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Biased agonism in drug discovery – is it too soon to choose a path?

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Abbreviations:

AT1R, angiotensin II type 1 receptor

ERK, extracellular signal-regulated kinase

GPCR, G protein-coupled receptor

OAB, overactive bladder syndrome

PTX, pertussis toxin

TPP, target product profile

ABSTRACT

A single receptor can activate multiple signaling pathways that have distinct or even opposite effects on cell function. Biased agonists stabilize receptor conformations preferentially stimulating one of these pathways and, therefore, allow a more targeted modulation of cell function and treatment of disease. Dedicated development of biased agonists has led to promising drug candidates in clinical development, such as the G protein-biased μ opioid receptor agonist oliceridine. However, leveraging the theoretic potential of biased agonism for drug discovery faces several challenges. Some of them are technical, such as techniques for quantitative analysis of bias and development of suitable screening assays. Others are more fundamental, such as the need to robustly identify in a very early phase which cell type harbors the cellular target of the drug candidate, which signaling pathway leads to the desired therapeutic effect and how these pathways may be modulated in the disease to be treated. We conclude that biased agonism has potential mainly in the treatment of conditions with a well-understood pathophysiology; in contrast, it may increase effort and commercial risk under circumstances where the pathophysiology has been less well defined, as is the case with many highly innovative treatments.

INTRODUCTION

It has been assumed historically that a given G protein-coupled receptor (GPCR) primarily couples to one G protein and signaling pathway, for instance angiotensin II type 1 receptors (AT1R), muscarinic M₃ receptors and α_1 -adrenoceptors receptors to G_q, muscarinic M₂ receptors, μ opioid receptors and α_2 -adrenoceptors to G_i, and β -adrenoceptors to G_s (Bylund et al., 1994; Caulfield and Birdsall, 1998; de Gasparo et al., 2000; Dhawan et al., 1996).

While exceptions from this rule have been reported early after the definition and classification of G proteins, it only became accepted in the past decade that coupling of a single GPCR to multiple G proteins is the rule and not the exception. Receptors typically coupling to G_q proteins can also couple to G_i proteins, for instance AT1R (Crawford et al., 1992), or G_s proteins, for instance α_{1B} -adrenoceptors (Horie et al., 1995). Conversely, typically G_i-coupled receptors such as M₂ muscarinic acetylcholine receptors can also couple to G_q (Schmidt et al., 1995) and typically G_s-coupled receptors such as β_2 - and β_3 -adrenoceptors to G_i (Cao et al., 2000) and/or G_q (Wenzel-Seifert and Seifert, 2000). Moreover, GPCRs can directly couple not only to G proteins but also to other signaling molecules such as arrestins (Peterson and Luttrell, 2017) or src (Cao et al., 2000). Apparently, the ‘classic’ or ‘canonical’ signaling pathway of a receptor is present in most if not all cell types, whereas the additional or ‘non-canonical’ signaling pathways can exhibit a more restricted presence. For instance, we have detected coupling to cAMP formation upon β -adrenoceptor stimulation, presumably via G_s, in every cell type we ever studied; in contrast, we only detected coupling to phosphorylation of extracellular signal-regulated kinase (ERK) via G_i in only some cell types. This does not necessarily mean that coupling to additional signaling pathways per se is restricted, but it may be too weak in many cell types to be quantified in a robust manner.

The term ‘biased agonism’, originally introduced by Jarpe (Jarpe et al., 1998), describes the phenomenon that a ligand preferentially activates one of several signaling pathways, whereas another agonist in the same system and acting on the same receptor preferentially activates another pathway (Patel et al., 2010). This phenomenon has also been referred to as ‘stimulus trafficking’ (Kenakin, 1995), ‘functional dissociation’ (Whistler et al., 1999), ‘biased inhibition’ (Kudlacek et al., 2002), ‘differential engagement’ (Manning, 2002), or ‘ligand-directed signaling’ (Michel and Alewijnse, 2007). Such preferential coupling translates into differential induction of receptor trafficking and gene transcription programs (Delgado-Peraza et al., 2016; Maudsley et al., 2015). Of note, the concept of biased agonism is not necessarily restricted to GPCRs and could also be applied other signaling processes where the ligand-activated molecule may bind to more than one other partner, for instance to steroid hormone receptors and other ligand-activated transcription factors (Michel et al., 2014).

