarinic receptor revealed an important role in memory and learning of the hippocampus in a β-arrestin-dependent manner. This may lead to the discovery of more efficient therapies against Alzheimer disease. In the pancreas, M_3-muscarinic receptor activation augments sustained insulin secretion by a G protein-independent but β-arrestin-dependent pathway through M_3-muscarinic receptor and protein kinase D1 activation (29). Selective activation of β-arrestin-mediated signaling will be a novel way to improve the control of blood glucose levels in diabetic patients.

3.6. GPR109A-biased agonists

GPR109A is the receptor for nicotinic acid and couples with $G_{i/o}$. Nicotinic acid is used for the treatment of hyperlipidemia. It decreases the amounts of plasma low density lipoprotein (LDL) and triglyceride and increases the level of plasma high density lipoprotein (HDL). The most common side effect of nicotinic acid is flushing. It has been reported that β-arrestin-1 mediates nicotinic acid–induced flushing, as nicotinic acid–induced flushing is greatly inhibited in β-arrestin-1-knockout mice (30). However, the lipolytic action of nicotinic acid was not affected in β-arrestin-1-knockout mice (30). Thus, lipolytic action of nicotinic acid is mediated by $G_{i/o}$, and flushing is mediated by β-arrestin-1. This also suggests that G protein-biased agonists could be better drugs for the treatment of hyperlipidemia. Several compounds are now reported to activate lipolytic action without affecting flushing (31). Clinical trials will answer the interesting question of whether G protein-biased agonists work better than nicotinic acid.

4. New advances in β-arrestin-dependent signaling pathways

It was reported that SII-AngII stimulation increases phosphorylation of 35 proteins. It was also found that SII-AngII changes the phosphorylation states of 38 protein kinases and three phosphatases (32). Another study reported that SII-AngII stimulation regulates 36% of phosphorylation reactions among 1,183 phosphorylations regulated by AT1R stimulation (33). These results suggest that β-arrestin-mediated / G protein-independent signaling may be more pronounced than previously recognized for AT1R and other G protein-coupled receptors as well. As these β-arrestin-biased signaling pathways have a high possibility of therapeutic benefit in many cases, development of β-arrestin-biased agonists could be one of the most promising strategies for the development of new therapeutic agents.

References

1 Premont RT, Gainetdinov RR. Physiological roles of G protein-coupled receptor kinases and arrestins. Annu Rev Physiol. 2007;69:511–534.

2 Rajagopal S, Rajagopal K, Lefkowitz RJ. Teaching old receptors new tricks: biasing seven-transmembrane receptors. Nature Rev Drug Discov. 2010;9:373–386.

3 Chen W, Kirkbride KC, How T, Nelson CD, Mo J, Frederick JP, et al. β-Arrestin 2 mediates endocytosis of type III TGF-β receptor and down-regulation of its signaling. Science. 2003;301: 1394–1397.

4 Lin FT, Daaka Y, Lefkowitz RJ. β-Arrestins regulate mitogenic signaling and clathrin-mediated endocytosis of the insulin-like growth factor I receptor. J Biol Chem. 1998;273:31640–31643.

5 Dasgupta P, Rastogi S, Pillai S, Ordonez-Ercan D, Morris M, Haura E, et al. Nicotine induces cell proliferation by β-arrestin-mediated activation of Src and Rb-Raf-1 pathways. J Clin Invest. 2006;116:2208–2217.

6 Shukla AK, Kim J, Ahn S, Xiao K, Shenoy SK, Liedtke W, et al. Arresting a transient receptor potential (TRP) channel: β-arrestin 1 mediates ubiquitination and functional down-regulation of TRPV4. J Biol Chem. 2010;285:30115–30125.

7 Shenoy SK, Drake MT, Nelson CD, Houtz DA, Xiao K, Madabushi S, et al. β-Arrestin-dependent, G protein-independent ERK1/2 activation by the β2 adrenergic receptor. J Biol Chem. 2006;281:1261–1273.

8 Rakesh K, Yoo B, Kim IM, Salazar N, Kim KS, Rockman HA. β-Arrestin-biased agonism of the angiotensin receptor induced by mechanical stress. Sci Signal. 2010;125:ra46.

9 Noor N, Patel CB, Rockman HA. β-Arrestin: a signaling molecule and potential therapeutic target for heart failure. J Mol Cell Cardiol. 2011;51:534–541.

10 Ahn S, Wei H, Garrison TR, Lefkowitz RJ. Reciprocal regulation of angiotensin receptor-activated extracellular signal-regulated kinases by β-arrestins 1 and 2. J Biol Chem. 2004;279:7807–7811.

11 Barnes WG, Reiter E, Violin JD, Ren XR, Milligan G, Lefkowitz RJ. β-Arrestin 1 and $G_{\alpha q/11}$ coordinately activate RhoA and stress fiber formation following receptor stimulation. J Biol Chem. 2005;280:8041–8050.

12 Anthony DF, Sin YY, Vadrevu S, Advant N, Day JP, Byrne AM, et al. β-Arrestin 1 inhibits the GTPase-activating protein function of ARHGAP21, promoting activation of RhoA following angiotensin II type 1A receptor stimulation. Mol Cell Biol. 2011; 5:1066–1075.

