Pharmacology & Therapeutics 121 (2009) 285–293

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

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

Associate editor: V. Watts

Physiological and pharmacological implications of beta-arrestin regulation

Cullen L. Schmid, Laura M. Bohn *

The Ohio State University College of Medicine, Department of Pharmacology and Neuroscience Graduate Studies Program, Columbus, OH 43210, United States

ARTICLE INFO

Keywords:
G protein coupled receptors
Seven transmembrane spanning receptors
GRK
Beta-arrestin
Functional selectivity
Knockout mouse models
Drug discovery
Receptor regulation
Physiology

ABSTRACT

G protein-coupled receptor-targeted drug discovery as well as "compound reassessment" requires the utilization of diverse screens to determine agonist efficacies and potencies beyond the scope of ligand binding and G protein coupling. Such efforts have arisen from extensive studies, both in cellular and animal models, demonstrating that these seven transmembrane domain-spanning, G protein-coupled receptors may engage in more diverse functions than their name suggests and particular focus is drawn to their interactions with beta-arrestins (βarrestins). As regulators, βarrestins are involved in dampening G protein-coupling pathways. βArrestins can also play pro-signaling roles in receptor mediated events and the coupling of receptors to βarrestins may be as important as their potential to couple to G proteins in the physiological setting. In the last decade, the development of βarrestin deficient mouse models has allowed for the assessment of the contribution of individual βarrestins to receptor function in vivo. This review will discuss the current literature that implicates βarrestins in receptor function in respect to physiological and behavioral responses observed in the live animal model.

Contents

1. Introduction

βArrestins (non-visual arrestins) are ubiquitously expressed proteins that were first described for their role in desensitizing G protein-coupled receptors (GPCRs). There are two βarrestins, namely, βarrestin1 and βarrestin2, which are also referred to as arrestin-2 and arrestin-3, respectively. As their names imply, βarrestins were first identified for their ability to "arrest" agonist-stimulated β2 adrenergic receptor (β2AR) signaling (Lohse et al., 1990) in a manner similar to arrestin regulation of rhodopsin. The canonical model of GPCR regulation by βarrestins also involves GPCR kinases (GRKs) which phosphorylate receptors and thereby serve to facilitate receptor-βarrestin interactions (Benovic et al., 1987; Sibley et al., 1987; Lohse et al., 1992; Pitcher et al., 1992). Upon complexing with receptors, βarrestins can serve as inhibitors of signal transduction by preventing further receptor coupling to G protein signaling cascades (for reviews see: Premont et al., 1995; Freedman & Lefkowitz 1996; Lefkowitz, 1998).

Specific examples of βarrestins serving as negative regulators of GPCR signaling are plentiful in cellular as well as animal model systems (Table 1) (for reviews see: Gainetdinov et al., 2004; Bohn et al., 2004a;

Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol. DOI, 2,5-dimethoxy-4-iodoamphetamine. CP 55,940, 5-(1,1-Dimethylheptyl)-2-[hydroxyl-2-(3-hydroxypropyl)cyclohexyl] phenol. UK-14,304, 5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline. 5-HTP, 5-hydroxy-L-tryptophan. α2AR, α2 adrenergic receptor. β2AR, β2 adrenergic receptor. βarrestins, beta-arrestins. βarr1-KO, βarrestin1 knockout. βarr2-KO, βarrestin2 knockout. BRET, bioluminescence resonance energy transfer. CNS, central nervous system. D2 DAR, D2 dopamine receptor. FRET, fluorescence resonance energy transfer. GPCR, G protein-coupled receptor. GRK, GPCR kinase. LPS, lipopolysaccharide. MOR, mu opioid receptor. MEF, mouse embryonic fibroblast. OVA, ovalbumin. PKC, protein kinase C. PP2A, protein phosphatase 2A. M100907, R(+)-alpha-(2,3-dimethoxyphenyl)-1-[2(4-fluorophenylethyl)]-4-piperidinemethanol. R-PIA, R(-)N6-(2-phenylisopropyl) adenosine. S1P, sphingosine-1-phosphate. 5-HT2AR, serotonin 2A receptor. SSRI, selective serotonin reuptake inhibitor. WT, wildtype

* Corresponding author. The Ohio State University College of Medicine, 460 W 12th Ave, 798 BRT, Columbus, OH 43210, United States. Tel.: +1 614 292 1303.
E-mail address: Laura.Bohn@osumc.edu (L.M. Bohn).

0163-7258/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2008.11.005

Table 1
Enhanced drug responsiveness in βarrestin1-KO and βarrestin2-KO mice

Proposed target	Drug/challenge	Model system	Phenotype	Reference
β_2 Adrenergic receptor	Isoproterenol	βarr1-KO mice	Stimulated increase in cardiac ejection fraction	Conner et al., 1997
	Albuterol	βarr2-KO mice	Increased bronchodilation	Deshpande et al., 2008
CB1 cannabinoid receptor	Δ9-THC	βarr2-KO mice	Enhanced antinociception and hypothermia	Breivogel et al., 2008
CXCR2	CXCL1	βarr2-KO mice	Increased neutrophil migration into air pouches	Su et al., 2005
	Excisional punch wounds	βarr2-KO mice	Increased neutrophil migration into wound bed Increased rate of wound re-epithelialization	
Mu opioid receptor	Morphine	βarr2-KO mice	Enhanced and prolonged antinociception and hypothermia	Bohn et al., 1999, 2000, 2004b
			Enhanced drug reinforcement	Bohn et al., 2003
			Reduced antinociceptive tolerance	Bohn et al., 2000, 2002
		βarr2 anti-sense (rat)	Reduced antinociceptive tolerance	Przewlocka et al., 2002
	Heroin	βarr2-KO mice	Enhanced and prolonged antinociception	Bohn et al., 2004b
Parathyroid receptor 1	Parathyroid hormone	βarr2-KO mice	Disrupted increase in bone mineral content and trabecular bone parameters and increased osteoclast number	Ferrari et al., 2005
Toll-like receptor 4	LPS and D-galactosamine	βarr2-KO mice	Increased susceptibility to endotoxin shock and enhanced expression of proinflammatory cytokines	Wang et al., 2006

THC: tetrahydrocannabinol; LPS: Lipopolysaccharides.

