Functional Signaling Biases in G Protein-Coupled Receptors: Game Theory and Receptor Dynamics

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Abstract: Pharmacotherapeutic targeting of G protein-coupled receptors (GPCRs) is perhaps the most important field of drug design, as agents designed to control these receptors constitute more than half of the pharmacopeia. Initially GPCRs were considered to be unitary entities, possessing all of their potential functionality in their characteristic heptahelical core. Early models of the functional activity of GPCRs considered them to possess just a simple 'on' or 'off' status. Recent research however has allowed us to realize that GPCR functionality is dependent upon many other proteins outside of the heptahelical core, on the site of GPCR expression in a tissue or a microdomain in a cell, and, most importantly, on the formation of differential 'active' states preferentially coupled to specific signal transduction structures. The recognition of such signaling diversity has facilitated the ability to appreciate and identify ligands for GPCRs that demonstrate a bias towards one signaling form of a receptor to another. However while potentially increasing our ability for selective signal targeting, our approach to understanding the physiological ramifications of systemic signaling manipulation is underdeveloped. This explosion in the complexity of GPCR signaling is now becoming familiar territory to receptor biologists, yet the application of this knowledge to drug design is relatively limited. This review will attempt to outline potential pitfalls and unseen benefits of using signaling bias in therapeutic design as well as highlighting new applications such as Game Theory for uncovering new therapeutic applications for biased agonists.

Keywords: G protein-coupled receptor, biased agonist, signaling, drug design, aging, allostasis.

INTRODUCTION

Heptahelical G protein-coupled receptors (GPCRs) are highly conserved transmembrane proteins ubiquitously expressed throughout eukaryotic organisms that can account for as much as 3 to 4% of the genome [1]. GPCRs likely evolved to facilitate survival of early lifeforms by enabling detection of an unprecedented variety of entities/ligands [2]. GPCRs can detect photons, odorants, tastants, amino acids, peptides, lipids, carbohydrates, simple chemicals and large complex proteins [3]. Interaction of these diverse ligands with the receptor effects the transmission of environmental information from outside a cell to the interior. GPCRs were initially considered to be relatively simple signaling entities that conveyed information transfer in a unidirectional manner. This initial appreciation considered that GPCRs acted in a simple linear fashion in which biological ligands interacted with the receptor to effect a positive biological action. Such ligands were historically referred to as agonists; hence compounds that were able to compete with the agonist-mediated stimulation of receptors were logically termed antagonists. Classically, agonist binding to the receptor (R) was thought to promote transition (through protein structural modification/stabilization) of the receptor from an "off" to an "on" state, capable of productively engaging heterotrimeric guanine nucleotide-binding (G) proteins, whose dissociated G_{α} and $G_{\beta\gamma}$ subunits in turn activate or inhibit various downstream effector molecules such as adenylate cyclases, phospholipases and ion channels. This early model of membrane receptor function was introduced in the late 1960s [4]. Subsequently, experimental evidence suggested that GPCR behavior was more complex, *e.g.*, the finding that β-adrenergic receptors exhibit two affinity states for agonists, the relative proportions of which were modulated by the presence of guanine nucleotides [5]. The dynamic model proposed to explain such activity predicted that in the presence of GDP, agonist binding promotes the formation of a stable ternary complex between agonist (H), GPCR (R), and the heterotrimeric G protein (G) that exhibits a high agonist binding affinity. In the absence of the G protein, or when the presence of GTP allows for receptor-catalyzed G protein activation, the H-R-G complex is dissociated, and the receptor resides in a low-affinity (H-R) state. Considerable research was then undertaken to understand the molecular-protein basis of this GPCR transitional activity. For example, by creating chimeras between the α_{1b} and β_2-adrenergic receptors it was demonstrated that some GPCR forms carrying relatively conservative substitutions in the C-terminal portion of the