Perhaps the best-known hypothesis for the molecular basis of biased agonism relates to the fact that each ligand stabilizes a specific conformation of a receptor (Costa-Neto et al., 2016; Kenakin and Miller, 2010; Kenakin and Morgan, 1989). This has been demonstrated using a variety of techniques, from NMR and DEER spectroscopy (Manglik et al., 2015) to stabilization of discrete conformations using allosteric nanobodies (Staus et al., 2016). As different receptor conformations are likely to exhibit different affinities for various G proteins or G protein vs. arrestin, it appears logical that ligands inducing different receptor conformations will also differentially affect coupling to specific G proteins, i.e. can exhibit biased agonism. Even minor chemical differences between ligands, e.g. their stereoisomers, may lead to preferential activation of distinct signaling pathways of the same receptor (Seifert and Dove, 2009). A structural basis for this is that distinct amino acids within a receptor are critical for coupling to G_s as compared to G_i proteins (Manglik et al., 2015).

Many cases of proposed biased agonism include receptor binding to arrestins (Peterson and Luttrell, 2017), which in turn is often linked to activation of ERK (Delgado-Peraza et al., 2016; Patel et al., 2010; Szakadati et al., 2015). Activation of ERK can also occur independent of arrestin, for instance via src (Cao et al., 2000). Moreover, it has been proposed that receptors primarily coupling to G_q or G_s proteins may activate ERK via G_i . An example of the latter are β_3 -adrenoceptors, which typically couple to G_s followed by activation of adenylyl cyclase and generation of cAMP but in some cell types can also cause (moderate) induction of ERK phosphorylation, which is proposed to involve activation of a pertussis toxin (PTX)-sensitive G protein, presumably G_i (Gerhardt et al., 1999; Soeder et al., 1999). However, the latter finding may not be robust, as it is based on the observation that less ERK phosphorylation was observed following pre-treatment with PTX, but the effects of PTX on basal ERK phosphorylation had not been assessed. Recent observation from our group confirm that PTX reduces ERK phosphorylation responses but also markedly lowers basal ERK phosphorylation; relative to this lowered basal value, β_3 -adrenoceptor ligands, if anything, yielded a greater relative enhancement of ERK phosphorylation than in the absence of PTX (Okeke et al., 2018). As this may also apply to other receptors, the true role of G_i proteins in ERK activation as alternative signaling pathway remains to be determined. Of note, ERK activation by G_q or G_s -coupled receptors may result from activation these G proteins (Lefkowitz et al., 2002).

Based on the molecular basis of biased agonism, the specific signaling pathway activated by a ligand depends on several factors (Kenakin and Christopoulos, 2013). Firstly, the bimolecular interaction between ligand and receptor favors a specific receptor confirmation. This confirmation in turn will favor binding to a given G protein, arrestin or other signaling molecule. These two properties together define ligand bias (Kenakin, 2015b). Second, the stoichiometric ratio of G proteins, arrestins and other signaling partners affects to which

degree they will be activated by a given receptor confirmation (Onfroy et al., 2017). Thus, high expression of one signaling partner may lead to preferential activation of this pathway even if the receptor confirmation has somewhat lower affinity for it. These stoichiometric ratios define system bias (Kenakin, 2015b). Third, stoichiometric ratios of G proteins and arrestins in given cell type or tissue can be modified by various physiological, pathological or iatrogenic factors. These effects define dynamic bias (Michel et al., 2014). Fourth, whether a given signaling pathway is stimulated by a ligand may be dominated by the intrinsic efficacy of that ligand for the pathway to be activated, which in turn depends on the relative affinity of the effector molecules for the receptor (Kenakin, 2015a). Of note, ligands may be weak partial agonists or even inverse agonists for one but strong agonists for another signaling pathway, for instance carvedilol at β_2 -adrenoceptors (Wisler et al., 2007) or L 748,337 at β_3 -adrenoceptors (Sato et al., 2008).

