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Allosteric binding cooperativity in a kinetic context

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Allosteric modulators are of prime interest in drug discovery. These drugs regulate the binding and function of endogenous ligands, with some advantages over orthosteric ligands. A typical pharmacological parameter in allosteric modulation is binding cooperativity. This property can yield unexpected but illuminating results when decomposed into its kinetic parameters. Using two reference models (the allosteric ternary complex receptor model and a heterodimer receptor model), a relationship has been derived for the cooperativity rate constant parameters. This relationship allows many combinations of the cooperativity kinetic parameters for a single binding cooperativity value obtained under equilibrium conditions. This assessment may help understand striking experimental results involving allosteric modulation and suggest further investigations in the field.

Keywords: allosteric modulation; binding kinetics; binding cooperativity; rate constant; cooperativity rate constant; residence time; GPCRs; heterodimer receptor

Allosteric modulation at equilibrium conditions

Allostery, in particular in G-protein-coupled receptors (GPCRs), is a research area of special interest to both academia and the pharmaceutical industry because of the known advantages (a ceiling effect level and greater GPCR subtype selectivity, among others) that allosteric modulators have with respect to orthosteric ligands.[1] In this study, we consider first the allosteric ternary complex receptor model, in which a receptor R bears two binding sites to which the orthosteric ligand A and the allosteric ligand B bind.[2,3] In the first instance, we do not consider how ligand binding translates into receptor function and we limit the analysis to a pure binding scenario in which either equilibrium or rate constants are used.

At equilibrium, the concentrations of the four receptor species present in the system are regulated by the corresponding K_1 to K_4 equilibrium dissociation constants (Fig. 1a).[2,3] We can change the notation and introduce the α and β binding cooperativity parameters (Fig. 1b). α measures the binding affinity of A to RB with respect to the free receptor R, whereas β measures the binding affinity of B to AR with respect to R. Only three of the four equilibrium constants are independent or, in other words, α is equal to β. From now on, we use α to denote the binding cooperativity parameter between the two ligands. α can be greater, lower, or equal to one, indicating, respectively, an increase, decrease or no effect on the affinity of each of the ligands for the receptor because of the presence of the other bound ligand. Binding cooperativities can be experimentally measured by different methods and allosteric modulators can be classified as positive, negative, or neutral depending on $\alpha > 1$, $\alpha < 1$, or $\alpha = 1$, respectively.[2] However, the mutual influence between the two ligands can be further examined when time is considered. For example, we can

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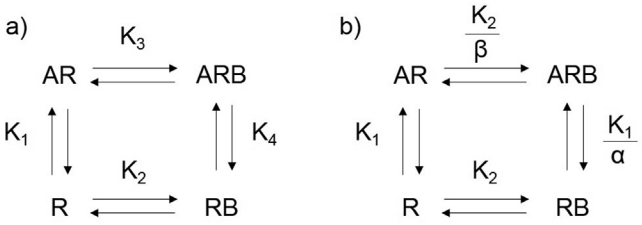

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FIG. 1

Allosteric ternary complex model. It is assumed that A is the agonist and B is the allosteric modulator. **(a)** K_1 to K_4 equilibrium dissociation constants are used: K_1 = [A][R]/[AR]; K_2 = [B][R]/[RB]; K_3 = [B][AR]/[ARB]; K_4 = [A][RB]/[ARB]. **(b)** K_3 and K_4 have been removed by including α and β binding cooperativity parameters, with $\alpha = K_1/K_4$ and $\beta = K_2/K_3$. It can be shown that only three of the four K_1 to K_4 constants are independent: if we substitute each equilibrium dissociation constant by its expression in terms of concentrations of receptor species, then $K_1/K_4 = K_2/K_3$ = [R][ARB]/([AR][RB]) (one equilibrium constant depends on the other three), or, in other words, $\alpha = \beta$. Adapted from [2].

ask whether it is possible to find a positive allosteric modulator (PAM) B that is more kinetically unstable in its binding site when A is present than when it is bound to the free receptor. If $\alpha > 1$, one would expect an increase in the affinity of each of the two ligands when the other is present, but this conclusion does not explain the behavior of the ligands and the mutual influence between them when they are already at the receptor binding site and only the processes of ligand–receptor dissociation are considered.

From equilibrium constants to rate constants: including the kinetic context

Equilibrium constants are the ratio between rate constants (Fig. 2a). The inclusion of rate constants in the analysis opens the discussion to binding kinetics, a pharmacological research area of major application in clinical and drug discovery research.[4,5] We follow the same rationale as in Fig. 1 but using rate constants (association: k_{+1} to k_{+4} and dissociation: k_{-1} to k_{-4}) (Fig. 2a) and their corresponding α_+, α_-, β_+, and β_- cooperativity rate constant parameters, with $\alpha_+ = k_{+4}/k_{+1}$, $\alpha_- = k_{-4}/k_{-1}$, $\beta_+ = k_{+3}/k_{+2}$, and $\beta_- = k_{-3}/k_{-2}$ (Fig. 2b). It can be shown that only seven of the eight rate constants are independent: $(k_{-1}\ k_{+4})/(k_{+1}\ k_{-4}) = (k_{-2}\ k_{+3})/(k_{+2}\ k_{-3})$, or, in other words, $\alpha_+/\alpha_- = \beta_+/\beta_-$.