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third intracellular loop (IC3) activated $G_{q/11}$ proteins in the absence of agonist [6]. This 'constitutive' (agonist-independent) activation of the resultant GPCR could even be engineered with a single point mutation in IC3 [6]. Through the use of such constitutively active receptor mutants some ligands, that were previously considered to be pure competitive antagonists when interacting with non-mutated receptors, were found to express a preference for the high affinity receptor state and suppress the constitutive receptor activity [7]. Ligands possessing these properties have since been termed "negative antagonists" or "inverse agonists". This and subsequent work, revolving around a wide variety of GPCR types confirmed the hypothesis that receptors typically exist in a spontaneous equilibrium between two conformations (active, R*; inactive, R) that differ in their ability to activate downstream G proteins [7]. In the native state, the receptor is maintained predominantly in the R conformation by intramolecular interactions within the transmembrane helical bundle; *i.e.*, the spontaneous equilibrium heavily favors the inactive R state [8]. Agonist binding, or selective mutagenesis, relieves these intramolecular constraints, allowing the receptor to "relax" into the R* conformation, facilitating productive G protein coupling. The extended ternary complex model developed to explain these phenomena proposed that the intrinsic efficacy of a receptor ligand is dictated by its ability to alter the equilibrium between R and R* [9]. In this model, '*full agonists'* stabilize the R* conformation, moving the equilibrium toward the active state to generate full receptor activation and maximal physiological responses; '*partial agonists'* have lower intrinsic efficacy than full agonists, thus generating a submaximal response and potential attenuation of '*full agonist*' activation; '*antagonists'* cannot discriminate in their binding between R and R*, produce no physiological response on either native or constitutively active receptors, but can block the response to agonists; and *inverse agonists* act as antagonists in non-constitutively active receptor systems but actively reduce receptor-mediated constitutive activity of GPCRs by preferentially moving the equilibrium to the inactive, R, state. The functional behavior of so-called "protean agonists", ligands that act as partial agonists in some systems and as inverse agonists in others, can still be accounted for within the original extended ternary complex model, assuming that the active receptor conformation produced by ligand binding possesses a lower efficacy than the spontaneously formed R* state [10]. Under conditions of low basal activity, *i.e.*, little or no spontaneously formed R*, such a ligand would behave as a partial agonist, whereas under conditions of high basal activity, it would behave as an inverse agonist. From such research and conceptual modeling it became clear that the relationship between receptor conformation and structure and its functional relationship with the interacting ligand was more complex than initially considered. The seminal work that first, and most eloquently, discussed this new complexity was that of Kenakin, who introduced the concept that agonists stabilize multiple distinct GPCR active states to mediate their full gamut of effects [11]. These distinct GPCR states (of a single specific receptor) differ in their ability to regulate separate associated downstream signaling pathways [3, 12, 13]. This form of signaling selectivity has been described in a manner of different ways, e.g. agonist trafficking, functional selectivity, selective signaling, and most recently biased agonism [13, 14].

AGONIST-RELATED BIAS IN GPCR SYSTEMS

Biased agonistic activity at GPCRs was first identified via selective activation, by different series of ligands, of distinct G protein pools associated with receptors such as the alpha adrenoceptors as well as serotonin and cannabinoid receptors [15-19]. At the most basic level the interaction of the GPCR with several G protein types, that differ in their physico-chemical structure, to assemble discrete GPCR-G protein units facilitates the eventual generation of *de facto* multiple active receptor states. In addition to different interacting G protein structures creating multiple active receptor states, the nature of the nucleotide (usually guanosine diphosphate) occupying the associated G protein can add an extra level of GPCR functional 'speciation' [20, 21]. Multiple G protein signaling activity for a single class of GPCR has now become a well-tested and clinically-relevant aspect of GPCR research and drug development [22-25].

It is now appreciated that GPCRs do not solely signal through their titular associated G proteins, but through a series of additional signaling factors that possess a stronger and more stable interaction with the receptor. Primary among these additional signaling factors is the arrestin class of molecules. The study of arrestin-mediated GPCR signaling has essentially pioneered the field of alternative GPCR signaling investigation and drug development [26-31, for review see 32]. In contrast to G protein-mediated signaling, arrestin-mediated processes often occur at post-desensitized receptors or those pre-coupled to the arrestin itself [26]. Via this productive non-G protein interaction, the functional arrestin clade of GPCR signaling forms incorporates a broad scope of additional signaling factors that were never initially considered to be affected by GPCR ligands, including Src-family kinases, E3 ubiquitin ligases, diacylglycerol kinases, phosphodiesterases, inhibitors of nuclear factor-κB and serine/threonine protein phosphatases [26, 33-38]. This form of arrestin-mediated GPCR signaling increases the potential signaling output spectrum that receptor activation can induce.

Research over the past decade has demonstrated another form of GPCR signal 'conditioning', *i.e.* the modulation of receptor expression, function, post-activation processing and ligand selectivity induced by stable protein-protein interaction of scaffolding or signal modulating factors such as NHERF (Na^+/H^+ exchange regulatory factor), RAMP (receptor activity-modulating protein), Pyk2 (proline-rich tyrosine kinase 2), diacylglycerol kinases, other GPCRs in heteromers and steroid receptor co-factors [39-45, for review see 46]. With these multiple and diverse GPCR signaling paradigms, it is highly unlikely that each of these receptor signaling modes would be assembled *de novo* for each ligand-induced signaling event. To maintain high signaling fidelity and rapid information transfer across the membrane a considerable degree of receptor-signal transduction protein-accessory protein pre-assembly is probable. Such stable receptor entities have been termed '*signalsomes*', each of these determine a specificity of signaling, *i.e.* reducing signal

