On slope factor of the operational model of agonism

Jan Jakubík

Institute of Physiology Czech Academy of Sciences, Prague, Czech Republic

jan.jakubik@fgu.cas.cz

Abstract

Although being a relative term, agonist efficacy is a cornerstone in the proper assessment of agonist selectivity and signalling bias. The operational model of agonism (OMA) has become successful in the determination of agonist efficacies and ranking them. In 1995, Black et al. introduced slope factor to the OMA that makes the OMA more flexible and allows for fitting steep as well as flat concentration-response curves. Here I opinion drawbacks of the slope factor implemented by Black et al. that affects relationships among parameters of the OMA. Instead, I propose the implementation of the Hill coefficient in the OMA that does not affect observed parameters. The OMA modified by the Hill coefficient is more practical in the determination of operational efficacies for agonism ranking and subsequent analysis, like quantification of signalling bias.

Introduction

Since its introduction in 1983, the operational model of agonism (OMA) has become a golden standard in an evaluation of agonism and subsequently also of signalling bias[1]. The OMA describes the response of the system as a function of ligand concentration using three parameters: 1) The equilibrium dissociation constant of agonist (K_A) at the active state of the receptor; 2) The maximal possible response of the system (E_{MAX}); 3) The operational efficacy of agonist (τ). Original OMA describes the dependence of functional response on the concentration of ligand by rectangular hyperbola (Eq. 2). In practice, positive cooperativity or positive feedback lead to steep and negative cooperativity or negative feedback lead to flat concentration-response curves that the OMA does not fit to. Therefore, an equation intended for the description of non-hyperbolic concentration curves (Eq. 5), particularly for systems where the number of receptors varies, was introduced by Black et al.[2]. Since then, Eq. 5 is used perpetually. Here I show that under certain conditions the equation Eq. 5 allows unrealistic parameters of concentration-response curves. This renders data analysis impossible under these conditions. Further, the slope factor affects the relationship between observed maximal response to agonist and operational

efficacy and the relationship between the observed half-efficient concentration of agonist and its equilibrium dissociation constant. This substantially complicates data analysis.

Therefore, I propose the use of the Hill coefficient as a slope factor to the original rectangular hyperbola of OMA instead of the slope factor by Black et al. The Hill–Langmuir equation was originally formulated to describe the cooperative binding of oxygen molecules to haemoglobin[3]. The Hill coefficient reflects the sense and magnitude of cooperativity among concurrently binding ligands. A value of the Hill coefficient lower than 1 indicates negative cooperativity that is manifested as a flat concentration-response curve. The smaller value of the Hill coefficient the flatter curve is. A value of the Hill coefficient greater than 1 indicates positive cooperativity that is manifested as a steep concentration-response curve. The greater value of the Hill coefficient the steeper curve is. In contrast to Black et al. slope factor, the proposed implementation of the Hill coefficient (Eq. 8) does not affect the centre or asymptotes of the hyperbola describing concentration-response curves. Therefore, the Hill coefficient does not affect relationships among three parameters of the OMA, K_A, E_{MAX} and τ. This makes OMA (Eq. 9) more practical in many ways.

Model analysis

In bi-molecular reaction, the dependence of ligand binding to the receptor is described by Eq. 1 where [A] is the concentration of ligand and K_A is its equilibrium dissociation constant that represents the concentration of ligand at which half of the total number of receptors is bound to the ligand and other half receptors are free.

$$Binding = \frac{[A]R_{total}}{[A]+K_A} \qquad \text{Eq. 1}$$

If the bound ligand is an agonist it activates the receptor. Various agonists produce a functional response of different strength. The OMA was postulated to introduce the "transducer" function linking agonist binding to its functional response. The OMA ranks agonists by operational efficacy τ. According to OMA the response of the system as a function of ligand concentration is defined by three parameters: The equilibrium dissociation constant of agonist (K_A) at the active state of the receptor that is specific to a combination of ligand and receptor. The maximal possible response of the system (E_{MAX}) that is specific to the system. And the operational efficacy of agonist (τ) that is specific to a combination of ligand and system. In the OMA, the rectangular hyperbolic dependence of functional response on concertation of agonist A is given by Eq. 2[1].

$$Response = \frac{[A]\tau E_{MAX}}{[A](\tau+1)+K_A} \qquad \text{Eq. 2}$$

For Eq. 2, the horizontal asymptote, the observed maximal response to agonist A (E'_{MAX}), is given by Eq. 3.

$$E'_{MAX} = \frac{\tau}{\tau+1} E_{MAX} \qquad \text{Eq. 3}$$

A more efficacious agonist (having a high value of parameter τ) elicits higher E'_{MAX} than less efficacious agonists (having a low value of parameter τ). The relationship between parameter τ and E'_{MAX} is hyperbolic meaning that two highly efficacious agonists (e.g., τ values 10 and 20) differ in E'_{MAX} values less than two weak agonists (e.g., τ values 0.1 and 0.2).

