Methods 92 (2016) 64–71

Contents lists available at ScienceDirect

Methods

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

In vivo veritas, the next frontier for functionally selective GPCR ligands

Jean Martin Beaulieu *

Dept. Psychiatry and Neuroscience, Faculty of Medicine, Laval University, Canada

ARTICLE INFO

Article history:
Received 2 July 2015
Received in revised form 22 August 2015
Accepted 24 August 2015
Available online 28 August 2015

Keywords:
G protein coupled receptor
Functional selectivity
Animal models
Beta-arrestin
Cell signaling

ABSTRACT

The realization that G-protein coupled receptors (GPCR) engage several cell signaling mechanisms simultaneously has led to a multiplication of research aimed at developing biased ligands exerting a selective action on subsets of responses downstream of a given receptor. Several tools have been developed to identify such ligands using recombinant cell systems. However the validation of biased ligand activity in animal models remains a serious challenge. Here we present a general strategy that can be used to validate biased ligand activity in vivo and supports it as a strategy for further drug development. In doing so, we placed special attention on strategies allowing to discriminate between G-protein and beta-arrestin mediated mechanisms. We also underscore differences between in vitro and in vivo systems and suggest avenues for tool development to streamline the translation of biased ligands development to pre-clinical animal models.

1. Introduction

The realization that G-protein coupled receptors (GPCR) can engage several cell signaling mechanisms simultaneously, or under different conditions, has led to a multiplication of research aimed at developing receptor ligands exerting a selective action on subsets of responses downstream of a given receptor [1–3]. Such biased or functionally selective ligands, could in theory activate or antagonize therapeutically relevant signaling pathways without causing unwanted side effects by perturbing other receptor functions [4,5]. Considering the already central position of GPCRs as pharmaceutical targets [6], biased ligands hold great promise for the development of cleaner and more effective pharmaceutical treatments of animal and human diseases.

However, this exciting potential has also increased the complexity of GPCR drug development. The prevalent model of GPCR signaling in the late 20th century was based on the postulate that a given GPCR is coupled preferentially to a single type of G protein. This working model allowed for relatively straightforward drug discovery via the identification of ligands showing good selectivity (e.g. binding) and efficacy (e.g. activation of G protein) in recombinant cell based systems [1]. Such an approach was then complemented by investigation in animal models to demonstrate among other parameters, the bioavailability, metabolism, efficacy and non-toxicity of a given drug candidate.

This simple view has since been rendered more complex by the discovery that GPCRs can couple to more than a single G protein. Furthermore, beta arrestins (βArr1 and βArr2) have also been shown to exert dual functions downstream of several GPCRs. On the one hand βArr1 and βArr2 are negative regulators of G protein coupling and participate in receptor internalization [7–9]. On the other hand, these proteins also act as scaffolds for kinases and phosphatases thus constituting bona fides mediators of GPCR signaling [10–12]. The interactions between these dual functions of βArr remain unclear. In addition, G protein and βArr mediated signaling cascades demonstrate different temporal dynamics in cell systems, which are exacerbated in vivo.

The realization of the complexity of GPCR signaling has led to the exponential development of elegant cell-based and mathematical models to study biased ligand activity [13–15]. These methods often rely on measurements of G protein activation, second messenger production or βArr recruitment, alone or in combination, to quantify different dimensions of pharmacological efficacy [1]. Methodological aspects of these methods are covered in several excellent articles published in this issue of Methods.

While powerful tools exist to identify biased ligands in vitro, the validation of biased activity in vivo remains more hazardous. In addition to traditional parameters such as toxicity or bioavailability, several factors can potentially complicate the use of biased GPCR ligands in vivo. For instance, a given biased ligand may be processed into a non-biased active metabolite. The action of a

Abbreviations: GPCR, G protein coupled receptors; βArr1, beta-arrestin 1; βArr2, beta arrestin 2; D2R, D2 dopamine receptor; GSK3, glycogen synthase kinase 3.

* Address: 2601, Chemin de la Canardière, Québec City G1J 2G3, Canada.
E-mail address: martin.beaulieu.17@ulaval.ca

http://dx.doi.org/10.1016/j.ymeth.2015.08.018
1046-2023/

ligand affecting the recruitment of βArr to a GPCR may also affect the coupling of this receptor to G proteins. Furthermore, interaction of a GPCR with other membrane proteins in a natural environment can conceivably affect receptor coupling and the biased selectivity of a compound in vivo [16,17]. This article will provide an overview of a general approach that can guide the validation of biased ligand activity in animal models and a base to support further drug development. In doing so, I will focus mostly on situations involving biased activity targeting βArr versus G protein mediated signaling. I will also identify major limitations of current approaches and suggest how new research tools may simplify in vivo validation of biased ligands activity.