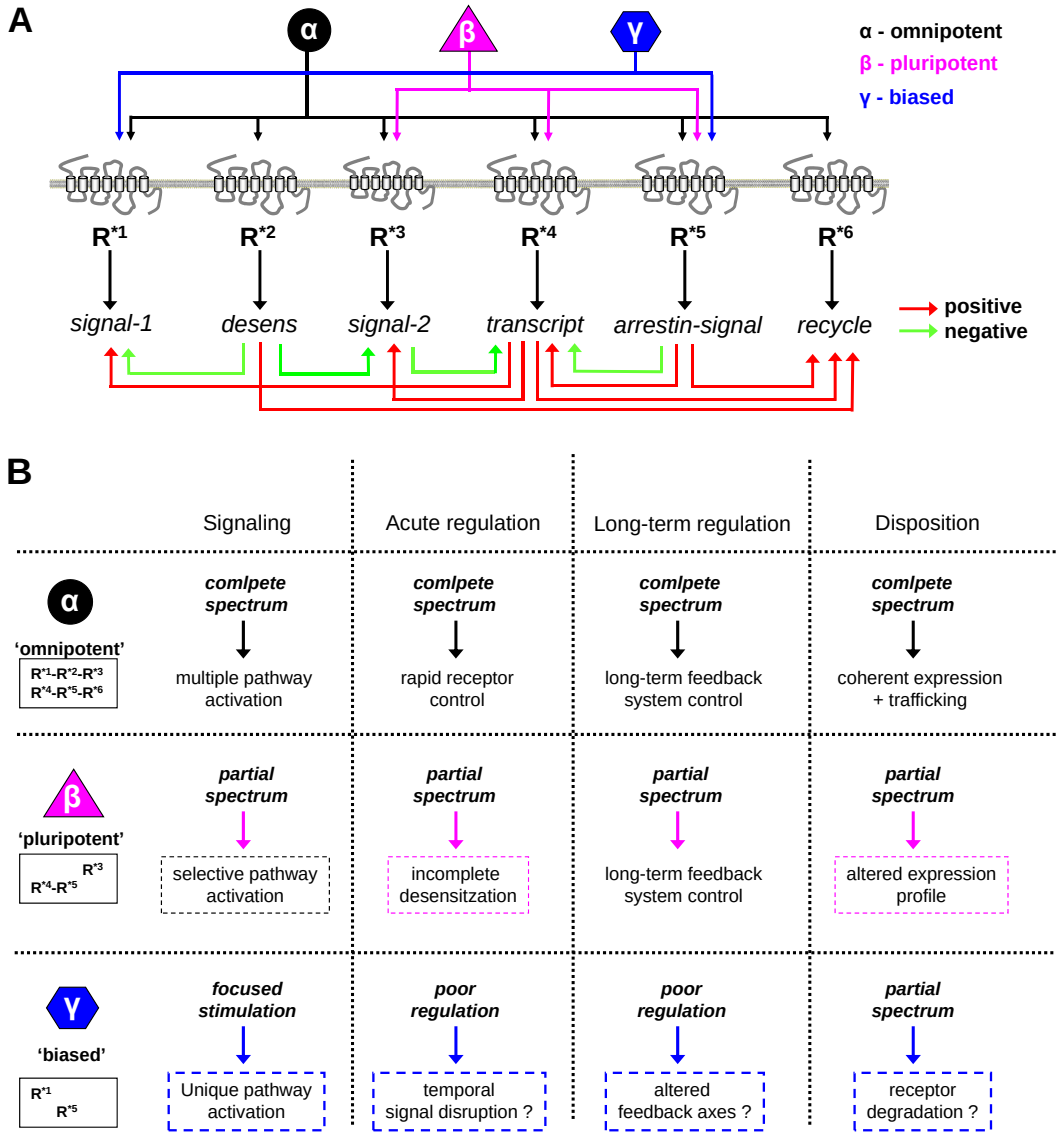

Fig. (1). Comparative functionality of biased versus non-biased GPCR ligands. (**A**) A single GPCR core can exist in 6 differential and stable active conformations (R^{*1} to R^{*6}). Three different forms of ligand (α, β, γ) that can all bind the different active states possess distinctive receptor isoform activation patterns (α-omnipotent, activates all forms; β-pluripotent, activates R^{*3}, R^{*4}, R^{*5}; γ-biased, activates R^{*1}, R^{*5}). Each of the stable receptor isoforms possess different preferential signaling activities once activated by the specific ligand (signal-1, signaling pathway 1; signal-2, signaling pathway 2; desens, receptor desensitization; transcript, transcriptional control of components affecting the receptor system; arrestin-signal, arrestin-specific receptor signaling pathway; recycle, pathways regulating receptor cellular disposition and resensitization). Each of these downstream cellular effects then can feed back to affect other forms of receptor responses either positively (red arrows) or negatively (green arrows). (**B**) Differential GPCR systemic effects of omnipotent versus pluripotent/biased agonists in an ideal system free of pathophysiology. The omnipotent ligand, α, efficiently controls GPCR systemic activity in an ideal system, while the pluripotent (β) and biased (γ) agonists mediate partial GPCR activity maintenance in the ideal system.

dilution post-receptor activation, and also introduce a potential for selectivity of ligand interaction [3, 47-50]. The likely existence of multiple, stable signalsome forms for each specific receptor type increases the potential for therapeutic exploitation of these divergent targets via biased agonism. Thus a biased agonist may be able to preferentially interact with functionally distinct targets containing the same heptahelical GPCR core, activating or inhibiting whichever signaling behavior the specific signalsome is 'hard-wired' to.

BIASED AGONISM AND CONTEXTUAL RESPONSE CONTROL

Through our improved appreciation of the pluridimensionality of GPCR signaling paradigms we have been able to identify signaling biases and preferences for diverse chemical ligand structures that induce differential downstream signaling effects via specific active GPCR states. These so-called 'biased agonists' have demonstrated

often selective pharmacological profiles, but the demonstration of actual distinct and beneficial physiological effects, compared to non-biased agonists, has been only a relatively new event. One of the best examples is the demonstration that a parathyroid hormone receptor (PTHR) agonist possessing strong arrestin-specific signaling bias exerts distinct and beneficial effects upon bone deposition compared to a conventional agonist that mimics the endogenous ligand for the receptor [29]. The ability of an arrestin-signaling specific PTHR ligand to effect functional bone deposition (with increases in resultant trabecular density) without causing additional bone resorption distinguishes it from the endogenous ligand activity.