In the same way as equilibrium constants are the ratio between rate constants, the binding cooperativity parameters (α and β) are the ratio between the corresponding cooperativity rate constant parameters ($\alpha = \alpha_+/\alpha_-$ and $\beta = \beta_+/\beta_-$). Given $\alpha = \beta$, this does not necessarily mean that $\alpha_+ = \beta_+$ and $\alpha_- = \beta_-$. The latter is a sufficient but not a necessary condition and, thus, the kinetic reciprocity between orthosteric and allosteric ligands occurs at the level of their cooperativity rate constant ratios and not their absolute values.

Let us consider the following example: $\alpha = \beta = 4$, with $\alpha_+ = 2$, $\alpha_- = 0.5$, $\beta_+ = 8$ and $\beta_- = 2$ for an orthosteric ligand A and an allosteric ligand B. These values imply a PAM in binding terms ($\alpha > 1$) and, thus, the equilibrium dissociation constants of both the orthosteric and the allosteric ligands decrease in the presence of the other bound ligand or, in other words, their affinities for the receptor increase in the presence of the other

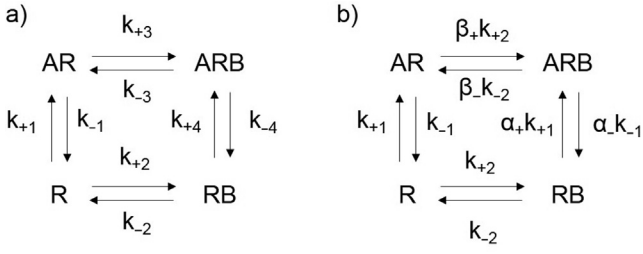

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FIG. 2

Allosteric ternary complex model. It is assumed that A is the agonist and B is the allosteric modulator. **(a)** The equilibrium dissociation constants in Fig. 1 in the main text are substituted by the corresponding association (k_{+1} to k_{+4}) and dissociation (k_{-1} to k_{-4}) rate constants: $K_1 = k_{-1}/k_{+1}$, $K_2 = k_{-2}/k_{+2}$, $K_3 = k_{-3}/k_{+3}$, $K_4 = k_{-4}/k_{+4}$. **(b)** k_{+3}, k_{-3}, k_{+4}, and k_{-4} have been removed by including α_+, α_-, β_+, and β_- cooperativity rate constant parameters, with $\alpha_+ = k_{+4}/k_{+1}$, $\alpha_- = k_{-4}/k_{-1}$, $\beta_+ = k_{+3}/k_{+2}$, and $\beta_- = k_{-3}/k_{-2}$. It can be shown that only seven of the eight k_{+1} to k_{+4} and k_{-1} to k_{-4} rate constants are independent: if we express each equilibrium dissociation constant in terms of concentrations of receptor species, then $K_1/K_4 = K_2/K_3$ = [R][ARB]/([AR][RB]) (Fig. 1 in the main text) and, by putting equilibrium constants in terms of rate constants, it can be seen that $(k_{-1}\ k_{+4})/(k_{+1}\ k_{-4}) = (k_{-2}\ k_{+3})/(k_{+2}\ k_{-3})$ (one rate constant depends on the other seven), or, in other words, $\alpha_+/\alpha_- = \beta_+/\beta_-$. Adapted from [3].

bound ligand. However, if we look at the α_- and β_- parameter values, we see opposite effects. Whereas the dissociation rate constant of the orthosteric ligand decreases when the allosteric ligand is bound (α_- = 0.5), that of the allosteric ligand increases when the orthosteric ligand is bound (β_- = 2). Moreover, if we look at the α_+ and β_+ parameter values, we see that both are > 1 although the effect of the orthosteric ligand on the association rate constant of the allosteric modulator (β_+ = 8) is greater than that of the allosteric modulator on the association rate constant of the orthosteric ligand (α_+ = 2).

Interestingly, for this particular $\alpha = \beta = 4$ value, many other combinations of values of the cooperativity rate constant parameters are possible, which indicates that different mechanistic hypothesis concerning microscopic events are compatible with a macroscopic outcome. For instance, the $\alpha = \beta = 4$ binding cooperativity value can also result from α_+ = 8, α_- = 2, β_+ = 0.8 and β_- = 0.2. Now, the effects that the allosteric modulator and the orthosteric ligand exert on each other are opposite in both the association and the dissociation rate constants (α_+>1 and β_+<1; α_->1 and β_-<1). See the discussion below on the relationship between residence time and agonist efficacy and [6] for an insightful review on the influence of allosteric modulators on the binding kinetics of the orthosteric ligand.

In this report[6], adenosine receptors were selected for analysis and, among others, adenosine A_3 allosteric modulators were examined. For purposes of illustration, two compounds are now taken. The first, VUF5455, behaved as an A_3 PAM by significantly retarding the dissociation rate of the agonist radioligand [^{125}I]-I-AB-MECA, from the adenosine A_3 receptor in a concentration-dependent manner.[7] Interestingly, its effect on the dissociation rate of the antagonist [^{3}H]-PSB-11 was insignificant.[6,7] These data illustrate the known dependence of allosteric modulation on the orthosteric ligand used, which is reflected in both equilibrium and kinetic assays.[1,8] The second compound, LUF6096, which bears a different chemical scaffold, also behaved as a PAM of the adenosine A_3 receptor.[9] Noteworthy, the compound was able to change the biphasic dissociation of [^{125}I]-I-AB-MECA from the receptor into a monophasic process, by slowing the kinetics of the agonist in the fast dissociating phase (k_{off_fast} = 0.089 to 0.035 min^{-1}). This effect was attributed to the stabilization of the receptor active conformation. This proposal was corroborated by a functional assay, in which LUF6096 significantly enhanced the intrinsic activity of Cl-IBMECA agonist.[6,9] These are two examples showing the effects of allosteric modulators on the dissociation rate constants of orthosteric ligands.

