Bioorganic & Medicinal Chemistry Letters 26 (2016) 241–250

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Digest paper

Biased agonism: An emerging paradigm in GPCR drug discovery

Zoran Rankovic [a,*], Tarsis F. Brust [b], Laura M. Bohn [b,*]

[a] Discovery Chemistry and Research Technologies, Eli Lilly and Company, 893 South Delaware Street, Indianapolis, IN 46285, USA
[b] Department of Molecular Therapeutics, and Department of Neuroscience, The Scripps Research Institute, Jupiter, FL 33458, USA

ARTICLE INFO

Article history:
Received 6 October 2015
Revised 4 December 2015
Accepted 8 December 2015
Available online 9 December 2015

Keywords:
Biased ligand
Functional selectivity
GPCR
Drug discovery

ABSTRACT

G protein coupled receptors have historically been one of the most druggable classes of cellular proteins. The members of this large receptor gene family couple to primary effectors, G proteins, that have built in mechanisms for regeneration and amplification of signaling with each engagement of receptor and ligand, a kinetic event in itself. In recent years GPCRs, have been found to interact with arrestin proteins to initiate signal propagation in the absence of G protein interactions. This pinnacle observation has changed a previously held notion of the linear spectrum of GPCR efficacy and uncovered a new paradigm in GPCR research and drug discovery that relies on multidimensionality of GPCR signaling. Ligands were found that selectively confer activity in one pathway over another, and this phenomenon has been referred to as 'biased agonism' or 'functional selectivity'. While great strides in the understanding of this phenomenon have been made in recent years, two critical questions still dominate the field: How can we rationally design biased GPCR ligands, and ultimately, which physiological responses are due to G protein versus arrestin interactions? This review will discuss the current understanding of some of the key aspects of biased signaling that are related to these questions, including mechanistic insights in the nature of biased signaling and methods for measuring ligand bias, as well as relevant examples of drug discovery applications and medicinal chemistry strategies that highlight the challenges and opportunities in this rapidly evolving field.

G protein-coupled receptors (GPCRs) are seven-transmembrane domain proteins that function to transmit signals into the intracellular space. It is estimated that nearly 36% of all FDA-approved drugs target at least one member of the GPCR gene family.[1,2] Classically, the categorization of GPCR-ligands has been done based on their efficacies for activation of G proteins, as full agonists, partial agonists, antagonists, or inverse agonists, depending on their abilities to elicit a receptor-mediated response. However, it has become evident that GPCRs can mediate multiple signaling outcomes. For instance, βarrestins, which have been long associated with receptor desensitization, can also lead to signaling events.[3]

Arrestins are cytosolic proteins originally discovered in the visual system, which include arrestin-1 (also referred to as visual arrestin), arrestin-2 (βarrestin1), arrestin-3 (βarrestin2) and arrestin-4 (termed a cone arrestin).[4,5] While arrestins-1 and -4 are localized in retinal rods and cones, molecular cloning revealed two ubiquitously expressed isoforms termed βarrestin1 and βarrestin2 due to their high homology with the visual arrestin and their potent functional regulation of the β_2 adrenergic receptor (β_2AR).[6–8] More recently, two additional families of arrestin-related proteins have been found to be broadly expressed in eukaryotes, namely, a group of vacuolar protein sort 26 (Vps26)-related proteins and αarrestins. Their tertiary structure is similar to that of the visual and βarrestins, although their physiological role and potential involvement in regulating GPCR signaling are still not fully understood.[9,10]

While arrestins were named based on their initially discovered ability to arrest (turn off) GPCR signaling, it is now evident that βarrestins regulate GPCR trafficking as well as G protein-independent signaling.[11–13] A general scheme for the βarrestin-mediated regulation of GPCR function is depicted in Figure 1. Agonist binding to GPCR stabilizes an active conformation(s) of the receptor that promotes its interaction with heterotrimeric G proteins, Gαβγ (Fig. 1a). This is followed by the GDP to GTP exchange at the Gα subunit, and the subsequent dissociation of G proteins (Fig. 1b). The dissociated G proteins interact with and modulate the activity of downstream effectors (e.g., adenylyl cyclase and phospholipases) that produce second messengers such as cAMP and inositol phosphate (G protein-dependent signaling).[14]

Since one activated receptor can sequentially couple to multiple G proteins with signal amplification occurring through to the enzymatic activity of receptor second messengers (e.g., cyclases and

* Corresponding authors.
E-mail addresses: rankovic_zoran@lilly.com (Z. Rankovic), lbohn@scripps.edu (L.M. Bohn).