Accepting the posit of pre-assembled distinct active GPCR states, it is highly likely that endogenous ligands, which would be required to support a variety of biological actions, have evolved the capacity to remain '*omnipotent*', *i.e.,* exert agonist effects across diverse active states of the same GPCR. These endogenous ligands would mediate a 'balanced' level of GPCR stimulation as they productively engage all of the distinct active states that mediate direct G protein or arrestin signaling, and also correctly engage reactive regulatory possesses such as receptor recycling, desensitization and transcriptional control of signaling factors/ligands/receptors associated with a ligand's receptor system ([51], Fig. **1**). Conversely, xenobiotic GPCR ligands are less likely to be able to replicate this GPCR omnipotence, and are thus more prone to demonstrate bias and potential 'imbalances' in signaling, particularly in the case of large peptide/protein-interacting GPCRs with complex ligand-interaction domains.

While this may be concerning from the optimistic viewpoint that biased agonists represent the future of GPCR pharmacotherapeutics, one should also consider that cellular physiological changes, *e.g*. caused by aging or disease, may themselves introduce imbalances into GPCR signaling. If disease-related cellular perturbations significantly affect the availability of signaling machinery or receptor scaffolding proteins, there may be an alteration in the balance/type of the GPCR active state conformations, hence a marked bias in the cellular response elicited by the omnipotent endogenous agonist. In such settings, endogenous ligands that are well-tuned to maintain balanced functional interactions with the majority of the active states may become maladaptive under pathophysiologic conditions.

Considering that cellular, tissue and organismal ligand-receptor signaling systems are in a constant state of flux, from a diurnal to lifespan extent, it is likely that physiological receptor systems operate at an allostatic, and not homeostatic, level [52, 53]. In generalized terms allostasis can be differentiated from homeostasis by the nature of the applied perturbation to the extant equilibrium, *i.e.* allostasis is literally the process of 'remaining stable by being variable', in which constant re-adjustment occurs time-and-again to consistent challenging perturbations. In contrast, homeostatic mechanisms can be considered as long-term overall adaptive responses to maintained, repeated and predictable perturbations. With acute stress events, disease pathophysiology, and progressively global physiological alterations, *e.g*. aging [54], the GPCR signaling repertoire/active state ensembles may be consistently in flux and therefore represent a problematic target for the omnipotent endogenous ligand. In this allostatic scenario the endogenous agent may come to act in an imbalanced manner as it is unable to accommodate changes in GPCR active state ensembles, while a biased agent may avoid such behavior and facilitate a more beneficial effect (Fig. **2**).