For Eq. 2, the inflexion point, the half-efficient concentration of agonist A (EC_{50}), is given by Eq. 3.

$$EC_{50} = \frac{K_A}{\tau+1} \qquad \text{Eq. 4}$$

The EC_{50} value is always lower than the K_A value. The K_A to EC_{50} ratio is greater for efficacious agonists than for weak agonists. Similarly to E'_{MAX}, the relationship between parameter τ and EC_{50} is hyperbolic. In contrast to E'_{MAX} values, the ratio K_A to EC_{50} ratio is more profound for two highly efficacious agonists (e.g., τ values 10 and 20) than two weak agonists (e.g., τ values 0.1 and 0.2).

In the case of linear relationship between agonist binding and functional response, the concentration-response curve (Eq. 2) has the same shape as the agonist-binding curve (Eq. 1). In practice, however, concentration-response curves steeper or flatter than the ones described by Eq. 2 are observed. In such cases, Eq. 2 does not fit experimental data. As stated by authors, Eq. 5 was devised for non-rectangular hyperbolic dependence of functional response on concertation of agonist[2].

$$Response = \frac{[A]^n \tau^n E_{MAX}}{[A]^n \tau^n + ([A] + K_A)^n} \qquad \text{Eq. 5}$$

For $[A] \geq 0$ and $n > 0$, Eq. 5 is, however, rectangular hyperbola with horizontal asymptote (E'_{MAX}) given by Eq. 6 and vertical asymptote equal to $-K_A/(\tau+1)$. From Eq. 5, the EC_{50} value is given by Eq. 7.

$$E'_{MAX} = \frac{\tau^n E_{MAX}}{1 + \tau^n} \qquad \text{Eq. 6}$$

$$EC_{50} = \frac{K_A}{(2 + \tau^n)^{\frac{1}{n}} - 1} \qquad \text{Eq. 7}$$

Evidently, the introduced slope factor **n** affects both the observed maximal response E'_{MAX} and half-efficient concentration of agonist EC_{50} (Figure 1A and C). The influence of the slope factor on E'_{MAX} is **bidirectional**. For operational efficacies $\tau > 1$, an increase in the value of slope factor increases E'_{MAX}. (Figure 1A and 2A blue lines). For operational efficacies $\tau < 1$, an increase in slope factor decreases E'_{MAX} (Figure 1C and 2A red lines). The effect of slope factor on E'_{MAX} is the most eminent for low values of operational efficacy τ, making the estimation of model parameters of weak partial agonists impractical.

