Chapter

Allosteric Modulators for GPCRs as a Therapeutic Alternative with High Potential in Drug Discovery

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Abstract

The superfamily of G protein-coupled receptors (GPCRs) consists of biological microprocessors that can activate multiple signaling pathways. Most GPCRs have an orthosteric pocket where the endogenous ligand(s) typically binds. Conversely, allosteric ligands bind to GPCRs at sites that are distinct from the orthosteric binding region and they modulate the response elicited by the endogenous ligand. Allosteric ligands can also switch the response of a GPCR after ligand binding to a unique signaling pathway, these ligands are termed biased allosteric modulators. Thus, the development of allosteric ligands opens new and multiple ways in which the signaling pathways of GPCRs can be manipulated for potential therapeutic benefit. Furthermore, the mechanisms by which allosteric ligands modulate the effects of endogenous ligands have provided new insights into the interactions between allosteric ligands and GPCRs. These new findings have a high potential to improve drug discovery and development and, therefore, creating the need for better screening methods for allosteric drugs to increase the chances of success in the development of allosteric modulators as lead clinical compounds.

Keywords: GPCRs, allosteric modulators, biased signaling, β-Arrestin, G-protein, orthosteric site, endogenous agonist

1. Introduction

Allosteric modulators are small molecules or peptides that by specifically interacting with the receptor can alter the affinity, and/or efficacy of the endogenous hormone or other orthosteric ligands including antagonists, and possibly even constitutive signaling by GPCRs. By modifying these pharmacological parameters, allosteric modulators can exert multiple effects on the signaling of GPCRs. Positive Allosteric Modulators (PAMs) potentiate the signaling of the receptor by increasing the affinity and/or efficacy of the endogenous ligand or other administered agonists. On the contrary, Negative Allosteric Modulators (NAMs) decrease the affinity and/or efficacy of the agonists. Biased Allosteric Modulators (BAMs) will direct the agonist response to a single signaling pathway [1, 2].

Before going deeper into pharmacological concepts, it is necessary to define fundamental parameters used to describe the activity of a ligand. Affinity refers to the

capacity of a ligand to bind to a receptor. The efficacy of a ligand, is the ability of a ligand to activate or amplify a response after binding to a receptor. Then Furchgott defined intrinsic efficacy as efficacy divided by the total receptor number, in hopes of defining a unique ligand-receptor value much like a ligand's affinity [3]. Earlier, Ariens had introduced the term 'intrinsic activity' of a ligand to explain the behavior of partial agonists [4]. Ariens proposed assigning the maximum response of the endogenous ligand a value of 1 or 100% and partial agonists were expressed as a fraction of this response. The discovery of inverse agonists, ligands that can shut down constitutive signaling by a GPCR expanded the scale from −1 to 1 (or − 100–100%). A ligand that does not produce a cellular response (i.e., zero efficacy) when bound to the orthosteric site of the receptor is termed an antagonist [5].

The site to which endogenous agonists bind to is defined as the orthosteric site. Allosteric modulators do not bind to this site. They bind to other sites and are thus termed allosteric sites [6]. Upon binding, modulators generally stabilize a pre-existing conformation or change the structural conformation of the receptor. This will often modulate the orthosteric site and can modify the effects of the agonists, or in theory, inverse agonists [1]. Allosteric modulators can also stabilize one of the multiple conformational states of the receptor [7].

Experimentally, allosteric modulation can be challenging because the allosteric modulators may affect the affinity and/or efficacy differently for each agonist (see agonist or probe dependence discussed below). For instance, different agonists that induce the same cellular response, after binding to the same receptor, can be differentially modulated by the same allosteric modulator [1]. Furthermore, they are usually difficult to screen for because they do not produce an effect by themselves and may not displace radiolabeled ligands used in binding assays.

Drugs that target the orthosteric site of G protein-coupled receptors (GPCRs) are currently the most common therapeutic tools. Allosteric binding sites (e.g., sites elsewhere on the receptors) are less well-defined and, therefore, less exploited clinically. Diversity in location, mechanism, and specificity of allosteric ligands are characteristics giving them a great potential to extend the range of the ways that drugs can modulate GPCR signaling.