One example of this is the maladaptive effects of endogenous catecholamines in chronic congestive heart failure (CHF). The endogenous 'omnipotent' ligands for adrenoceptors (epinephrine (EPI), norepinephrine (NE)) evolved to regulate inotropic, chronotropic, and vasomotor responses during an acute 'flight or flight' response in healthy individuals; temporarily increasing cardiac output while redirecting blood flow to skeletal muscle. In CHF, as cardiac ventricular function progressively declines, systemic hypotension and attenuated renal perfusion also elicit a compensatory elevation in EPI/NE in an attempt to boost cardiac performance [55]. However in CHF this hyperadrenergic state is continuous, not transient. As a result the cardiac and vascular adrenergic receptor systems desensitize, regulatory proteins upregulate and adverse cardiac remodeling occurs over time [56, 57].

In this setting it is the combination of excess 'omnipotent' ligands (EPI/NE) and altered functional receptor ensembles that contributes to the maladaptive physiologic response. While global antagonism of beta adrenergic receptor signaling has been shown to improve cardiac contractile function and confer survival advantage in chronic CHF [58, 59], one might imagine that a 'biased' ligand that blocks deleterious signals originating from endogenous 'omnipotent' ligands while permitting transduction of beneficial signals, might be uniquely efficacious at restoring signal balance to a skewed system. Data from murine genetic models of CHF induced by excessive beta1 adrenergic suggest that 'arrestin pathway-selective' beta 1 receptor agonists might confer benefit by permitting arrestin-dependent 'transactivation' of epidermal growth factor receptors, which exerts a putative cardioprotective effect, while blocking harmful cyclic adenosine monophosphate production arising from excessive G protein activation [60].

Another primary target of EPI/NE in the cardiovascular system are alpha adrenoceptors and data suggest that receptor signalsome constitution, and balance between signalsome isoforms, may be strongly involved in blood pressure regulation [61]. The creation of $alpha_{1D}$-adrenoceptor signalsomes containing multiple syntrophin molecules appear vital for adrenoceptor-mediated vascular tone [61, 62]. Syntrophins are membrane-associated proteins expressed mainly in skeletal muscle, cardiac muscle, and brain that form functional complexes with the carboxyl-termini domains of structural proteins including dystrophin, utrophin and dystrobrevins [63-66]. The five syntrophin isoforms, α1, β1, β2, γ1 and γ2 [67-69] all contain two pleckstrin homology domains, a PDZ (PSD-95/SAP-90, Discs-large, ZO-1 homologous) domain, and a syntrophin unique domain [68, 69], which facilitate their roles in anchoring membrane proteins and in signal transduction organization. In skeletal muscle, syntrophins are expressed