2. A general approach to study biased ligand activity in vivo

2.1. General principles, seven basic criteria

In a perfect world, biased ligand development should be undertaken with a knowledge of which receptor signaling mechanism represents a valid therapeutic target. Several parameters such as GPCR interaction partners and drug metabolism may also affect the functional selectivity of a GPCR ligand in vivo. Furthermore, functional selectivity in vivo and relevance to disease treatment should be demonstrated to support further drug development and clinical trials. In order to achieve this, I propose that validation of biased ligand activity as a valid drug target and of biased ligands as promising drug candidates should follow seven basic criteria. These would allow to establish that a given compound has biased activity in vivo and that such activity could be functionally implicated in the therapeutic effects of drugs targeting a given GPCR in vivo.

Seven basic criteria:

(1) The targeted cell signaling mechanism must be affected in disease or by less selective clinically effective drugs.
(2) Modulation of the targeted signaling mechanism must be dependent on the targeted receptor in vivo.
(3) Modulation of the targeted cell signaling mechanism by the receptor must be dependent of a specific effector in vivo.
(4) Biological effects of clinically effective drugs must be dependent of the targeted cell signaling mechanism.
(5) Modulation of the targeted cell signaling mechanism must replicate relevant drug actions in models with predictive validity.
(6) Biased ligands must engage targeted signaling mechanism specifically downstream of the targeted receptor.
(7) Biased ligands must replicate relevant drug action in models with predictive validity.

The following subsections constitute an explanation of the basis of these seven criteria and propose approaches allowing to establish that a given compound meets these in vivo. For the sake of simplicity, development of compounds targeting βArr2 mediated signaling downstream of the dopamine D2 receptor (D2R) [18] will be used as a working example (Fig. 1). This modality of dopamine receptor signaling has been shown to result in the formation of a signaling complex comprised at least of βArr2, the kinase Akt and protein phosphatase 2A [11]. The formation of this complex results in an inactivation of Akt following D2R activation. Research projects have targeted this modality of D2R signaling for the development of new antipsychotics [19–21].

2.1.1. The targeted cell signaling mechanism must be affected in disease or by less selective clinically effective drugs

One of the postulates supporting the development of biased GPCR ligands is that such compounds would allow to separate therapeutically positive from negative consequences of GPCR signaling. This implies that relevant target GPCRs are known for a given condition and that a biased ligand would make acting on this GPCR more effective and/or safe. In order for this to occur, it could be important to establish that a given signaling mechanism may be implicated in disease causation or that it may participate in the effect of drugs that are already effective to treat a given condition, which is our criterion #1.

Establishing contribution to disease causation may be difficult and constitutes a separate endeavor. That being said, information about cell signaling mechanisms relevant to a given pathology may already be available from existing literature. In contrast, establishing the effects of a drug on a subset of signaling responses in vivo is much more feasible. Both chronic and acute administration of a selection of drugs known to act on a therapeutic mechanism should be considered for this stage. However, acute treatment should be tested first since chronic effects may result from long-term adaptations several steps downstream from the action of a ligand on its receptor.

For acute administration, it is important to keep in mind that different cell signaling responses may occur in response to different drug doses and that βArr and G protein mediated signaling display different temporal dynamics. Moreover, one should also consider that in contrast to simple in vitro systems GPCR ligands in vivo compete with a natural agonist and may affect natural regulatory mechanisms thus complicating data analysis. More information on this is provided in Section 2.2.

To address these limitations one approach is to identify measurable cell signaling outcomes that are associated to different effectors for the same GPCR. In the case of D2R, one can use pAkt (Th308) as a readout for βArr2 mediated and DARPP32 (Th34) for Gi/cAMP mediated signaling mechanisms (Fig. 1). Specific guidance for the design of in vivo cell signaling experiments interrogating protein phosphorylation is provided in Section 3. However, cell signaling readouts used for this type of evaluation may differ across different systems and may also include measurement of second messenger levels or other responses that would be specific to a given type of GPCR signaling mechanism.

Independently of the experimental setup, cell signaling readouts will have to be evaluated at different drug doses and over different time periods. As a rule of thumb, the response of G protein mediated signaling events to receptor agonists or antagonists generally occurs in a matter a minutes (e.g. 5–30 min) following drug administration, conditional on the pharmacokinetics. This can be much slower (e.g. 30–120 min) for events depending of βArr. It is thus probable that the acute response of different cell signaling mechanisms to a non-biased GPCR ligand will occur at different time points in vivo. Measurement of signaling outcomes at a single time point may thus provide an illusion of functional selectivity and that should be avoided. In order to minimize animal use, a simple strategy can be to perform a time course study using a super-optimal dose of each drug. When an optimal time point has been identified for each GPCR signaling mechanism a top-down dose response study can then be conducted for these time points only.

2.1.2. Modulation of the targeted signaling mechanism must be dependent of the targeted receptor in vivo

Therapeutically effective drugs often act on several GPCRs at the same time. This is particularly true in psychopharmacology. For instance the second-generation anti-psychotic clozapine has been shown to activate Akt in vivo [22]. This action of clozapine may result from its antagonistic action on D2R. However, clozapine is also an antagonist of 5HT2A receptors [23], which have been shown to regulate Akt mediated cell signaling in vivo [24]. This exemplifies the need to establish that therapeutically effective drugs modulate the pathway of interest by acting on the GPCR