2. Advantages of allosteric modulators

Allosteric modulators with no intrinsic efficacy will usually only exert their effects in the presence of an endogenous agonist. Thus, they can selectively tune cellular responses in tissues where the endogenous agonist exerts its physiological or pathophysiological functions. As a result, temporal and spatial aspects of the endogenous agonist signaling can be chronically maintained or even corrected in pathological states. Also, the saturation of allosteric binding sites limits itself the action of the allosteric modulator and the effect on the function of the agonist. This excellent property of the agonist overcomes the overdosing of a drug, making the allosteric modulators much safer than classical drugs [8].

A great benefit of using allosteric ligands for therapeutic applications is their huge potential to achieve greater selectivity at subtypes of GPCRs [8]. This could be due to greater diversity in the amino acid sequence of the allosteric binding sites compared to the orthosteric binding pocket. Another possibility is via selective cooperativity between the allosteric and orthosteric binding sites at a given receptor subtype. In addition, in some GPCRs, where the orthosteric binding site is not clear or its structure is poorly defined, the allosteric binding site might be a good alternative to target with small molecules; this has been observed in receptors with long

peptidic ligands and is frequently found with the class B GPCRs (which are GPCRs characterized of having a long N-terminal extracellular domain). In general, the advantages of allosteric modulators apply regardless of the specific therapeutic area or the tissue where the receptor is being targeted.

2.1 The challenge of agonist dependence

Radioligand binding and functional assays have particular advantages when they are used to screen for allosteric modulators of GPCRs. Nevertheless, the extent and direction (that is positive or negative) of the interaction between the allosteric and orthosteric ligand for receptors with more than one endogenous ligand will depend on the orthosteric agonist interacting with the receptor; this phenomenon is known as probe or agonist dependence (**Figure 1**). Agonist dependence makes more complex the identification and pharmacological characterization of allosteric modulators. Particularly, the case of aplaviroc (an allosteric modulator for CC-chemokine receptor 5) showed no effect in the binding of CCL5 to the receptor but totally prevented the binding of CCL3 [9].

Agonist dependence, or better known as probe dependence, can seriously affect potential therapeutics and also the development of allosteric ligands. In the ideal situation, the endogenous ligand would be used as a prototypic ligand in the high throughput screening process during drug development. However, in a real situation, the endogenous agonists, most of the time are unsuitable and susceptible to rapid degradation along the drug screening process and even more during *in vivo* studies. For this reason, highly stable agonists are preferentially used. Therefore, the allosteric ligand is first tested with the natural ligand on an early stage of the process in order to conclude if the achieved effects are equivalent to those registered with the prototypical agonist.

In order to better illustrate this concept a good example would be when a PAM has the ability to increase the cholinergic neuronal transmission in Alzheimer's disease [10], but the endogenous ligand acetylcholine is of rapid degradation making it unsuitable for drug screening. Cholinergic substitute ligands such as carbachol or pilocarpine are used for screening, and the stable analog oxotremorine is used to validate the effects of muscarinic acetylcholine receptor (mAChR) allosteric modulators *in vivo* [11]. However, the effects of the PAM LY2033298 are dependent on the orthosteric agonist (carbachol or pilocarpine) being used [12, 13] and these effects could lead to unexpected profiles of the allosteric ligand in later stages of the drug discovery process.

The characteristic of some allosteric ligands to have differential probe dependence on multiple receptor subtypes is an additional consideration that can have significant implications for the drug screening process. For instance, LY2033298 was reported to be a specific positive allosteric modulator of the M4 mAChR. Accordingly, PAM LY2033298 increased acetylcholine-mediated calcium responses at the M4 mAChR but not for the rest of the four receptor subtypes [14]. This allosteric modulator has also been shown to have high positive cooperativity with the surrogate orthosteric ligands oxotremorine and tetramethylammonium at the M2 mAChR [13]; this positive cooperative effect is similar to that observed with LY2033298 and oxotremorine at the M4 mAChR. This is an example of how the probe-dependent interaction of oxotremorine at both the M2 and M4 receptor subtypes can confound experimental interpretation of the effect of the allosteric ligand *in vivo* [11]. This highlights the need to understand the probe dependence of allosteric ligands at related receptors to ensure a robust target validation.