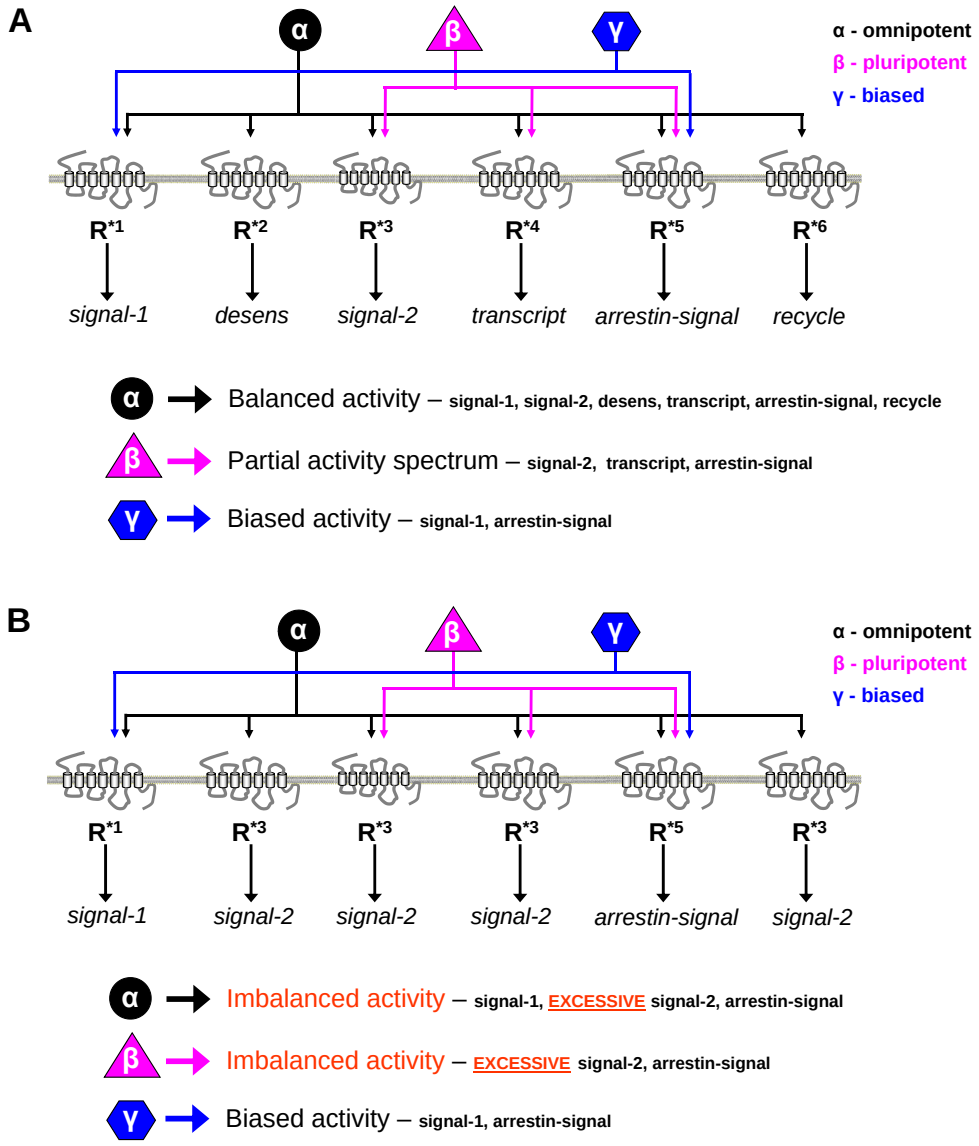

Fig. (2). Biased agonism in ideal and non-ideal physiological systems. (**A**) Omnipotent endogenous ligand (α) activity mediates balanced GPCR activity control while pluripotent (β) and biased agonists (γ) offer limited GPCR system control. (**B**) In a non-ideal (pathophysiological system) induced by aberrant active state R*3 overexpression and domination of other R* isoforms, the omnipotent (α) and pluripotent (β) agonists do not support GPCR system integrity while the biased agonist (γ) retains signaling activity without excessive system activation.

both at the sarcolemma and the neuromuscular junctions, while in neuronal tissue a similar cellular-communication integrative function is served in the synapse. Recent work has demonstrated that the formation of alpha-adrenoceptor signalsomes with differential syntrophin (α, β1, β2) content affects both adrenergic receptor regulation of vascular tone regulation, and the interaction affinity and selective signaling efficacies of artificial EPI/NE analogs [63]. With the recognized perturbation of syntrophin-related molecules in cardiovascular disorders [70-72], it is likely that in pathophysiological conditions the excessive ligand (EPI/NE) stimulation of altered adrenoceptor ensembles could contribute to continued cardiovascular dysregulation, while selective xenobiotics with preferences for different syntrophin-adrenoceptor isoforms offer specific benefit.

GAME THEORY APPLIED TO GPCR DYNAMICS

GPCR systems might be envisioned as constantly jostling between specific or non-specific ligand interactions and shifting receptor ensembles, while simultaneously adapting to varying rates of receptor desensitization and recycling and transcriptionally regulated changes in the relative abundance of system components, imposed by a changing extracellular environment. While biased agonists may cause a discrete stimulatory action on a subset of receptor states they may still interact (non-productively) with other receptor states competing with other ligands. The actions of these multiple stimuli will also eventually feed back and affect the original ligand-receptor balance via changes in intermediary cell metabolism and transcriptional activation. This suggests that