13 Nunes KP, Rigsby CS, Webb RC. RhoA/Rho-kinase and vascular diseases: what is the link? Cell Mol Life Sci. 2010;67: 3823–3836.

14 Gao H, Sun Y, Wu Y, Luan B, Wang Y, Qu B, et al. Identification of β-arrestin2 as a G protein-coupled receptor-stimulated regula-

tor of NF-κB pathways. Mol Cell. 2004;14:303–317.
15 Witherow DS, Garrison TR, Miller WE, Lefkowitz RJ. β-Arrestin inhibits NF-κB activity by means of its interaction with the NF-κB inhibitor IκBα. Proc Natl Acad Sci U S A. 2004;101: 8603–8607.
16 Buchanan FG, Gorden DL, Matta P, Shi Q, Matrisian LM, DuBois RN. Role of β-arrestin 1 in the metastatic progression of colorectal cancer. Proc Natl Acad Sci U S A. 2006;103:1492–1497.
17 Kang J, Shi Y, Xiang B, Qu B, Su W, Zhu M, et al. A nuclear function of β-arrestin1 in GPCR signaling: regulation of histone acetylation and gene transcription. Cell. 2005;123:833–847. Erratum in: Cell. 2006;124:645.
18 Shin J, Johnson JA. β-Blocker pharmacogenetics in heart failure. Heart Fail Rev. 2010;15:187–196.
19 Noma T, Lemaire A, Prasad SV, Barki-Harrington L, Tilley DG, Chen J, et al. β-Arrestin-mediated β_1-adrenergic receptor transactivation of the EGFR confers cardioprotection. J Clin Invest. 2007;117:2445–2458.
20 Kim IM, Tilley DG, Chen J, Salazar NC, Whalen EJ, Violin JD, et al. β-Blockers alprenolol and carvedilol stimulate β-arrestin-mediated EGFR transactivation. Proc Natl Acad Sci U S A. 2008; 105:14555–14560.
21 Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM, et al. Independent β-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. Proc Natl Acad Sci U S A. 2003;100:10782–10787.
22 Violin JD, DeWire SM, Yamashita D, Rominger DH, Nguyen L, Schiller K, et al. Selectively engaging β-arrestins at the angiotensin II Type 1 receptor reduces blood pressure and increases cardiac performance. J Pharmacol Exp Ther. 2010;335:572–579.
23 Rajagopal K, Whalen EJ, Violin JD, Stiber JA, Rosenberg PB, Premont RT, et al. β-Arrestin2-mediated inotropic effects of the angiotensin II type 1A receptor in isolated cardiac myocytes. Proc Natl Acad Sci U S A. 2006;103:16284–16289.
24 Gesty-Palmer D, Luttrell LM. 'Biasing' the parathyroid hormone receptor: a novel anabolic approach to increasing bone mass? Br J Pharmacol. 2011;164:59–67.
25 Gesty-Palmer D, Flannery P, Yuan L, Corsino L, Spurney R, Lefkowitz RJ, et al. A β-arrestin-biased agonist of the parathyroid hormone receptor (PTH1R) promotes bone formation independent of G protein activation. Sci Transl Med. 2009;1:1ra1.
26 Seifert R, Schneider EH, Dove S, Brunskole I, Neumann D, Strasser A, et al. Paradoxical stimulatory effects of the "standard" histamine H_4-receptor antagonist JNJ7777120: the H_4 receptor joins the club of 7 transmembrane domain receptors exhibiting functional selectivity. Mol Pharmacol. 2011;79:631–638.
27 Suwa E, Yamaura K, Oda M, Namiki T, Ueno K. Histamine H_4 receptor antagonist reduces dermal inflammation and pruritus in a hapten-induced experimental model. Eur J Pharmacol. 2011; 667:383–388.
28 Poulin B, Butcher A, McWilliams P, Bourgognon JM, Pawlak R, Kong KC, et al. The M3-muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. Proc Natl Acad Sci U S A. 2010;107:9440–9445.
29 Kong KC, Butcher AJ, McWilliams P, Jones D, Wess J, Hamdan FF, et al. M3-muscarinic receptor promotes insulin release via receptor phosphorylation/arrestin-dependent activation of protein kinase D1. Proc Natl Acad Sci U S A. 2010;107:21181–21186.
30 Benyó Z, Gille A, Kero J, Csiky M, Suchánková MC, Nüsing RM, et al. GPR109A (PUMA-G/HM74A) mediates nicotinic acid–induced flushing. J Clin Invest. 2006;115:3634–3640.
31 Shen HC, Ding F-X, Deng Q, Wilsie LC, Krsmanovic ML, Taggart AK, et al. Discovery of novel tricyclic full agonists for the G-protein-coupled niacin receptor 109A with minimized flushing in rats. J Med Chem. 2009;52:2587–2602.
32 Xiao K, Sun J, Kim J, Rajagopal S, Zhai B, Villén J, et al. Global phosphorylation analysis of β-arrestin-mediated signaling downstream of a seven transmembrane receptor (7TMR). Proc Natl Acad Sci U S A. 2010;107:15299–15304.
33 Christensen GL, Kelstrup CD, Lyngsø C, Sarwar U, Bøgebo R, Sheikh SP, et al. Quantitative phosphoproteomics dissection of seven-transmembrane receptor signaling using full and biased agonists. Mol Cell Proteomics. 2010;9:1540–1553.