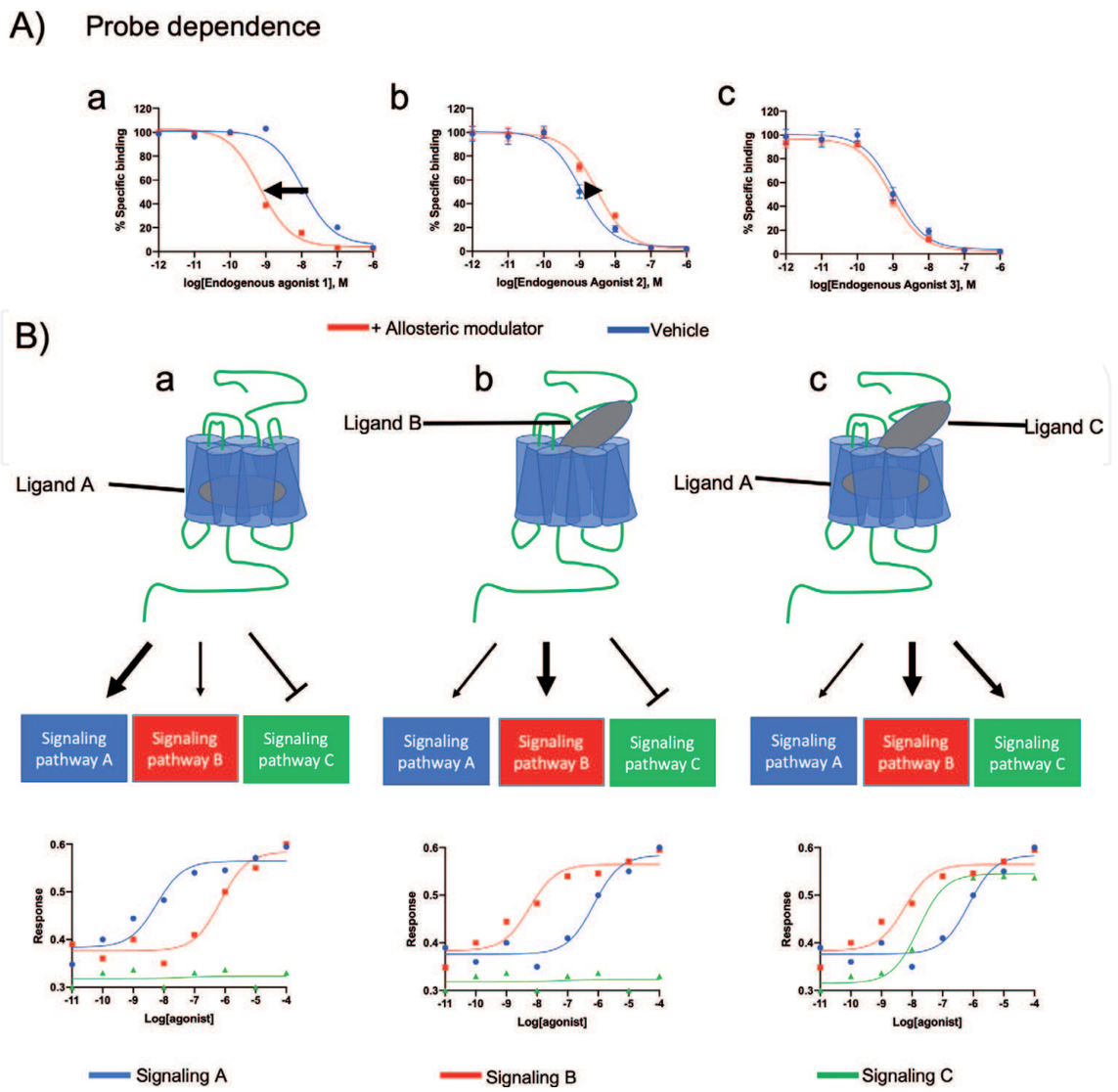

Figure 1.
Agonist dependence and biased agonism. (A) Agonist dependence of an allosteric ligand is shown with several endogenous agonists. (Aa) An allosteric modulator potentiates the ability of endogenous Agonist-1 to inhibit the binding of an antagonist to a human GPCR. (Ab) An allosteric modulator displaying weak or almost neutral cooperativity with endogenous Agonist-2. (Ac) An allosteric modulator displaying neutral cooperativity with endogenous Agonist-3. (B) Biased agonism is the capacity of different agonists to differentially activate the same GPCR, producing specific sets of signaling pathways. Changes in efficacy or potency by different agonists are indicators of potential biased agonism for a given GPCR. (Ba) Allosteric modulator-A showing stronger potency for signaling-A than for signaling-B, and not showing effect at signaling-C. (Bb) Allosteric modulator-B displaying higher potency for signaling-B than for signaling-A. (Bc) A biased agonist in complex with a GPCR can by itself preferentially activate to a unique set of signaling pathways and the interaction of the ligand-GPCR complex with an allosteric ligand will affect the signaling bias of the GPCR. Ligand-C is co-bound with an allosteric modulator-A and it potentiates the stimulus towards pathway-B and generates activity on signaling-C, however it down regulates signaling-A. This can be observed by a change in potency and efficacy between signaling-B and signaling-C in comparison with signaling-A.