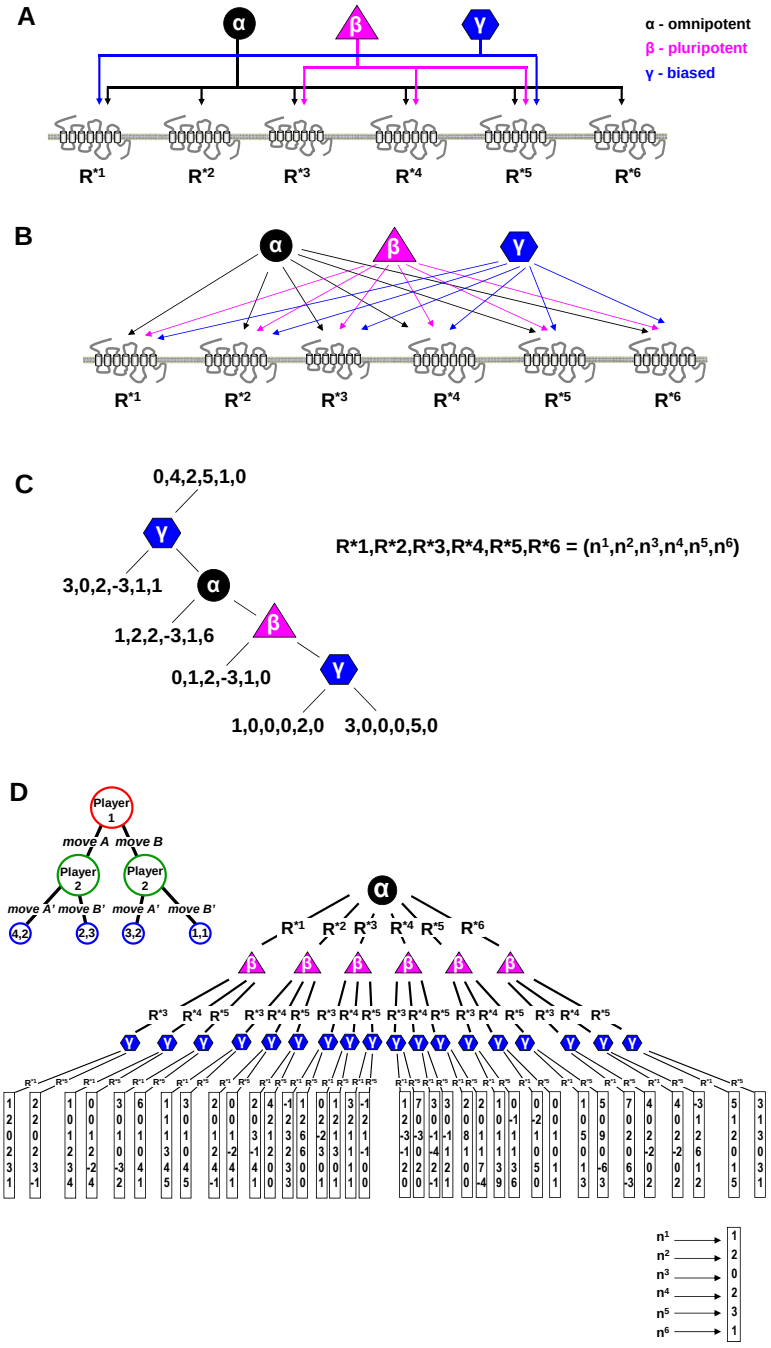

Fig. (3). Game-based interactions between biased and non-biased agonists in GPCR systems. (**A**) Three different forms of ligand (α, β, γ) that can all bind the different active states possess distinctive receptor isoform activation patterns (α-omnipotent, activates all forms; β-pluripotent, activates R^{*3}, R^{*4}, R^{*5}; γ-biased, activates R^{*1}, R^{*5}). (**B**) All three forms of receptor ligand can compete for interaction with the receptors and therefore compete with each other for the subsequent control of the downstream and regulatory feedback effects of receptor stimulation. Therefore ligand-receptor interaction, receptor stimulation of signaling pathways and cellular feedback effects on receptor-ligand activity are all linked the actions on the R^{*1} to R^{*6} isoforms. (**C**) The resultant effects (positive or negative) at these receptors within a sequential game scenario that ligands generate (n^1 to n^6) can constitute a series of simple conditional outcomes in a simplified sequential game of ligand function. Each receptor effect of one of the ligands causes an eventual numerical effect on all of the isoforms using the following matrix: n^1,n^2,n^3,n^4,n^5,n^6. (**D**) Competition amongst ligands for multiple receptor forms may develop into a 'combinatorial' game scenario – exemplified by a theoretical Player 1 (red circle) versus Player 2 (green circle) effect tree in which different 'move' choices (move A, or move B) affect the end point results (blue circles). In a GPCR-based game system we can analogize the 'players' to the ligands (α, β, γ) and the 'moves' are the receptor choices (R^{*1}-R^{*6}) they make. The resultant functional effects of this game (n^1-n^6) are therefore represented by multiple matrices controlled by the separate ligand-receptor choices that affect each other.

there will be a higher-order functional relationship of expected results of receptor-ligand engagement in this highly competitive forum. The formalistic appreciation of such complex results-based systems in an allostatic environment may be aided by recent advances in Game Theory equilibria.