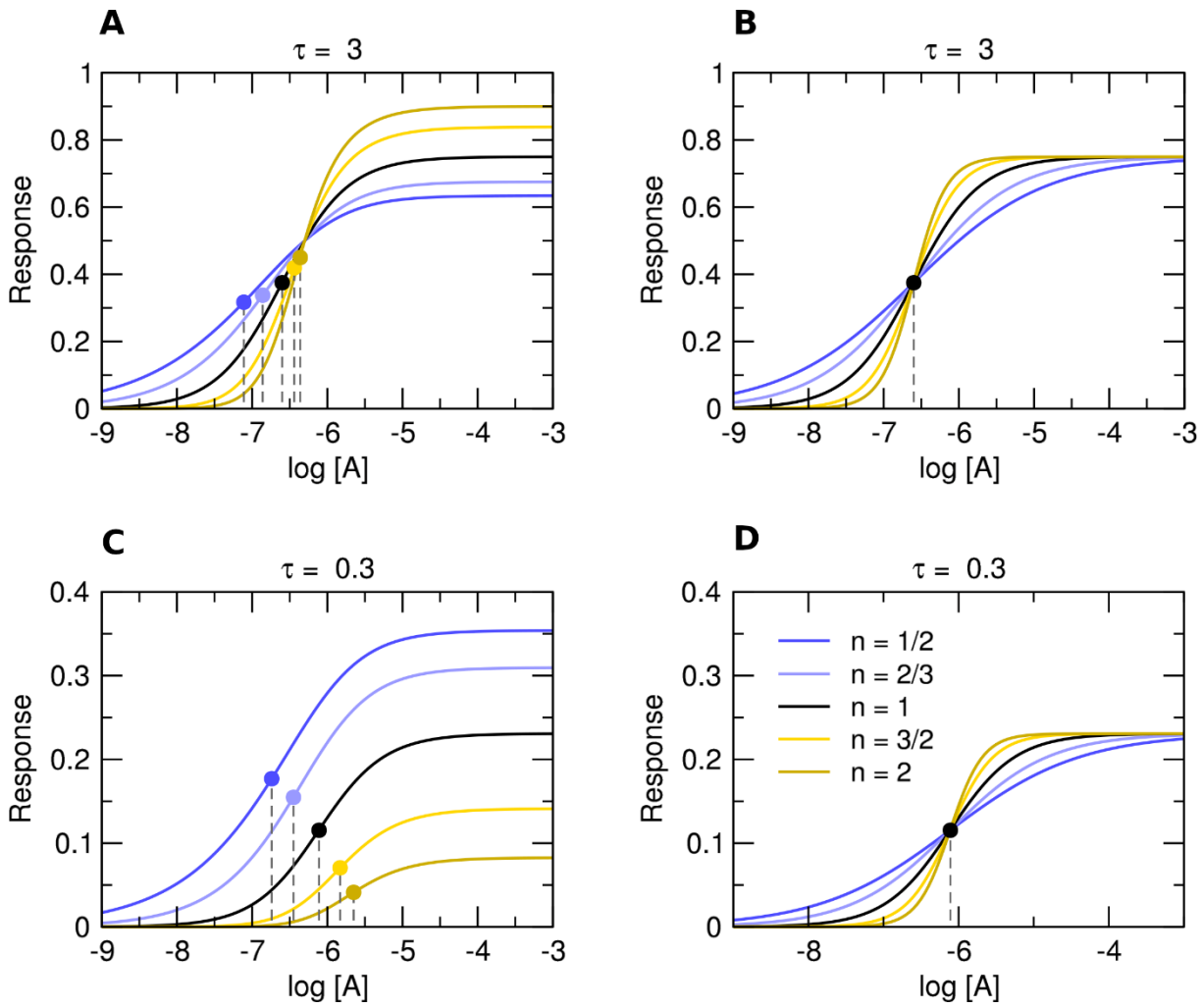

Figure 1 Theoretical concentration curves
Theoretical response curves according to Eq. 5, left and Eq. 9, right. Simulation parameters: $E_{MAX} = 1$; $\tau = 3$ (top) or $\tau = 0.3$ (bottom); $K_A = 10^{-6}$. Values of slope factors are listed in the legend.

An increase in the value of the slope factor increases the EC_{50} value (Figure 2B). Again, the effect of the slope factor on the EC_{50} value is more eminent at low values of operational efficacy τ (red lines). Paradoxically, any combination of operational efficacy τ and slope factor fulfilling the inequality in Figure 2C (blue area) results in EC_{50} values **greater** than K_A (e.g., Figure 1C,

yellow lines). For example, $EC_{50} > K_A$ applies if $\tau = 0.5$ and $n > 1.6$, or if $\tau = 1$ and $n > 1.6$, or if $\tau = 1.5$ and $n > 2.15$. The upper asymptote of inequality is 2. Thus, the possibility of $EC_{50} > K_A$ applies to $\tau < 2$.