Probe dependence is irrelevant in many physiological systems because the therapeutically targeted GPCRs have only one endogenous ligand. However, as we mentioned above, receptors can also respond to several endogenous agonists, under physiological conditions and disease. It is important to mention few more examples like the case of the chemokine receptors [15], melanocortin receptors [16], parathyroid hormone receptor 1 [17], relaxin receptors [18], calcium-sensing receptors [19], calcitonin and calcitonin-like receptors [20], as well as glucagon and glucagon-like peptide 1 (GLP1) receptors [21] and galanin receptors. In these examples, agonist dependence represents a huge challenge in the development of allosteric ligands as well as their therapeutic application.

2.2 Biased agonism in allosteric modulation

Distinct ligands can show different capacities to differentially activate signaling pathways from a GPCR by inducing different structural conformations [8, 22]; this effect is termed as biased signaling (also known as biased agonism see **Figure 2**). Examples of ligands that produce biased signaling include classical orthosteric adrenoceptor antagonists and inverse agonists (also known as beta-blockers) that antagonize receptor-mediated cyclic AMP production but promote cAMP response element-mediated gene transcription [23].

Research of carvedilol, an adrenoceptor antagonist, has shown to be a superior therapeutic, as compared to other adrenoceptor antagonists, for heart failure therapy [24] Despite that the drug was shown to be an inverse agonist for Gαs dependent signaling, it was also observed that carvedilol exerts partial agonism inβ -arrestin-dependent extracellular signal-regulated kinase 1 (ERK1) and ERK2 phosphorylation [25]. Based on these observations we can hypothesize that a different set of efficacies in different signaling pathways determines the final therapeutic outcome of GPCR ligands. Biased signaling has been widely studied for orthosteric ligands, however, there is also the possibility that many, if not all allosteric ligands, will exert biased signaling properties when the receptor is co-bound to the agonist.

At the present time, the terms biased agonism and allosteric modulation are usually considered to be different pharmacological phenomena. However, both events share in common that they are due to ligand-specific conformational changes in the GPCR that implicates a change in the three-dimensional structure of the GPCR. Having as a result that some specific signaling pathways can be either positively or negatively regulated.

For instance, the allosteric modulators of parturition (PDC113.824) induce biased signaling when an orthosteric ligand is co-bound to the prostaglandin F2α receptor. In mouse models, this compound acts as a negative allosteric modulator of prostaglandin F2α receptor-mediated cytosolic calcium oscillations and myometrial contraction. Specifically, PDC113.824 uncouples the receptor from the Gα12–RHO–ROCK (RHO-associated protein kinase) signaling pathway, but still significantly increases the phosphorylation of ERK1 and ERK2 Gαq dependent [26]. An auto-antibody for the calcium-sensing receptor that produces acquired hypocalciuric hypercalcemia by selectively increasing Gαq-dependent signaling and inhibiting

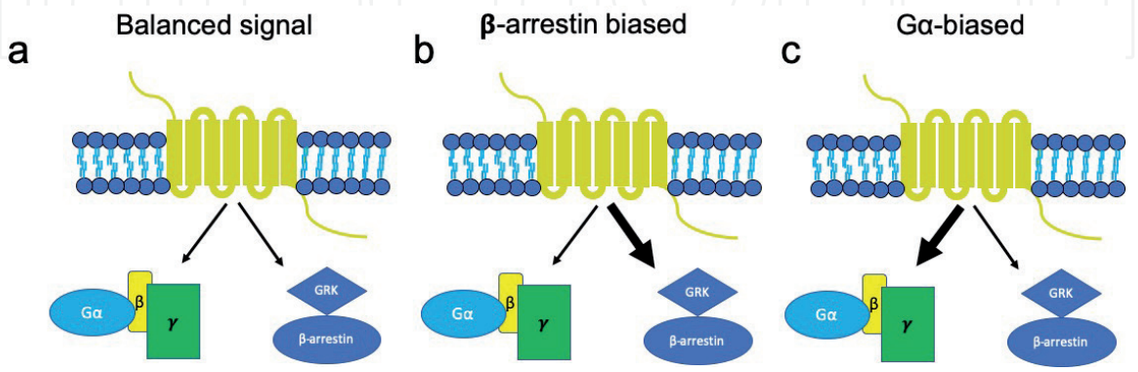

Figure 2.
Biased agonism is the ability of a receptor-ligand complex to selectively activate its downstream signaling pathways. (a) A balanced signal that stimulates both signaling pathways, G-protein dependent or β-arrestin dependent, equally in the same manner. (b) A biased ligand acting on the receptor as an agonist of one pathway (e.g., β-arrestins) while acting as an antagonist of another pathway (e.g., through heterotrimeric G proteins). (c) A biased ligand acting on the receptor as an agonist of one pathway (e.g., through heterotrimeric G proteins) while acting as an antagonist of another pathway (e.g., beta-arrestins).

Gαi-dependent signaling [27]. This is only one example of how an allosteric modulator can induce biased signaling that results in disease.

Based on these examples is that we can see the need to deeply understand the effect of allosteric modulation at different signaling pathways, as positive and negative regulation of every pathway from the receptor, does not always generate a beneficial therapeutic effect.

In theory, the operational model describes that biased agonism via allosteric modulation is a pathway-dependent variation in the signaling produced by the agonist in such a way that is not correlated with the intrinsic efficacy of the agonist or allosteric modulators [28]. However, in real conditions, biased signaling by an allosteric modulator is when the allosteric ligand potentiates one pathway but decreases the other one, as we previously described.

For a better understanding of this pharmacological phenomenon, we can continue describing more examples in this regard. Another very good example is the case of the allosteric modulation of the muscarinic receptor M4 subtype (M4 mAChR). Increasing signaling at M4 mAChR by the allosteric modulator LY2033298 generated significant changes in the grade of positive cooperativity in various signaling cascades of this receptor [29]. An additional case to exemplify this is the biased allosteric modulation of the GLP1 receptor. Some allosteric ligands can potentiate cAMP production and having a smaller effect on β-arrestin dependent signaling [30, 31].

Currently, most allosteric modulators have been discovered following screening assays; such as those used to identify agonists and antagonists, instead of a thoughtful rational process. The development of novel allosteric modulators that can induce biased agonism has the potential of specifically targeting therapeutic signaling pathways and excluding off-target pathways providing in this way a novel mechanism of biased agonism and produce new drugs with fewer side effects. However, we also face the problem that poses a big challenge in drug discovery and development of allosteric ligands and is that for the vast majority of GPCRs, it is still not well understood which set of signaling pathways efficacies will produce the desired therapeutic effect. The most important issue in the development of biased allosteric ligands is required a full understanding of the molecular determinants and also structural signatures that will lead to biased signaling on a receptor.

2.3 Agonist dependence in allosteric modulation and biased agonism

Many GPCRs have more than one endogenous agonist in such a way that their action is differentially regulated by the same allosteric modulator, having, as a result, a phenomenon called probe dependence. For instance, in the case of GLP1 receptor, there are two PAMs (compound 2 and BETP (4-(3-benzyloxyphenyl)-2-ethylsulfinyl-6-(trifluoromethyl) pyrimidine)) that show agonist dependence; where they increased the affinity of the oxyntomodulin (an endogenous agonist) by 18–25 times respectively. In contrast, none of them had no effect on four more ligands of GLP1 receptor [30, 32]. Interestingly, these allosteric modulators induced biased signaling during GLP-1 activation by oxyntomodulin. Observing an increase in Gαs activation, β-arrestin1/2 recruitment and insulin secretion, but they did not show any allosteric effect in ERK1/2 activation. The capacity of a modulator to regulate all or only some endogenous ligands in a pathway-dependent manner could not only seriously affect the development of novel allosteric modulators but also their therapeutics.

2.4 Implications in drug screening

Currently, allosteric screening routinely relies on seeking compounds that regulate the actions of the main endogenous ligand. Conversely, screening against

alternative endogenous ligands, even if they have lower affinity or efficacy, may yield new leads that might not be possible to identify if screening for cooperative effects is only performed using the main endogenous ligand.