The promise of biased agonism

As different G proteins and arrestins can modulate different signaling pathways, which in some cases may even have opposite effects on cell function, it is obvious that a ligand exhibiting biased agonism may yield distinct cellular responses as compared to a reference agonist. Some of these signaling responses may be desirable whereas others are undesirable, depending on the clinical condition under consideration. Thus, biased agonism in principle offers the possibility to selectively modulate one cellular/tissue response activated by a given receptor. For obvious reasons, this potential new avenue for selective modulation of cell and tissue function has generated considerable excitement.

The most informative, but perhaps up to now only example how the potential of biased agonism can be leveraged for the development of novel therapeutics is the discovery of opioid

receptor agonists that exhibit analgesic effects but are associated with little constipation and/or respiratory suppression. Initial work had demonstrated that β -arrestin 2 knock-out mice or mice or rats injected with β -arrestin 2 interfering RNAs exhibited enhanced analgesia in response to opioid receptor agonists but less tolerance development and little constipation or respiratory suppression (Kelly, 2013; Raehal et al., 2011). This suggested that μ opioid receptor agonists biased for G protein activation but having little arrestin-mediated effects may exhibit a beneficial profile in the treatment of pain. Based on such findings, a team at Trevena has developed oliceridine (formerly known as TRV 130), a μ opioid receptor agonist (DeWire et al., 2013). Oliceridine exhibited robust G protein activation with a potency and efficacy similar to that of morphine, but caused far less arrestin recruitment and receptor internalization. It was a potent analgesic in mice and rats but caused less gastrointestinal dysfunction and respiratory suppression than morphine at equally analgesic doses. A clinical phase II study confirmed that oliceridine is a potent analgesic drug in patients (Viscusi et al., 2016), and the FDA has granted breakthrough therapy status to this drug. Oliceridine produced similar analgesia as compared to morphine but caused fewer adverse events in a phase IIB study (Singla et al., 2017). However, presently available clinical data rely on short-term administration, i.e. are unsuitable to determine whether the reduced desensitization, constipation and respiratory depression also occur with chronic treatment. In a different approach, other investigators have used the crystal structure of μ opioid receptors and docking studies with over 3 million molecules to identify another ligand with strong bias for the G protein as compared to arrestin pathways (Manglik et al., 2016) but the leading ligand identified in this study has not yet been tested clinically. Biased agonists have also been described for κ opioid receptors (White et al., 2014), but the relevance for this subtype in analgesia remains unclear.

The unfulfilled promise of biased agonism

AT1R are modulators of many cardiovascular and renal functions, antagonists at these receptors have beneficial effects in corresponding disease and are clinically established drugs (Michel et al., 2016), but the clinically used AT1R antagonists do not exhibit biased agonism (Michel et al., 2013). However, experimental AT1R antagonists (Szakadati et al., 2015) and analogs of the endogenous agonist angiotensin II (Domazet et al., 2015) exhibit biased agonism. Therefore, investigators at Trevena also developed biased agonists at AT1R. They reasoned that the optimal ligand should be a potent antagonist for G protein activation via AT1R but a biased agonist promoting arrestin recruitment. Based on these consideration, they have identified TRV 027 (formerly known as TRV 120027), which inhibited angiotensin-stimulated G protein signaling and stimulated arrestin recruitment and activated several kinase pathways, including ERK, src and endothelial NO synthase phosphorylation (Violin et al., 2010). Similar to clinically used AT1R antagonists, TRV 027 reduced blood pressure but unlike the unbiased antagonists increased cardiac performance. This compound showed promising results in a dog model of congestive heart failure (Boerrigter et al., 2012) but a clinical phase II study (BLAST-AHF) failed to meet its composite primary endpoint consisting of (i) time from baseline to death through day 30, (ii) time from baseline to heart failure re-hospitalization through day 30, (iii) the first assessment time point following worsening heart failure through day 5, (iv) change in dyspnea visual analogue scale (VAS) score calculated as the area under the curve (AUC) representing the change from baseline over time from baseline through day 5, and (v) length of initial hospital stay (in days) from baseline (Pang et al., 2017).