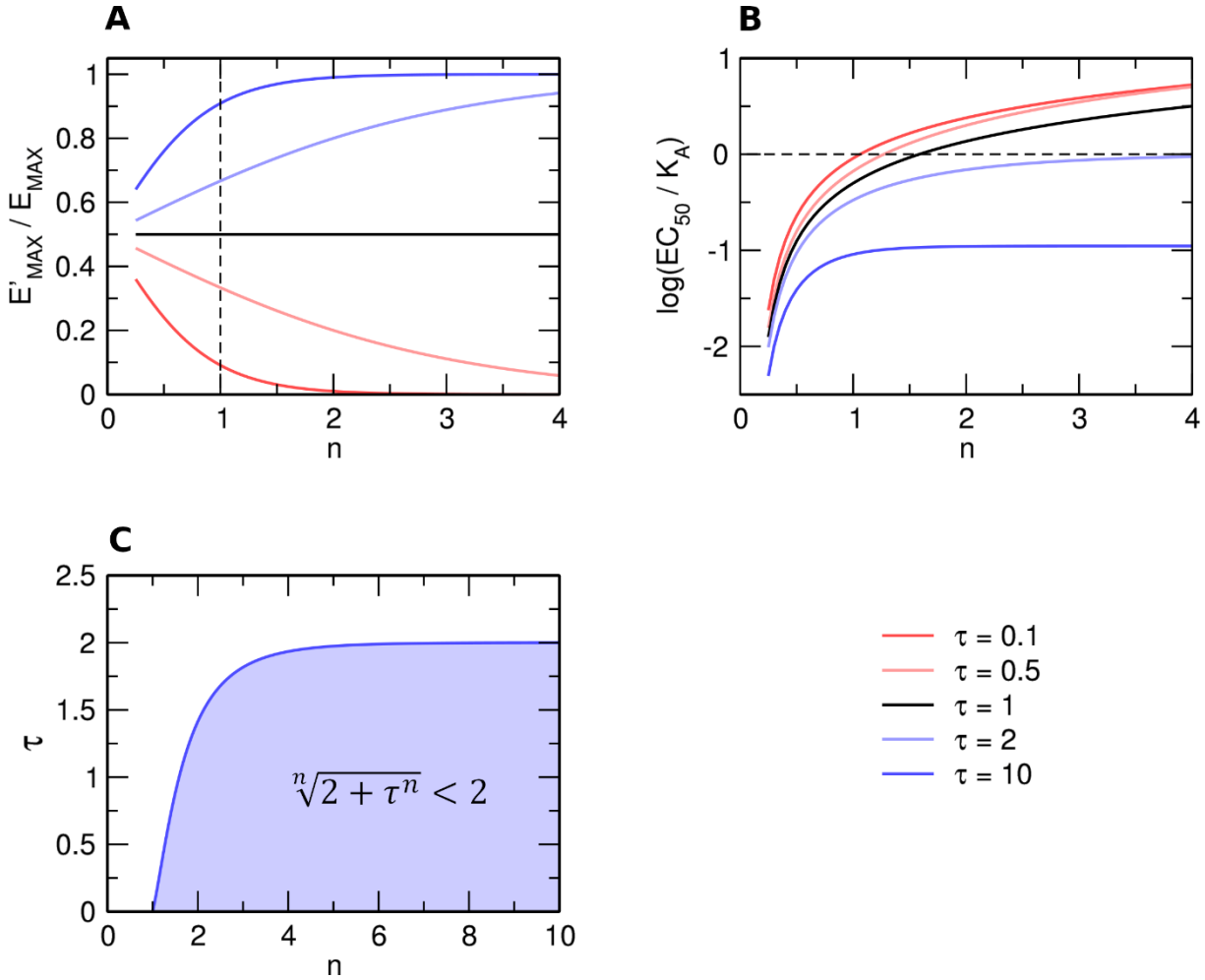

Figure 2 Analysis of Eq. 5
A, Dependency of observed E'_{MAX} to system E_{MAX} ratio (ordinate) on slope factor n (abscissa) and operational efficacy τ (legend). B, Dependency of EC_{50} to K_A ratio (ordinate) on slope factor n (abscissa) and operational efficacy τ (legend). C, Inequality plot of slope factor **n** (abscissa) and operational efficacy τ (ordinate) yielding half-efficient concentration EC_{50} greater than equilibrium dissociation constant K_A.

The operational efficacy τ may be also considered as a measure of "receptor reserve". In the system with a relatively small capacity of a functional response output, the full agonist reaches its maximal response before reaching full receptor occupancy. Thus, the agonist EC_{50} value is lower than its affinity for the receptor. The smaller occupancy fraction is needed for the full response to a given agonist the greater is difference between agonist EC_{50} and K_A values. According to OMA (Eq. 2), the relation between EC_{50} and K_A is described by Eq. 4. The greater value of operational efficacy τ, the smaller EC_{50} value and greater the difference from K_A. Thus,

the value of operational efficacy τ is a measure of receptor reserve of a given agonist at a given system. In a system with a large capacity of functional output, agonists do not have a receptor reserve and must reach full receptor occupancy to elicit full signal. In such a system, even full agonists have small operational efficacies. Thus, the parameter τ is specific to a combination of ligand and system.