The possibility that an allosteric modulator will antagonize or potentiate the effects of an endogenous agonist without affecting other endogenous agonists would be seen as a therapeutic advantage, only in the case that each agonist exerts a different physiological effect. For example, according to previous studies some CCR5 allosteric modulators prevent the interaction of HIV-1 to CCR5, inhibiting in this way the infection [33]. However, it has not been well understood, whether inhibiting the chemokine physiological function of CCR5, would be desirable from a therapeutic point of view. During AIDS treatment, it is highly desirable the availability of allosteric modulators that prevent the HIV-1 entry without affecting CCR5 internalization by chemokines, since CCR5 plays a key role in favorable protection in the progression of AIDs after HIV-1 infection [34].

Probe dependent effects and the capacity of allosteric ligands to induce allosteric bias could be used to regulate GPCR physiological function in such a way that the signaling pathways that lead to favorable physiological outputs can be selectively targeted.

2.5 GPCR structure and allosteric modulation

All GPCRs are involved in nearly all physiological functions in humans and are the target of intense drug discovery efforts [35, 36]. Recent structures of GPCRs bound to allosteric modulators have revealed that the receptor surface is characterized by diverse cavities and crevices that may serve as binding sites for allosteric modulators [37]. This supports the notion that GPCRs are structurally flexible and they can be regulated by different allosteric ligands through a wide variety of mechanisms [38–43]. The vast majority of these structures have been solved with NAMs, which stabilize receptors in their inactive states [37]. Currently, only a single structure of an active GPCR bound to a small-molecule PAM has been described, the M2 muscarinic acetylcholine receptor with LY2119620 [44]. Thus, mechanisms of PAMs and their potential binding sites remain unexplored.

2.6 Therapeutic relevance

Receptor subtypes have orthosteric sites that are similar in its tridimensional structure and sometimes even in their amino acid sequence since mutations within this site, may especially decrease receptor function with detrimental consequences for the system. This can be harmful in complex systems and thus, evolution does not frequently favor such changes. In contrast, allosteric binding sites are less critical for receptor function and this is why they often have great structural variation between receptor subtypes. Moreover, in contrast to orthosteric ligands, allosteric drugs have the potential of being highly specific by only targeting a very specific set of receptor subtypes. But also, it is worth to highlight that the same allosteric site might be structurally different across species, having as a consequence differential effects of the same allosteric ligand between species [45].

Allosteric ligands cannot activate or inactivate receptors. Specifically, allosteric action will depend on endogenous ligands like neurotrophins, hormones, nucleotides or lipid moieties whose levels in the organism are tightly regulated. This can lower overdose risk relative to similarly acting orthosteric drugs. It may also allow a strategy where large enough doses that saturate all the receptors of the target tissue can be administrated to prolong the drug effect [5]. These characteristics enable receptors to be activated at specific times (i.e., in response

to a physiological stimulus) with the difference of the constant activation by an orthosteric agonist [45].

Allosteric ligands regulate the responses already existing within tissues and making possible the drug response on specific tissue. Contrary to orthosteric ligands where they produce a less targeted effect within the organism since they bind to every receptor they can, affecting multiple tissues expressing the target receptor [1].

Some allosteric modulators have also been shown to lack the desensitizing effect that some agonists. Nicotinic acetylcholine receptors, for example, quickly desensitize in the presence of agonist drugs but maintain normal function in the presence of PAMs [46].

3. Conclusions

A huge number of drugs with fewer side effects are being developed using allosteric targets. Only two types of screening strategies are the principal approach in drug discovery; phage display and high-throughput screening. It is foreseen that complex computations will be conducted in years to come in order to gain better insights about the binding pockets within the receptors for which allosteric modulators can potentially be designed [47–49]. Information from crystal structures of receptors bound to different ligands would provide structural insights about the conformational changes that occur upon ligand binding. These studies would be fundamental during a rational drug design of these kind of ligands. In the last few years, we have seen a great advance in the design of novel allosteric modulators and it will possibly intensify even more in the near future. Progress in drug delivery will help to obtain further spatial specificity of therapeutic drugs, and as a result, we expect will translate into identifying significantly increased number of new drug possibilities that are more effective and with fewer side effects [50, 51]. However, no matter how selective such drugs can be designed, they cannot equalize the spatiotemporal basis of specificity that occurs naturally in our systems. Therefore, every single advance in allosteric drug discovery that promotes the homeostasis of our biological systems, will significantly contribute to the goals of developing more effective drugs with fewer side effects.

Acknowledgements

This work was supported by grants from the National Institute of Health, the American Heart Association, and Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation.

Conflict of interest

The authors declare no conflict of interest.

DOI: http://dx.doi.org/10.5772/intechopen.91838

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