http://dx.doi.org/10.1016/j.bmcl.2015.12.024
0960-894X/

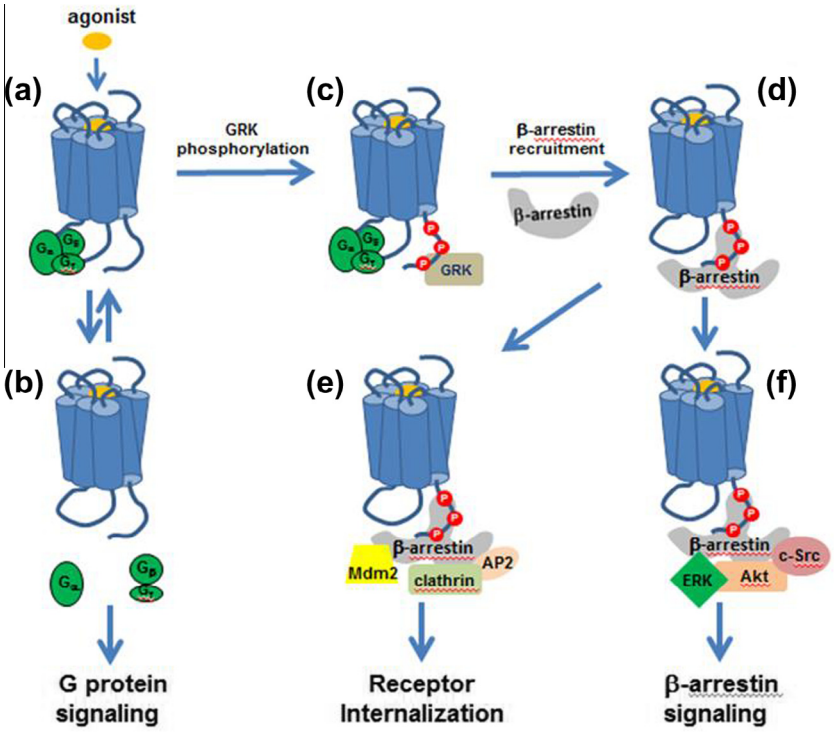

Figure 1. Paradigms of GPCR-mediated signaling and multiple roles of βarrestins: binding of an agonist (a) results in activation of signaling pathways by G proteins (b), as well as βarrestins (f), in addition to desensitization and internalization by βarrestins (d and e).

phospholipases), desensitization mechanisms have evolved to turn off the potentially deleterious effects of sustained signaling.[15] Desensitization is considered a two-step process, which starts with phosphorylation of the agonist-occupied GPCR by the second messenger-dependent protein kinases, such as protein kinase A or C (PKA or PKC), or by a corresponding GPCR Kinase (GRK, Fig. 1c).[4] This triggers the second desensitization step, which involves the recruitment of βarrestin to active phosphorylated receptors and the consequent steric hindrance of further G protein activation (Fig. 1d).[16] The initial discovery of the critical involvement of βarrestins in GPCR desensitization was subsequently followed up by studies that indicated their important role in receptor internalization. It has been found that the receptor-bound βarrestins interact with several key components of the receptor endocytotic process (Fig. 1e), including the adaptor-protein2 (AP2) complex,[17] the clathrin heavy chain,[18] and the E3 ubiquitin ligase Mdm2.[19] The complex formed upon the binding of clathrin/AP2 to the receptor-bound arrestin translocates to the clathrin-coated pit, which is then sequestered off the plasma membrane and hindered from subsequent stimuli.[20]

While the receptor–G protein interaction that takes place upon receptor activation is unstable and brief, the receptor–arrestin complex can be relatively stable and exist on a time scale of minutes to hours.[21,22] Analysis of the agonist-mediated arrestin translocation to multiple GPCRs identified two major classes of receptor–arrestin complexes, based on their strength and longevity. Class A complexes are transient and receptor–βarrestin interactions rapidly dissociate upon receptor internalization, as exemplified by the β_2 adrenergic, μ-opioid, endothelin type A, dopamine D_1, and α_1 adrenergic receptors. Receptors in this class are rapidly recycled back to the plasma membrane. In contrast, Class B complexes have more stable receptor–βarrestin interactions that persist even as the receptor undergoes endosomal sorting. Receptors in this class are sequestered in endosomes and recycled slowly or undergo degradation, for example, the angiotensin II type 1A, neurotensin 1, vasopressin V_2, thyrotropin-releasing hormone, and substance P receptors.[23]

Recent βarrestin crystallographic,[24] mutation,[25] and biophysical studies[26] suggest that βarrestins undergo extensive conformational changes upon binding to the phosphorylated GPCR. In their basal cytosolic receptor-unbound state, arrestins are elongated molecules, which consist of two (N- and C-) domains (Fig. 2a) and the C-terminus anchored in a polar core between them, unavailable for interaction with partner proteins (Fig. 2b).[27]

The recently reported crystal structure of βarrestin1 in complex with a fully phosphorylated 29-amino-acid carboxy-terminal (derived from the vasopressin receptor-2 (V_2Rpp) and shown to functionally and conformationally activate βarrestin1) revealed extensive conformational changes in the C-terminus, which is released and becomes available for interactions with clathrin and AP2 (Fig. 2c).[24] The V_2Rpp–βarrestin1 crystal structure also revealed a 20° twist between the N- and C-domains. A similar 20° rotation has been observed in the crystal structure of the mouse visual arrestin bound to a constitutively active form of human rhodopsin.[28] It has been suggested that the twisting movement of the two domains is part of the general mechanism by which arrestins, upon activation, may expose an additional interface for interacting with their numerous binding partners.

Over the past decades, a growing list of binding partners has implicated βarrestins in a number of important cellular functions in addition to GPCR desensitization and trafficking. In particular, arrestins have been found to link activated GPCRs to signaling effectors such as the Src family tyrosine kinases,[29] components of the extracellular signal-regulated kinase 1/2 (ERK 1/2) and the c-Jun N-terminal kinase 3 (JNK3),[30] mitogen-activated protein kinase (MAPK) cascades,[31] Akt,[32,33] and effectively convey the G protein-independent signaling (Fig. 1f). Because arrestin binding can uncouple G protein from the activated receptor, the