As recognized in [6], the influence of allosteric modulators on association rate constants of orthosteric compounds has been less investigated, which indicates that further work is needed to cover the entire kinetic space. However, the occurrence of receptor–receptor interactions in GPCRs adds a layer of complexity to the concept of allosterism.

Allostery in a heteromeric context

Allostery can arise not only from the interaction between an orthosteric and an allosteric ligand within a single receptor protein, but also from the interaction between two or more orthosteric ligands in an oligomeric receptor. Given its analogy with the previous case, at least at the mathematical level, we consider the model of receptor heterodimerization. Previously,[10] we proposed a mathematical model for receptor heterodimerization. Fig. 3a shows an adaptation of the binding part of the model in which kinetic constants for the binding of ligands A and B to the corresponding R_1 and R_2 receptor protomers in the heterodimer are included. Figs. 2 and 3 depict two models describing allostery between two ligands. Although the models are different in terms of protein structure (one or two proteins, respectively), they are equivalent to each other from the point of view of the kinetic parameters involved. This means that, in the absence of structural information about the allosteric interactions between the two bound receptor–ligands, binding experimental data are compatible with both a monomeric and a heterodimeric receptor. Therefore, the discussion above on the variability of cooperativity kinetic parameter values within a common $\alpha_+/\alpha_- = \beta_+/\beta_-$ ratio is also valid.

At this point, it is worth comparing heterodimeric receptors with homodimeric receptors. We see that a homodimeric receptor is a particular case of a heterodimeric receptor with $R_1 = R_2 = R$ or, in other words, a homodimeric receptor is a limit-

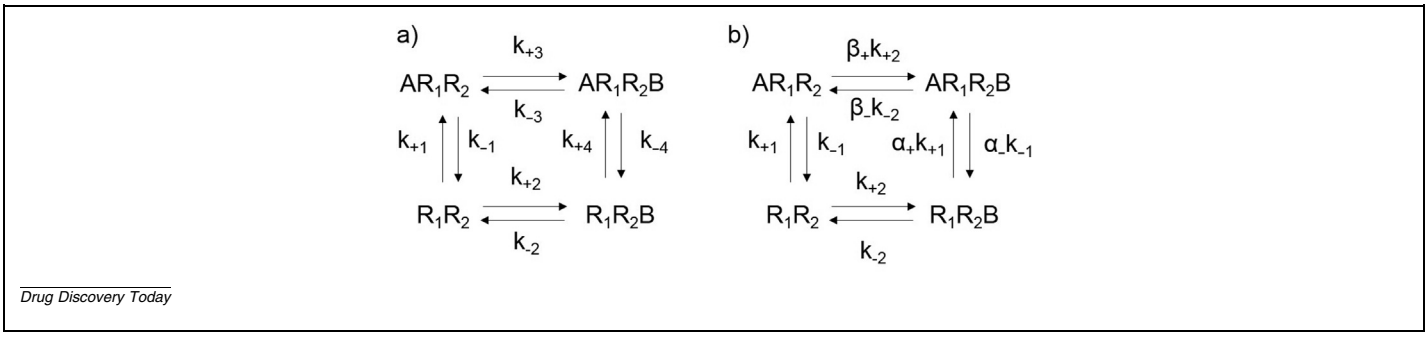

FIG. 3

Heterodimer receptor model considering only the binding part. A is a ligand selective for R_1 and B is a ligand selective for R_2. **(a)** k_{+1} to k_{+4} association and k_{-1} to k_{-4} dissociation rate constants are used. **(b)** k_{+3}, k_{-3}, k_{+4} and k_{-4} have been removed by including α_+, α_-, β_+, and β_- cooperativity rate constant parameters, with $\alpha_+ = k_{+4}/k_{+1}$, $\alpha_- = k_{-4}/k_{-1}$, $\beta_+ = k_{+3}/k_{+2}$, and $\beta_- = k_{-3}/k_{-2}$. Proceeding analogously as in Fig. 2 in the main text, it can be shown that only seven of the eight k_{+1} to k_{+4} and k_{-1} to k_{-4} rate constants are independent, $(k_{-1}\,k_{+4})/(k_{+1}\,k_{-4}) = (k_{-2}\,k_{+3})/(k_{+2}\,k_{-3})$, or, in other words, $\alpha_+/\alpha_- = \beta_+/\beta_-$. Adapted from [10].

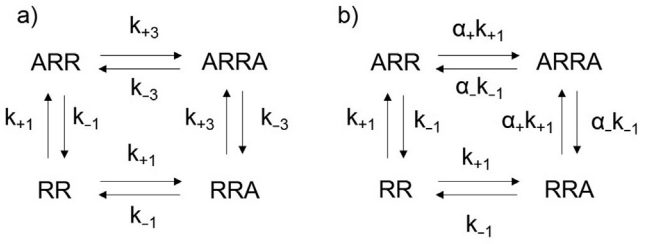

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FIG. 4

Homodimer receptor model constructed from the heterodimer model shown in Fig. 3 in the main text by considering $R_1 = R_2 = R$ and $A = B$. **(a)** The set of rate constants present in Fig. 3a in the main text is reduced because $k_{+2} = k_{+1}$, $k_{-2} = k_{-1}$, $k_{+4} = k_{+3}$ and $k_{-4} = k_{-3}$. **(b)** k_{+3} and k_{-3} have been removed by including α_+ and α_- cooperativity rate constant parameters, with $\alpha_+ = k_{+3}/k_{+1}$ and $\alpha_- = k_{-3}/k_{-1}$. Comparing Fig. 3b in the main text with **(b)**, it can be seen that $k_{+2} = k_{+1}$, $k_{-2} = k_{-1}$, $\alpha_+ = \beta_+$ and $\alpha_- = \beta_-$.

ing case of a heterodimeric receptor in which the two protomers progressively resemble each other until finally they are the same.[11] If, in addition, only one ligand species (say A) is included, then the previous relationship between cooperativity rate constants is simplified ($\alpha_+ = \beta_+$ and $\alpha_- = \beta_-$) and only two cooperativity rate constants (α_+ and α_-) contribute to the homodimer receptor system[12] (Fig. 4).

In functional terms, a heterodimer receptor with two orthosteric sites is more complex than a monomeric receptor with an orthosteric site and an allosteric site. In the heterodimer, at least two signaling pathways are present, one for each protomer, leading to a more complex functional scenario. The present study is mainly aimed at binding. However, it appears clear that the mutual influence in binding kinetics that ligands can have on each other could affect their respective functional responses. The potential variability in cooperativity kinetic parameter values might be obscured under equilibrium conditions but can be determinant when equilibrium is not present. In this regard, it can be interesting to consider whether complex pharmacological problems, such as that described in [13] can be reanalyzed through binding kinetics.

In [13], the potential relationship of the 5HT2A-mGlu2 heteromer with schizophrenia was postulated. For this heteromer, a Gq signaling pathway is linked to the 5HT2A protomer, whereas a Gi signaling pathway is linked to the mGlu2 protomer. In healthy circumstances, a determined Gi-Gq balance is present, which is disrupted by a decrease in Gi and an increase in Gq signaling under schizophrenia conditions. The authors found that, in general, dominant (strong) agonists enhance signaling through the protomer they target as part of the heteromer but inhibit signaling of the heteromeric receptor partner. By contrast, inverse agonists inhibit signaling through the protomer they target as part of the heteromer but enhance signaling of the heteromeric receptor partner.[13] Thus, to restore normal balance in patients with schizophrenia, an mGlu2 dominant agonist would be appropriate to increase Gi signaling and additionally decrease Gq signaling. In the same way, a serotonin 5HT2A inverse agonist would be appropriate to decrease Gq signaling and additionally increase Gi signaling. Furthermore, a combination of the two ligands would synergistically favor the desired effect.[13] This behavior was modeled in [14] by using a heterodimer model under equilibrium conditions.[10] To do so, proper values for the parameters describing receptor function under equilibrium conditions were chosen; in particular, values either greater than one or lower than one for the functional cooperativities in their respective Gi or Gq signaling pathways were chosen.[14] A second layer of complexity comes from considering the mutual influence between the receptors through ligand binding. Thus, if we are including a combination of two ligands, that is, a strong agonist A for mGlu2 and an inverse agonist B for 5HT2A, a binding cooperativity $\alpha > 1$ would favor the binding of the two ligands. Interestingly, and as discussed above, a single α value can be obtained from different (α_+, α_-) and (β_+, β_-) combinations. This variability in association and dissociation cooperativity rate constants can yield striking and unexpected results for those cases not restricted to equilibrium conditions, which could provide new insights into the biological problem. Furthermore, this mechanistic knowledge can help design the appropriate protocol for combination drug therapy in neurologic and psychiatric diseases.[15]

Residence time, agonist efficacy, and allosteric interactions

Binding kinetics and, consequently, the time factor are conceptual pieces in the mechanism of drug action that should not be neglected in pharmacological research and pharmaceutical development. The time factor is present in an explicit way through the concept of residence time.[16] If we define residence time as the time a ligand spends at the receptor-binding site, many different combinations of cooperativity rate constant values can be obtained from different ligands with similar equilibrium dissociation constants. In the above proposed case ($\alpha = \beta = 4$, with $\alpha_+ = 2$, $\alpha_- = 0.5$, $\beta_+ = 8$, and $\beta_- = 2$), if no other factors are considered, there would be expected an increase in the residence time of ligand A because of the presence of ligand B ($\alpha_- < 1$) and a decrease in the residence time of ligand B because of the presence of ligand A ($\beta_- > 1$).

There are studies in the literature showing the effect of allosteric modulators on the binding kinetics of orthosteric ligands. As examples, we can mention values included in Table 7 of [6] showing the

decrease in the dissociation rate constant of the adenosine A_3 [^{125}I]-I-AB-MECA agonist exerted by some allosteric modulators, namely, 43% (VUF5455),[7] 46% (DU124183),[17] 58% (2-AG),[18] and 47% (HMA).[19] Interestingly, the HMA allosteric modulator increased 1.6-fold the dissociation rate constant of the [^{3}H]-PSB-11 antagonist.[19]

Residence time is expected to be positively correlated with agonist efficacy because the longer an agonist remains bound to the receptor, the more cycles of G-protein activation it can catalyze.[8] This proposal has been proven in some receptor systems, such as the M_3 muscarinic acetylcholine,[20] the adenosine A_{2A},[21] the adenosine A_3,[9] and the β_2 adrenergic[22] receptors, but not in others, such as the adenosine A_1[23] and dopamine D_2 [24] receptors (see [8] for a discussion). These discrepancies can be a consequence of the intrinsic complexity of the relationship between efficacy and residence time when, for example, allosteric effects coming from lipid–receptor[25,26] or receptor–receptor [11,27] interactions might be present (reviewed in [8]). These interactions might differently modulate agonist-binding kinetics, yielding a functional result that is the product of a combination of association and dissociation rate constants. In this regard, dissociation rate constants determine the time ligands spend in the receptor binding site. Yet, before dissociating from the receptor, the ligand must bind to it. Thus, dissociation and association rate constants influence drug action and should be considered together.[28,29] On the one hand, high association rate constants can be useful in pharmacological therapy to allow fast association of the drug to its target.[30,31] On the other hand, although dissociation rate constants might be fundamental for drug action, this is not always the case because, as found in [32], the prolongation of binding owing to a long drug–target residence time can only occur when the binding dissociation is slower than the pharmacokinetics (PK) elimination. PK is beyond the scope of the present report, which is limited to the study of cooperativity from a binding kinetics perspective and applied to two receptor models: a ternary complex receptor model and a heterodimer model.

Moreover and to make the picture more complex, when relating residence time with efficacy, the former should include only the time the ligand spends bound to active receptor conformations, such as, for instance, the results in [9] and reviewed in [6], in which the adenosine A_3 receptor allosteric compound LUF6096 specifically stabilized the active conformation of the receptor with a concomitant increase in the intrinsic efficacy of orthosteric agonist Cl-IBMECA. In this regard, we have to take into account that the receptor species included in Figs. 1–4 represent macroscopic terms, including populations of different receptor conformations and states. Thus, inactive and active receptor species, either free or ligand bound, are present in the system. Moreover, if we attribute the observed receptor effect to receptor–G protein interactions, G protein-bound receptors are also implicitly included in the receptor terminology.

The question arises on how the schemes in Figs. 1–4 represent this molecular variety. For the sake of simplicity, we denote R as the free receptor and LR as the ligand-bound receptor in the figures. When using mathematical models of receptor function, it is said that a stimulus is provided by each of the receptor species through the product of the concentration of the considered receptor species and the corresponding intrinsic efficacy ε ($S_R = \varepsilon_R[R]$ and $S_{LR} = \varepsilon_{LR}[LR]$); where, if L is an agonist then $\varepsilon_{LR} > \varepsilon_R$; if L is an inverse agonist then $\varepsilon_{LR} < \varepsilon_R$; and if L is a neutral antagonist $\varepsilon_{LR} = \varepsilon_R$. Then, these stimuli are summed up ($S = S_R + S_{LR}$) and converted into effect through the transducer function $E = E_m S/(S + K_E)$, with E_m the maximum possible effect and K_E the transducer parameter.[33] Yet, and speaking in molecular terms, for the receptor to generate a stimulus, it is necessary that an active conformation is formed. If R and LR now denote inactive receptor conformations, R* and LR* are the corresponding active ones. If, in addition, the G protein is the transducer protein involved in the signaling pathway, we can accept that R*G and LR*G represent the receptor species responsible for the produced stimuli and, subsequently, for the observed effect. We can consider the binding kinetics concept through the association and dissociation rate constants of the different species of the system and, most importantly, of those related with the generated stimuli. Thus, we can consider that a low LR* dissociation rate constant (high residence time of the ligand in the active receptor complex) would be beneficial to allow the binding of the G protein. By contrast, if we focus our attention on the free receptor, initial receptor stimulus through R*G precoupling would be increased by an agonist with a high association rate constant for this receptor–G protein complex, because the intrinsic efficacy of an agonist–receptor complex (ε_{LR}) is higher than that of the free receptor (ε_R) (see [34,35] for detailed descriptions of GPCR kinetics). These are two examples of microscopic events showing how either decreasing or increasing dissociation or association ligand–receptor rate constants, respectively, can both increase the efficacy of the system. These changes in ligand–receptor rate constants both will lead to a decrease in the ligand–receptor equilibrium dissociation constant. The increase in efficacy will result only if a concomitant increase in the efficiency of G protein activation is part of the process.

To quantitatively illustrate these concepts, a simulation of the biological response under the heterodimer receptor model depicted in Fig. 3 was performed.[10] To this end, the transducer function $E/E_m = S/(K_E + S)$ proposed above was used, with the total stimulus S defined as $S = \varepsilon[R_1R_2] + \varepsilon_A[AR_1R_2] + \varepsilon_B[R_1R_2B] + \varepsilon_{AB}[AR_1R_2B]$. The model has the complexity of including two ligands, A and B, which are selective for protomers R_1 and R_2, respectively. We assume that both ligands are in excess with respect to the total receptor concentration. The model includes constitutive receptor activity through the ε parameter and ligands A and B are agonists, neutral antagonists, or inverse agonists depending on the values of their intrinsic efficacies, ε_A and ε_B, compared with ε. The doubly bound receptor has an intrinsic efficacy, ε_{AB}, defined as $\varepsilon_A\varepsilon_B\delta$. In a similar way to the binding cooperativity α, δ can be greater than, lower than, or equal to one, thus reflecting the mutual allosteric interaction between the two ligands at the functional level (see [10] for a detailed description of the heterodimer model).

For the sake of simplicity, we examined the biological effect of changing the concentration of various agonists in the presence of a constant concentration of allosteric modulators (Fig. 5). To analyze the effect of binding kinetics on the transducer function, some parameters were

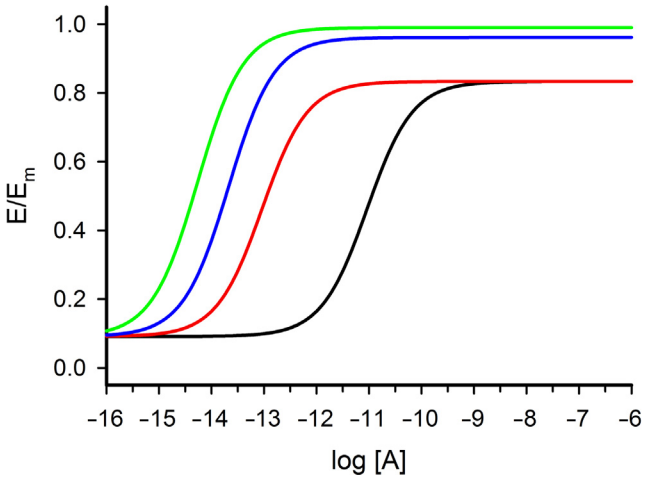

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FIG. 5

Simulation of the E/E_m fractional effect resulting from the heterodimer binding kinetics model depicted in Fig. 3 in the main text. The translation of binding into function is made through the relationship $E/E_m = S/(K_E + S)$, with the total stimulus S defined as $S = \varepsilon[R_1R_2] + \varepsilon_A[AR_1R_2] + \varepsilon_B[R_1R_2B] + \varepsilon_{AB}[AR_1R_2B]$, where ε, ε_A, ε_B, and $\varepsilon_{AB} = \delta\varepsilon_A\varepsilon_B$ are the intrinsic efficacies of the free receptor, the singly bound A and B receptors, and the doubly bound receptor, respectively; δ measures the functional interaction between A and B, and K_E is the transduction factor of stimulus into effect.[10] The reference curve is the black curve, which includes the following functional parameter values: $\chi = [R_T]/K_E = 1$, $\varepsilon = 1$, $\varepsilon_A = 10$, $\varepsilon_B = 10^{-1}$, and $\delta = 5$ and the following binding kinetics parameter values: $k_{+1} = 10^7$, $k_{-1} = 10^{-1}$, $k_{+2} = 10^7$, $k_{-2} = 10^{-2}$, $k_{+4} = 2*10^7$, $k_{-4} = 10^{-3}$, $k_{+3} = 4*10^7$, $k_{-3} = 2*10^{-4}$, with M^{-1} s^{-1} and s^{-1} units for association and dissociation rate constants, respectively. Ligands A and B are an agonist and an inverse agonist, respectively, because their intrinsic efficacies are greater and lower than that of the free receptor, ε, respectively. Using cooperativity rate constant parameters, it can be seen that $\alpha_+ = k_{+4}/k_{+1} = 2$, $\alpha_- = k_{-4}/k_{-1} = 10^{-2}$, $\beta_+ = k_{+3}/k_{+2} = 4$ and $\beta_- = k_{-3}/k_{-2} = 2*10^{-2}$. Moreover, it is found that $\alpha_+/\alpha_- = \beta_+/\beta_- = 200$. The allosteric compound B is present at 10^{-6} M fixed concentration. Red curve: the dissociation rate constants k_{-4} and k_{-1} are decreased with respect to the black curve, that is, $k_{-4} = 10^{-5}$ and $k_{-1} = 10^{-3}$. The values for the cooperativity rate constant parameters are the same. The decrease in the k_{-4} and k_{-1} dissociation rate constants translates into a decrease in the dissociation equilibrium constant of A for R_1 and, consequently, into an increase in the potency of A, which is reflected in the displacement of the red curve to the left with respect to the black one. Note: the red curve can be equally obtained by an increase in the association rate constants k_{+4} and k_{+1}, with respect to the black curve, that is, $k_{+4} = 2*10^9$ and $k_{+1} = 10^9$. Blue curve: taking the black curve as a reference, k_{-4} and k_{-1} are changed in the same way as for the red curve, that is, $k_{-4} = 10^{-5}$ and $k_{-1} = 10^{-3}$, but, in addition, there is an increase in the intrinsic efficacy associated with ligand A, $\varepsilon_A = 50$. As a result, there is an increase in the potency and efficacy of the system with a displacement of the black curve both left and upwards (the maximum response is increased). Green curve: taking the black curve as a reference, k_{-4} and k_{-1} are changed in the same way as in the red curve, that is, $k_{-4} = 10^{-5}$ and $k_{-1} = 10^{-3}$, but, in addition, δ is changed from 5 to 100. As a result, there is an increase in the potency and efficacy of the system with a displacement of the black curve both left and upwards (the maximum response is increased). Note: mathematical expressions of the maximum response (the limiting value of E/E_m as [A] or [B] increase) can be found in [14].

changed with respect to a reference condition (Fig. 5, black curve). First, we considered the effects of either decreasing the dissociation rate constant of ligand A through k_{-1} and k_{-4} rate constants or increasing the association rate constant of ligand A through k_{+1} and k_{+4} rate constants (Fig. 5, red curve). The values used were chosen so that the $(k_{-1}\ k_{+4})/(k_{+1}\ k_{-4}) = (k_{-2}\ k_{+3})/(k_{+2}\ k_{-3})$, or, in other words, $\alpha_+/\alpha_- = \beta_+/\beta_-$ condition was satisfied. We can see that a change in binding kinetics has an effect only on the observed potency of ligand A (shift to the left with respect to the reference curve) with no change in efficacy (maximum response). However, as discussed above, a change in the asymptotic maximum effect can be observed if the change in binding involves a change in intrinsic efficacy. To illustrate this, the blue curve in Fig. 5 was obtained from the red curve by assuming an increase in ε_A that might result from either a decrease of the dissociation rate constants or an increase of the association rate constants of ligand A. Both changes might increase the biological response: in the former case, by assuming active AR_1R_2 and AR_1R_2B receptor states in which a low dissociation rate constant of ligand A favors the binding of the G protein; in the latter case, by assuming active R_1R_2 and R_1R_2B receptors coupled to G proteins in which ligand A presents a high association rate constant for the complexes. The maximum response displayed by the agonist A can also be increased through the mutual allosteric effect between ligands A and B. To show this point, the green curve in Fig. 5 was obtained from the red curve by assuming an increase in the δ parameter from 5 to 100. This increase in δ leads to an increase in ε_{AB} and, as a result, an increase in the concentrations of active AR_1R_2B receptor states. To generalize the concept of allosterism, we draw attention again to the conceptual similarities between the allosteric ternary complex receptor model and the heterodimer receptor model, where δ is present in both (see [36] for a discussion on operational models of allosterism).

As mentioned above, some, but not all, experimental studies have found a correlation between residence time and efficacy. In particular, we can recall on a study on the adenosine A_{2A} receptor,[21] in which the authors found a correlation between the residence time of an agonist and its functional efficacy in two assays; they also found that, compared with the equilibrium affinity, the receptor residence time of the A_2 receptor agonist had a much bet-

ter correlation to its intrinsic efficacy. Similarly, in a study of the M_3 muscarinic acetylcholine receptor involving seven agonists,[20] the authors did not find a relationship between agonist efficacy and the equilibrium binding affinity. However, when efficacy was compared with the dissociation rate constant, a high correlation was found, suggesting a relationship between the duration of agonist binding at the receptor and the intrinsic efficacy.[20]

Apart from experimental studies, a typical field in which the discussion on residence time makes particular sense is molecular dynamics (MD) simulations. In the case of a PAM in the context of binding to the receptor ($\alpha > 1$), one would expect that, in those MD simulations including both the orthosteric and the allosteric ligands, the stability of ligand–receptor interactions of both ligands in their binding sites would increase compared with their simulations in the absence of the partner ligand. However, if this were not the case, we can now understand, based on the above discussion on α_- and β_- values, that a variety of results can be obtained from the dynamic interactions between orthosteric and allosteric ligands that can be compatible with a single cooperativity binding parameter obtained at equilibrium conditions.

Concluding remarks

Inclusion of cooperativity in a binding kinetic model of allosterism has enabled us to find a mathematical expression ($\alpha_+/\alpha_- = \beta_+/\beta_-$) that links the cooperativity rate constants of the orthosteric and allosteric ligands. The expression shows that many different combinations of kinetic allosteric effects are possible for a particular value of the α binding cooperativity parameter obtained under equilibrium conditions. This assessment could help understand striking experimental results involving allosteric modulation and suggest further investigations in the field. Furthermore, the fact that allosteric modulators can exert pathway-specific effects leads to the concept of biased allosteric modulation,[37] a chemical space in which kinetically oriented drug discovery programs can help in the search for new pharmacological therapies.

Data availability

Data will be made available on request.

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Declaration of interests

The authors declare no competing interests.

References

1 A. Christopoulos, T. Kenakin, G protein-coupled receptor allosterism and complexing, Pharmacol Rev 54 (2002) 323–374.
2 F.J. Ehlert, Estimation of the affinities of allosteric ligands using radioligand binding and pharmacological null methods, Mol Pharmacol 33 (1988) 187–194.
3 S. Tucek, J. Proska, Allosteric modulation of muscarinic acetylcholine receptors, Trends Pharmacol Sci 16 (1995) 205–212.
4 D.A. Sykes, L.A. Stoddart, L.E. Kilpatrick, S.J. Hill, Binding kinetics of ligands acting at GPCRs, Mol Cell Endocrinol 485 (2019) 9–19.
5 D. Guo, L.H. Heitman, A.P. Ijzerman, The role of target binding kinetics in drug discovery, ChemMedChem 10 (2015) 1793–1796.
6 D. Guo, L.H. Heitman, A.P. IJzerman, Kinetic aspects of the interaction between ligand and G protein-coupled Receptor: the case of the adenosine receptors, Chem Rev 117 (2017) 38–66.
7 Z.G. Gao, J.E. Van Muijlwijk-Koezen, A. Chen, C.E. Müller, A.P. Ijzerman, K.A. Jacobson, Allosteric modulation of A(3) adenosine receptors by a series of 3-(2-pyridinyl)isoquinoline derivatives, Mol Pharmacol 60 (2001) 1057–1063.
8 J.R. Lane, L.T. May, R.G. Parton, P.M. Sexton, A. Christopoulos, A kinetic view of GPCR allostery and biased agonism, Nat Chem Biol 13 (2017) 929–937.
9 L.H. Heitman, A. Göblyös, A.M. Zweemer, R. Bakker, T. Mulder-Krieger, J.P. van Veldhoven, et al., A series of 2,4-disubstituted quinolines as a new class of allosteric enhancers of the adenosine A3 receptor, J Med Chem 52 (2009) 926–931.
10 B. Zhou, J. Giraldo, Quantifying the allosteric interactions within a G-protein-coupled receptor heterodimer, Drug Discov Today 23 (2018) 7–11.
11 B. Zhou, J. Giraldo, An operational model for GPCR homodimers and its application in the analysis of biased signaling, Drug Discovery Today 23 (2018) 1591–1595.
12 C. White, L.J. Bridge, Ligand binding dynamics for pre-dimerised G protein-coupled receptor homodimers: linear models and analytical solutions, Bull Math Biol 81 (2019) 3542–3574.
13 M. Fribourg, J.L. Moreno, T. Holloway, D. Provasi, L. Baki, R. Mahajan, et al., Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs, Cell 147 (2011) 1011–1023.
14 J. Giraldo, B. Zhou, D. Roche, C. Gil, J. Ortiz, I. Lans, et al., Analysis of the function of receptor oligomers by operational models of agonism, in: T.E. Kenakin (Ed.), Comprehensive Pharmacology, Elsevier, Amsterdam, 2022, pp. 337–359.
15 I. Gilron, T.S. Jensen, A.H. Dickenson, Combination pharmacotherapy for management of chronic pain: from bench to bedside, Lancet Neurol 12 (2013) 1084–1095.
16 R.A. Copeland, D.L. Pompliano, T.D. Meek, Drug-target residence time and its implications for lead optimization, Nat Rev Drug Discov 5 (2006) 730–739.
17 Z.G. Gao, S.G. Kim, K.A. Soltysiak, N. Melman, A.P. IJzerman, K.A. Jacobson, Selective allosteric enhancement of agonist binding and function at human A3 adenosine receptors by a series of imidazoquinoline derivatives, Mol Pharmacol 62 (2002) 81–89.
18 J.R. Lane, M.W. Beukers, T. Mulder-Krieger, A.P. Ijzerman, The endocannabinoid 2-arachidonylglycerol is a negative allosteric modulator of the human A3 adenosine receptor, Biochem Pharmacol 79 (2010) 48–56.
19 Z.G. Gao, N. Melman, A. Erdmann, S.G. Kim, C.E. Müller, A.P. Ijzerman, et al., Differential allosteric modulation by amiloride analogues of agonist and antagonist binding at A(1) and A(3) adenosine receptors, Biochem Pharmacol 65 (2003) 525–534.
20 D.A. Sykes, M.R. Dowling, S.J. Charlton, Exploring the mechanism of agonist efficacy: a relationship between efficacy and agonist dissociation rate at the muscarinic M3 receptor, Mol Pharmacol 76 (2009) 543–551.
21 D. Guo, T. Mulder-Krieger, A.P. Ijzerman, L.H. Heitman, Functional efficacy of adenosine A_2A receptor agonists is positively correlated to their receptor residence time, British J Pharmacol 166 (2012) 1846–1859.
22 E.M. Rosethorne, M.E. Bradley, K. Gherbi, D.A. Sykes, A. Sattikar, J.D. Wright, et al., Long receptor residence time of C26 contributes to super agonist activity at the human beta2 adrenoceptor, Mol Pharmacol 89 (2016) 467–475.
23 J. Louvel, D. Guo, M. Soethoudt, T.A. Mocking, E.B. Lenselink, T. Mulder-Krieger, et al., Structure-kinetics relationships of Capadenoson derivatives as adenosine A1 receptor agonists, Eur J Med Chem 101 (2015) 681–691.
24 C.K. Herenbrink, D.A. Sykes, P. Donthamsetti, M. Canals, T. Coudrat, J. Shonberg, et al., The role of kinetic context in apparent biased agonism at GPCRs, Nat Commun 7 (2016) 10842.
25 A. Bruzzese, C. Gil, J.A.R. Dalton, J. Giraldo, Structural insights into positive and negative allosteric regulation of a G protein-coupled receptor through protein-lipid interactions, Sci Rep 8 (2018) 4456.
26 Ó. Díaz, J.A.R. Dalton, J. Giraldo, Revealing the mechanism of agonist-mediated cannabinoid receptor 1 (CB1) activation and phospholipid-mediated allosteric modulation, J Med Chem 62 (2019) 5638–5654.
27 K.J. Gregory, J. Giraldo, J. Diao, A. Christopoulos, K. Leach, Evaluation of operational models of agonism and allosterism at receptors with multiple orthosteric binding sites, Mol Pharmacol 97 (2020) 35–45.
28 G. Vauquelin, Link between a high k (on) for drug binding and a fast clinical action: to be or not to be?, MedChemComm 9 (2018) 1426–1438
29 G. Vauquelin, Effects of target binding kinetics on in vivo drug efficacy: koff, kon and rebinding, British J Pharmacol 173 (2016) 2319–2334.

30 N. Yin, J. Pei, L. Lai, A comprehensive analysis of the influence of drug binding kinetics on drug action at molecular and systems levels, Mol Biosyst 9 (2013) 1381–1389.
31 A. Schoop, F. Dey, On-rate based optimization of structure-kinetic relationship–surfing the kinetic map, Drug Discovery Today Technol 17 (2015) 9–15.
32 G. Dahl, T. Akerud, Pharmacokinetics and the drug-target residence time concept, Drug Discov Today 18 (2013) 697–707.
33 D. Roche, D. Gil, J. Giraldo, Mechanistic analysis of the function of agonists and allosteric modulators: reconciling two-state and operational models, Br J Pharmacol 169 (2013) 1189–1202.
34 R.S. Stein, F.J. Ehlert, A kinetic model of GPCRs: analysis of G protein activity, occupancy, coupling and receptor-state affinity constants, J Recept Signal Transduct Res 1–15 (2014).
35 L.D. Shea, R.R. Neubig, J.J. Linderman, Timing is everything the role of kinetics in G protein activation, Life Sci 68 (2000) 647–658.
36 J. Giraldo, Operational models of allosteric modulation: caution is needed, Trends Pharmacol Sci 36 (2015) 1–2.
37 L.M. Slosky, M.G. Caron, L.S. Barak, Biased allosteric modulators: new frontiers in GPCR drug discovery, Trends Pharmacol Sci 42 (2021) 283–299.

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