Many reasons may potentially explain why a novel drug fails to reach its primary endpoint in a clinical proof-of-concept study. However, it is noteworthy that the clinically most advanced drug candidate based on biased agonism is an agonist at μ opioid receptors. This is a

mechanism of action that has been known for more than a century and numerous clinical and preclinical investigations have elaborated on the properties of morphine and how it decreases pain, causes tolerance and induces constipation and respiratory depression. Thus, the analgesic properties of opioid receptor agonists may be one of the best understood mechanisms in all of pharmacology. This is not likely to be the case for drug candidates that are based on novel targets.

The challenge for drug discovery

Two technical obstacles exist for leveraging the promise of biased agonism for drug discovery. Firstly, quantification of bias is not a trivial thing. Several useful approaches have been developed (Gundry et al., 2017; Kenakin, 2015a; Luttrell et al., 2015; Onaran et al., 2017; Stott et al., 2016) with $\Delta\Delta\log(\tau / K_A)$ or $\Delta\Delta\log(E_{\max} / EC_{50})$ being perhaps the most useful tools currently available (Winpenny et al., 2016), but it has recently been demonstrated that the “kinetic context” at the level of ligand-receptor and receptor-pathway kinetics is also a key consideration which further complicates interpretation of data (Klein Herenbrink et al., 2016; Lane et al., 2017). Identification of suitable screening assays for biased agonism, particularly high-throughput assays, is not trivial either, but there is theory to address this (Luttrell et al., 2015) and examples of practical implementation (McAnally et al., 2017; Winpenny et al., 2016). For reasons of scope, these obstacles will not be discussed further here.

In our view, the biggest challenge for drug discovery based on biased agonism is establishing the correct target product profile (TPP), that is determining how effective the ligand to be developed should be for which signaling pathway. We illustrate this challenge largely based on the example of drug discovery for β_3 -adrenoceptor agonists, a novel drug class for the

treatment of the overactive bladder syndrome (OAB) (Chapple et al., 2014; Ohlstein et al., 2012).

The signaling response to a receptor ligand depends on a combination of factors attributable to the ligand and the cell type/tissue in which it acts (ligand and system bias, respectively) (Kenakin, 2015b), and any changes this system may undergo in a pathological setting (dynamic bias) (Michel et al., 2014). Thus, the TPP of the lead compound for development must make assumptions which cell type harbors the molecular target responsible for desired and potential adverse effects, which signaling pathways mediate such effects and how this may be modulated in disease. Most β_3 -adrenoceptor agonists that have entered clinical development originally had been selected for the treatment of type 2 diabetes and obesity at a time when little knowledge was available about biased agonism and its implications; development for OAB was a repurposing endeavor (Michel and Korstanje, 2016). When repurposing studies for the OAB indication began, it had been assumed that the cellular target is the smooth muscle cell in the urinary bladder detrusor and that it mediates its desirable effects by increasing intracellular cAMP concentrations. Therefore, primary and secondary screens for suitable compounds in various companies were based on cAMP generation and relaxation of isolated detrusor strips in an organ bath, respectively, for instance for mirabegron (Takasu et al., 2007), ritobegron (Maruyama et al., 2012), solabegron (Hicks et al., 2007) or vibegron (Moyes et al., 2014). While one of these compounds has successfully undergone clinical development (Chapple et al., 2014), this may have been pure luck. Thus, while such drugs were already in clinical development, it became clear that cAMP generation plays a minor if any role in mediating detrusor smooth muscle relaxation by β -adrenoceptor agonists (Frazier et al., 2005; Uchida et al., 2005). Perhaps even more importantly, it is now increasingly being questioned whether the detrusor smooth muscle cell is indeed the cellular target of this drug class or rather is indirectly modulated via the urothelium, afferent nerves or

other structures (Michel, 2015). Therefore, even with today's knowledge it is difficult to say which cell type (system bias) and which signaling pathway (ligand bias) would be the optimal target for the treatment of OAB.

Moreover, β_3 -adrenoceptor ligands for the treatment of OAB must be agonists and based on their mode of action are assumed to provide symptom relief but not cure, indicating that long-term treatment may be required. Desensitization is a general issue with extended treatment with GPCR agonists, and biased agonism may affect speed and extent of desensitization (Raehal et al., 2011), including those of β -adrenoceptors (Giminez et al., 2015). Therefore, it would be interesting to know whether the β_3 -adrenoceptor agonists used or intended for OAB treatment differ with regard to biased agonism and how this affects their susceptibility for desensitization. Whether any of the clinically tested β_3 -adrenoceptor agonists is a biased agonist remains unknown, but multiple experimental β_3 -adrenoceptor ligands are biased agonists (Evans et al., 2010). However, recent data show that both cAMP formation and ERK phosphorylation can undergo agonist-induced desensitization when expressed in Chinese hamster ovary cells, but that the pattern of desensitization differs between the two signaling pathways (Okeke et al., 2018).

The above may sound a rather theoretical example since effective drugs have emerged.

However, it illustrates how lack of pathophysiological knowledge increases risk in defining a TPP. If neither the cell type nor the signaling pathway leading to desired therapeutic effect is known with certainty, it remains a high-stakes gamble to define the desirable molecular properties of a drug development candidate, i.e. whether it should be a biased agonist and, if so, for which signaling pathway. Only early translational approaches (most likely based on animal models) will be able to test whether a TPP based on biased agonism is viable.

Strategies for translational pathway validation have been reported (Rominger et al., 2014), but

do not address the validity of the inherent assumptions about validity of the model being used for the human target tissue and its alterations in disease.

Animal models still play a key role in target validation activities for many disease states, particularly through the widespread use of knock-out mouse models. It is not common, however, for the degree of agonist bias to be studied at different species orthologs of the human receptor. The often-tacit assumption that the pathway bias of a particular compound is maintained in other species presents another potential risk when ascribing the required degree of bias for a particular disease. This can be exemplified by studies on the histamine H₄ receptor. JNJ7777120 was the first selective histamine H₄ antagonist described and has been critical in defining a role for the H₄ receptor in a variety of allergic and inflammatory processes (Thurmond et al., 2008). In 2011, however, it was discovered that although JNJ7777120 was an antagonist/inverse agonist at the human H₄ receptor-mediated G_{αi} pathway, it was a partial agonist for the recruitment of β-arrestin to the human H₄ receptor (Rosethorne and Charlton, 2011). Furthermore, it was able to induce a prolonged ERK activation. While this unexpected biased agonism at the human receptor clearly complicates the interpretation of previous studies that assumed pure antagonism, the waters were muddied further when the activity of JNJ7777120 was tested in a number of species orthologs of the H₄ receptor. Surprisingly, and in stark contrast to the human receptor, JNJ7777120 was a partial agonist at the G_{αi} pathway from the mouse, rat and dog H₄ receptor (Schnell et al., 2011). This suggests that the beneficial effects of JNJ7777120 in the mouse (Thurmond et al., 2004) may be via H₄-mediated G_{αi} activation, rather than inhibition, potentially leading to the wrong choice of pathway for treating human disease. These species differences also raise concerns over interpretation of safety studies that often utilize the rat and dog as preferred species for the evaluation of toxicology. Thus, biased agonism simply being a probe-dependent form of

allostery and allosteric effects being species-dependent, it should not be surprising that biased agonism observed in one species does not necessarily translate to others.

System bias, i.e. the stoichiometric ratios between relevant signaling molecules, and dynamic bias, i.e. their possible alterations in disease and/or with treatment, are key in establishing the optimal TPP. As indicated above, the signaling pathway being activated by a ligand depends on its intrinsic properties (ligand bias) and those of the cell type which is targeted (system bias). A key element that influences system bias is the stoichiometric ratio of the different signaling molecules that are able to bind to activated receptor conformations (Onfroy et al., 2017), which is likely to differ considerably between cell types and tissues. To highlight this point, we have analyzed data on mRNA expression of several thousand genes across a panel of 31 human tissues (Uhlen et al., 2015). This analysis shows that the ratio between expression of G_s , G_i and arrestin is highly variable between tissues (Figure 1). While these data are based on mRNA expression and we do not know how this translates into functional protein in those tissues, it is safe to assume that a similar lack of correlation will hold true at the protein level and also when cell types rather than tissues are analyzed. Moreover, if differential expression of these three elements exists across human tissues, it is likely that similar differential expression exists in animal models as compared to patients.

To further complicate matters, expression of these various signaling components within a given cell type of tissue can be modulated by disease. For instance, congestive heart failure (the condition in which TRV 027 did not meet its primary endpoint) is characterized by a desensitization and down-regulation of β_1 -adrenoceptors (with less if any of β_2 -adrenoceptors), down-regulation of G_s , and up-regulation of G_i , β -arrestin-1 and G-protein-coupled receptor kinases (Brodde, 2007). Thus, a signaling pathway that may have been

important in healthy tissue may be less or more prominent in disease tissue. We have proposed to call such alterations of the signalosome ‘dynamic bias’ (Michel et al., 2014).

Conclusions

While it is clear that correctly assigning the required bias for a new receptor is currently very difficult, there are several technological advances that promise to shed more light on the discrete signaling pathways activated in disease. In particular, novel imaging approaches to dissect individual pathways in living cells, tissues and animals will allow better matching of the kinetics and signal strength to a particular phenotypic response. FRET-based imaging biosensors have been developed that can monitor the spatiotemporal characteristics of signaling pathways (e.g. calcium, cAMP, phosphorylated ERK) in single cells and even subcellular compartments (Halls et al., 2015; Lohse et al., 2012). More exciting still is the recent use of genetically encoded versions of these sensors to measure spatiotemporal signaling at a whole organ level in living animals (Jones-Tabah et al., 2017; van Unen et al., 2015). Using a microendoscopic implant, signaling via PKA and ERK_{1/2} has been imaged in the striatum of mice undergoing behavioral testing (Goto et al., 2015; Yamaguchi et al., 2015), representing a step-change in our ability to monitor therapeutically relevant signaling pathways in their physiological context.

The concomitant coupling of a single receptor to multiple signaling pathways and the selectivity for one of them that can theoretically be achieved by biased agonists is an attractive concept for drug discovery. However, definition of a sound TPP requires a lot of assumptions on system bias and dynamic bias, most importantly the cell type mediating the desired response and adverse responses, the signaling pathway causing them and how they behave quantitatively in the disease to be treated. As such knowledge typically is not available

for highly innovative targets at the time lead identification and optimization takes place, we feel that targeted development of biased agonists will be limited to a rather small number of conditions and even then, only in the discovery of second or third generation medicines.

Footnotes section

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Conflict of interest

MCM is a former employee of Boehringer Ingelheim; he also has received consultancy honoraria from Dr. Wilmar Schwabe and Velicept Therapeutics, and is a shareholder of the latter company. SJC is a founding Director and Chief Scientific Officer of Excellerate Bioscience, and a former employee of Novartis.

Author contributions:

MCM has generated the initial draft of the manuscript. Both authors have jointly developed the outline of the manuscript, searched the literature for relevant work, have revised the initial draft for critical content and approved the final version.

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LEGENDS TO THE FIGURES

Figure 1: Comparison of relative mRNA expression in a panel of 31 human tissues for G_s (GNAS), G_{i2} (GNAI2) and β -arrestin (ARRB2). All data expressed in FKPM and means of 2-7 individual subjects. Based on (Uhlen et al., 2015).

Figure 1:

