Operational models of allosteric modulation: caution is needed

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The operational model of allosteric modulation and allosteric agonism [1-5] is being used routinely to assess the functional effects of allosteric modulators. Because the meaning of parameters and concepts often loses precision after extensive use, it may be timely to revise some particularities of the model to preclude possible misinterpretations. To do so, I will use the equation for the model as expressed in [4]

$$E = \frac{E_m(\tau_A [A](K_B + \alpha\beta[B]) + \tau_B [B]K_B)^n}{([A]K_B + K_A K_B + K_A[B] + \alpha[A][B])^n + (\tau_A [A](K_B + \alpha\beta[B]) + \tau_B [B]K_A)^n} \quad (1)$$

where E_m is the maximum effect of the system; n, a parameter related with the slope of the curves; [A] and [B], the concentrations of the agonist A and the allosteric modulator B, respectively; τ_A and τ_B, the operational efficacies of A and B, respectively; K_A and K_B, the dissociation constants of A and B, respectively; and α and β, the cooperativity parameters governing binding and function, respectively. Values of α and β greater, equal and lower than 1 indicate positive, neutral and negative cooperativity, respectively.

I will not comment on all the parameters but only in a particular one the operational functional cooperativity β. The intrinsic efficacy of the receptor complexed with both the orthosteric agonist A and the allosteric compound B was defined [4] as $\varepsilon_{AB}=\varepsilon_A\beta$, where ε_A denotes the intrinsic efficacy of A and β is a coupling factor that describes the ability of B to alter the signalling capacity of A when bound to the same receptor in a ternary ARB complex. This definition of of ε_{AB}, which does not include the intrinsic efficacy of B, is useful for data fitting because decreases the number of parameters of the model. However, it makes β not a pure cooperativity factor but a hybrid parameter embodying both intrinsic efficacy and functional cooperativity. To uncouple these properties, ε_{AB} was defined in an alternative mathematical model [6,7] as $\varepsilon_{AB}= \varepsilon_A\varepsilon_B\delta$,

where ε_B denotes the intrinsic efficacy of B and δ describes the activation cooperativity between A and B in the ARB complex (note that δ does not measure the influence of B upon A but the mutual cooperative effects between A and B). By making equal both expressions of ε_{AB}, we see that $\beta=\varepsilon_B\delta$ includes in its definition the intrinsic efficacy of the allosteric compound. The latter relationship shows that for a fixed δ effect those allosteric compounds displaying higher intrinsic efficacy will yield higher estimates of the β cooperativity parameter, if data are fitted with Equation 1. Remarkably, this was what it was found in a recent study on the functional activity of some mGlu4 positive allosteric modulators [8]. In this respect, a discussion on the correlation between the β parameter and the efficacy of allosteric ligands can be found in [9].

A reflection of the efficacy character that is included in β can be seen in the expression of the asymptotic maximum response of an agonist (Top) in the presence of a fixed concentration of an allosteric modulator. If we take the limit of the effect E as [A] increases in Equation 1 we obtain

$$\mathrm{Top} = \lim_{[A]\to\infty} E = \frac{E_m}{1+\left(\frac{K_B + \alpha[B]}{\tau_A\left(K_B + \alpha\beta[B]\right)}\right)^n} \qquad (2)$$

It is worth noting that τ_B is not included in the expression for Top effect, when it should logically appear. The reason for this is that the efficacy of the allosteric modulator B is implicitly included in the definition of β.

I will comment now a second matter. In an attempt to overcome the data fitting problems arising from the many parameters included in Equation 1, the simplified Equation 3 was proposed [10],

$$E = \frac{E_m([A](K_B + \alpha\beta[B]) + \tau_B[B][EC_{50}])^n}{[EC_{50}]^n(K_B + [B])^n + ([A](K_B + \alpha\beta[B]) + \tau_B[B][EC_{50}])^n} \quad (3)$$

which was derived from Equation 1 under the following assumptions

(i) $[A] << K_A$, and then $[A] + K_A \approx K_A$

(ii) $\alpha[A] << K_A$, and then $\alpha[A] + K_A \approx K_A$

(iii) $\tau_A{}^n >> 1$, and then the location parameter of A in the absence of B in the operational model of agonism [11], $[EC_{50}] = \frac{K_A}{\left(2 + \tau_A{}^n\right)^{1/n} - 1}$, simplifies to $[EC_{50}] \approx \frac{K_A}{\tau_A}$.

All these assumptions relay on A being a full agonist. Additionally, assumption (ii) includes the property that the binding cooperativity α cannot be much greater than 1. It can be seen that Equation 3 reduces the number of parameters present in Equation 1 by considering jointly K_A and τ_A through $[EC_{50}]$ and α and β through the composite cooperativity parameter αβ.

It is worth noting that because of the above assumptions, Equation 3 has a limitation in its use; that is, it is circumscribed to concentration-effect curves whose maximum responses (Top) do not differ from the maximum response of the system (E_m).

If we calculate the asymptotic Top value from Equation 3 we have

$$Top = \lim_{[A]\to\infty} E = E_m \quad (4)$$

which, contrary to Equation 2 and to the original formulation of the operational model [11], matches E_m in all cases. Thus, Equation 3 cannot be used either for orthosteric compounds A displaying partial agonism or for allosteric compounds B increasing or decreasing the maximum response yielded by compounds A.

To briefly summarize, operational models of allosteric modulation and allosteric agonism [4,10] have proved useful for the quantification of the function of allosteric compounds; however, the pharmacological conditions imposed in some parameter definitions or the simplifications included in some equation derivations may lead to misleading conclusions if not used properly. Thus, caution is needed not to make interpretations from the models beyond the conditions and limitations inherent to them.

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