Gurevich & Gurevich 2006; Premont & Gainetdinov, 2007). In addition to mediating receptor desensitization, βarrestins can facilitate recruitment and interactions between GPCRs and signaling partners. In this capacity, βarrestins can serve as positive mediators of receptor signaling to downstream targets. Evidence for GPCRs coupling to βarrestins to transduce receptor signaling has also been widely demonstrated in cellular models (for reviews see: Luttrell et al., 1999; Luttrell, 2002; Lefkowitz & Shenoy, 2005; DeWire et al., 2007). Studies in mouse models also support a pro-signaling role for βarrestins (particularly βarrestin2) and these reports are summarized in Table 2.

Arguably, the most studied GPCR is the β2AR. In vitro studies with this receptor have been instrumental in demonstrating the diverse and pleiotropic roles that βarrestins can play in determining agonist-induced receptor responses. The β2AR has been shown to interact with both βarrestin1 and βarrestin2 upon agonist stimulation (Attramadal et al., 1992) and such interactions result in decreased responsiveness to agonist over time. The removal of βarrestins by early anti-sense studies (Mundell et al., 1999), later siRNA studies (Ahn et al., 2003), as well as studies utilizing mouse embryonic fibroblasts devoid of both βarrestin1 and βarrestin2 (Kohout et al., 2001), confirm that βarrestins play a critical role in promoting this waning effect on G protein-coupling and adenylyl cyclase stimulation following agonist activation of the β2AR. Similar studies have been performed for multiple GPCRs of diverse classes and together, these findings support the canonical model wherein the agonist-activated GPCR becomes phosphorylated by GRKs which subsequently increases the binding affinity of the receptor for βarrestins.

βArrestin interactions with activated GPCRs can be detected by co-immunoprecipitation (Groer et al., 2007), confocal microscopy (Barak et al., 1997), bioluminescence resonance energy transfer (BRET) (Hamdan et al., 2005), and fluorescence resonance energy transfer (FRET) (Drake et al., 2008) assays. Such developments, including enzyme complementation assays (von Degenfeld et al., 2007), have facilitated high throughput screens for assessing drug-induced βarrestin-receptor interactions. Looking forward, the interactions between βarrestins and GPCRs may be realized for ultimately determining relative drug efficacies in vivo (Claing & Laporte, 2005; Violin & Lefkowitz, 2007; DeWire et al., 2007).

2. βArrestin regulation of GPCRs in vivo

While cellular model systems have been particularly useful for determining which receptors can possibly interact with βarrestins, in many cases, the question remains as to whether such interactions are pharmacologically and physiologically relevant. Assessing βarrestin function in vivo is challenging as there are no selective inhibitors of βarrestins. Some attempts have been made to develop selective inhibitors to GRKs as a means to prevent subsequent βarrestin recruitment, yet the degree of selectivity for these kinase inhibitors may not exclude inhibition of other serine/threonine kinases involved in alternate signaling cascades.

To overcome these limitations, Drs. Robert J. Lefkowitz and Marc G. Caron of Duke University, undertook the challenge of generating gene knockout mice deficient in βarrestin2. At that same time, the

Table 2
Decreased drug responsiveness in βarrestin1-KO and βarrestin2-KO mice

Proposed target	Drug/challenge	Model system	Phenotype	Reference
α_2 Adrenergic receptor	UK 14,304	βarr2-KO mice	Disrupted increase in sedation	Wang et al., 2004
Chemokine receptors	Airway challenge	βarr2-KO mice	Reduced T lymphocyte accumulation and asthmatic response	Walker et al., 2003
Dopamine receptors (direct)	Apomorphine	βarr1-KO mice	Reduced climbing behavior	Gainetdinov et al., 2004
		βarr2-KO mice	Reduced climbing behavior	Gainetdinov et al., 2004
			Reduced hyperlocomotor activity	Beaulieu et al., 2005
Dopamine receptors (indirect)	Amphetamine	βarr2-KO mice	Reduced hyperlocomotor activity	Beaulieu et al., 2005
	Lithium	βarr2-KO mice	Disrupted reduction in locomotor activity and anti-depressant-like behaviors	Beaulieu et al., 2008
GABA receptors	Ethanol	βarr2-KO mice	Reduced ethanol intake and preference and decreased ethanol-induced locomotion	Bjork et al., 2008
LPA, Protease-activated and S1P receptors	High fat diet	βarr2-KO mice	Reduced aortic atherosclerosis and decreased prevalence of atheroma smooth muscle cells	Kim et al., 2008
Mu opioid receptor	Morphine	βarr2-KO mice	Reduced hyperlocomotor activity	Bohn et al., 2003
			Reduced constipation and respiratory suppression	Raehal et al., 2005
	Loperamide	βarr2-KO mice	Reduced constipation	Raehal et al., 2005
Serotonin 2A receptor	5-hydroxy-l-tryptophan	βarr2-KO mice	Reduced head twitch response	Schmid et al., 2008

LPA: Lysophosphotidic Acid; S1P: sphingosine-1-phosphate; UK14,304: 5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline.