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 M_3 receptors and α_1-adrenoceptors receptors to G_q, muscarinic M_2 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 M_2 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 confirmation 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 confirmations are likely to exhibit different affinities for various G proteins

or G protein vs. arrestin, it appears logical that ligands inducing different receptor

confirmations 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

stochiometric 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 stochiometric

ratios define system bias (Kenakin, 2015b). Third, stochiometric 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 H_4

receptor. JNJ7777120 was the first selective histamine H_4 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 H_4 receptor-mediated $G_{\alpha i}$

pathway, it was a partial agonist for the recruitment of β-arrestin to the human H_4 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 H_4

receptor. Surprisingly, and in stark contrast to the human receptor, JNJ7777120 was a partial

agonist at the $G_{\alpha i}$ pathway from the mouse, rat and dog H_4 receptor (Schnell et al., 2011). This

suggests that the beneficial effects of JNJ7777120 in the mouse (Thurmond et al., 2004) may

be via H_4-mediated $G_{\alpha i}$ 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 stochiometric 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 stochiometric 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

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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.

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Footnotes section

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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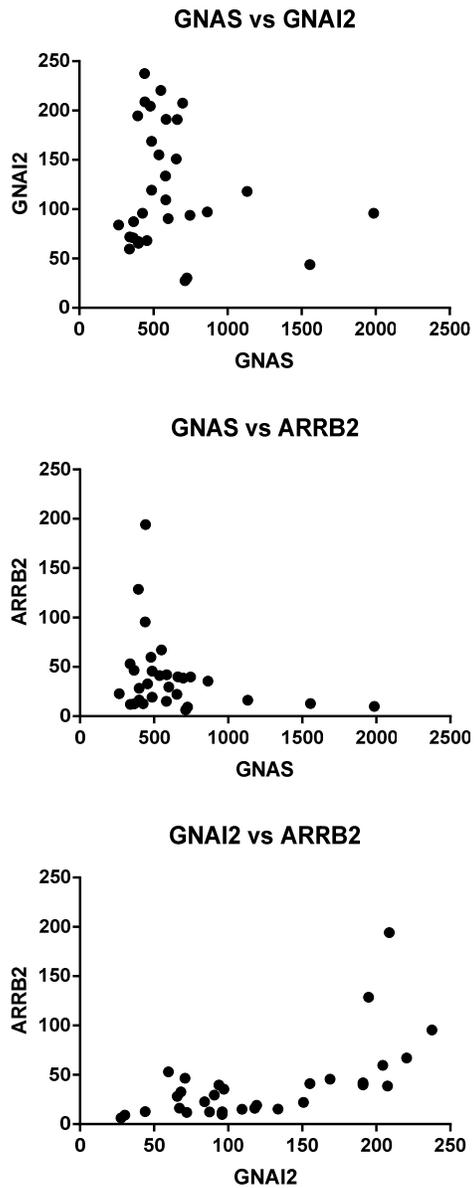
LEGENDS TO THE FIGURES

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:

GNAS vs GNAI2

GNAS vs ARRB2

GNAI2 vs ARRB2