Game theories represent a recently developed field of mathematical systems analysis. Principles of Game Theory are employed to consider the results of model strategic situations (games) in which the choice of actions of a unitary factor or agent, and the resultant loss or benefit to that factor/agent, are affected by the choices of factors/agents [73]. Mathematical models of dynamic systems created using Game Theorems have been applied to gross biological phenomena such as species competition [74] and complex physiological processes such as neural network communication [75]. Game Theory was initially developed to analyze competitions in which one factor/agent achieves success at the detriment of the other factor/agent ('*zero sum game*'), however subsequent modifications have been introduced to demonstrate potential collateral benefits of competition that were not initially apparent [76]. Game Theory is employed to define and study the dynamic equilibria in these games. One of the most notable examples of Game Theory applied to strategic equilibria in biological systems is the '*Nash equilibrium*' or so-called '*Prisoner's Dilemma*' [76]. In an equilibrium situation, each factor/agent in the game has adopted a strategy that cannot improve his outcome (optimal gain/loss ratio), given the strategic choices of the other involved factors/agents. Complex physiological systems, such as GPCR-ligand systems, clearly consist of multiple forms of equilibria, from R to R* conversions, to the equilibria between ratios of different GPCR ensembles within a single cell. As GPCR systems (comprised of ligands, receptors, transduction systems and reactive responses) themselves both comprise and attempt to control physiological allostasis, the components of this are likely to compete with each other to maintain, for example, neurotransmission, endocrine axes, and sensory perceptive mechanisms. Therefore if we consider the nature of ligand-receptor functional interactions, the ligands themselves could be considered as competing agents that will affect the resultant effects of other ligands by selectively or non-selectively activating discrete active receptor states. This competition is likely to occur at multiple levels, firstly at the GPCR occupation level, secondly at the liberation of second messenger signaling molecules and thirdly at the post-receptor modulatory level (desensitization, recycling and transcriptional control of multiple system components) (Fig. **3**). In this context we can hypothesize that the relative levels, degree of competition, and sequential nature of receptor-ligand interaction are all vital for understanding the '*gestalt*' of the impact biased agonists upon physiological systems. In such a complex system it is likely that the interactions of ligands with receptor isoforms creates a type of '*combinatorial game*' in a multiagent system environment as opposed to a more simple sequential format (Fig. **3**). To generate true '*Nash*-type' equilibria all factors/agents within a game attempt to achieve their optimal strategy, or allostasis in a biological context. Cellular signaling systems linked to complex and labile physiological processes that are controlled by multiple ligands represent an extreme form of factor/agent competition for equilibria. Games in which the difficulty of finding an optimal strategy stems from the multiplicity of potential moves an agent/factor ('player') can make are called combinatorial games, *e.g*. chess. In the context of ligand-receptor interactions we could refer to the ligand as the player that then makes 'moves' by functionally interacting with specific GPCR isoforms. As signaling events induced by GPCR ligands can exert profound effects on the ligand-receptor systems themselves, the resultant effects of such complex game interactions demonstrate a higher and higher level of inter-connectivity (Fig. **3D**).

From a physiological standpoint the ability of a functional GPCR system to reach its ideal result (*Nash equilibrium*) will reflect the maintenance of hormone feedback pathways, efficient metabolism of nutrients and cognitive actions that facilitate survival. In physiological systems exposed to a pathological perturbation, as we have stated before, the endogenous ligand may be disadvantaged in the 'game' and may not be able to resurrect allostasis, while introduction of a biased ligand may more effectively compete against the disease-modified system and reduce pathological progression. In this context, our increased appreciation of biased agonist function at GPCRs may allow us to ameliorate disease programs that are resistant to, or abetted by, evolutionarily derived 'omnipotent' ligands. It is relatively easy to imagine modern day scenarios in which this form of signaling behavior may be applicable, *e.g.* juvenile obesity and advanced aging. The prevalence of easily available high calorie-density foods and reduced exercise has dramatically elevated juvenile obesity in westernized countries [77]. The average lifespan in such westernized cultures has also altered significantly in recent decades [78]. Both of these new physiological states introduce significant changes to metabolic function that have occurred outside of endogenous genetic control, therefore in these cases it is likely that endogenous ligand control of GPCR systems is unable to maintain allostasis. Perhaps there are multiple aspects of these two physiological paradigms that may be more efficiently controlled by the rational application of xenobiotic biased agonists that are able to more effectively control cellular signaling in a distorted competitive game.

CONCLUSIONS

The appreciation of GPCR functionality at the molecular level has advanced exponentially in the last two decades. Investigation of GPCR function has facilitated the discovery of pharmacotherapeutic treatments for nearly every disease and pathophysiological situation. The discoveries outlined in this review concerning the ability to selectively target additional levels of GPCR targets may expand our current pharmacopeia several fold. The generation of biased GPCR ligands that can induce specifically designed physiological effects is likely to improve clinical efficacy while simultaneously improving compliance, via the minimization of deleterious 'off-target' effects. In the ever-evolving field of GPCR biology there is usually only one true consistent finding, *i.e.* there is always an extra level of GPCR signaling complexity waiting to be uncovered.

CONFLICT OF INTEREST

All of the authors have no conflict of interest.

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