Nevertheless, for agonists that elicit at least some response in a given system, the parameter τ must be greater than 0. Then according to Eq.4 of the operational model of agonism, the EC_{50} value must be smaller than the K_A value. Taken together, Eq. 5 cannot be applied to a steep response curve (n>1) of an agonist with the low parameter τ that would result in the EC_{50} value greater than the K_A value. In principle, the validity of Eq. 5 is questionable.

Hill equation incorporates the Hill coefficient as a slope factor to rectangular hyperbola[3]. The major advantage of the Hill coefficient as a slope factor is that it allows for a change in the eccentricity of the hyperbola without changing centre (EC_{50}) and asymptotes (E'_{MAX}). The Hill–Langmuir equation published in 1910 can be formulated as Eq. 8.

$$Response = \frac{[A]^{nH} E'_{MAX}}{[A]^{nH} + EC_{50}{}^{nH}} \qquad \text{Eq. 8}$$

Where nH is the Hill coefficient. Substitution of E'_{MAX} by Eq. 3 and EC_{50} by Eq. 4 gives Eq. 9.

$$Response = \frac{[A]^{nH} \frac{\tau}{\tau+1} E_{MAX}}{[A]^{nH} + (\frac{K_A}{\tau+1})^{nH}} \qquad \text{Eq. 9}$$

As expected, the Hill coefficient does not influence maximal observed response E'_{MAX} either half-efficient concentration of agonist EC_{50} (Figure 1B and D).

Implications

Biased agonists stabilize specific conformations of the receptor leading to non-uniform modulation of individual signalling pathways[4]. To measure an agonist bias correctly, the parameters τ and K_A must be determined and $\log(\tau/K_A)$ values of tested and reference agonist compared at two signalling pathways[5]. Alternatively, biased signalling may be inferred from the comparison of the ratio of intrinsic activity (E'_{MAX}/EC_{50}) of tested agonist to the intrinsic activity of reference agonist at two signalling pathways[6]. It is evident from the analysis of Eq. 5, that as far as the EC_{50} value is dependent on parameters n and τ (Figure 1A vs. C), relative intrinsic activities cannot be compared to judge possible signalling bias unless the parameter n is equal to 1 for both tested and reference agonist. Thus, in the case the parameter n is not equal to 1 for

both tested and reference agonist, the parameters τ and K_A must be obtained. In the case of Eq. 2, all three parameters (τ, K_A, and E_{MAX}) are inter-dependent[7]. In the case of Eq. 5, all four parameters (τ, K_A, E_{MAX}, and n) are inter-dependent. Thus, the parameters τ and K_A cannot be obtained by direct fitting of Eq. 2 or Eq. 5 to the single concentration-response data. In contrast, in Eq. 9 neither value of E'_{MAX} nor the value of EC_{50} is affected by the Hill coefficient (Figure 1B and D). The parameters E'_{MAX} and EC'_{50} can be readily obtained by fitting Eq. 8 to the single concentration-response data and relative intrinsic activities can be compared regardless of Hill coefficient value.

Limitations

The major criticism of the Hill equation is its parsimonious character. It is relatively simple and its parameters are easy to estimate. However, as a model, it is just an approximation. In an experiment, the slope of the concentration-response curve different from unity may be a result of the feedback mechanism or allosteric cooperativity. Positive feedback (e.g., a stronger signal is amplified more than a weak signal) or positive cooperativity results in steep concentration-response curves. Negative feedback (e.g., a stronger signal is amplified less than a weak signal) or negative cooperativity results in flat concentration-response curves. As a simple example, in the case of equipotent homotropic cooperativity between two sites, the binding of an agonist A to each site is given by Eq. 10.

$$Binding = \frac{[A]}{[A]+K_A\frac{[A]+K_A}{\alpha[A]+K_A}} \qquad \text{Eq. 10}$$

Where α is the factor of binding cooperativity. Values of α greater than 1 denote positive cooperativity. Values of α smaller than 1 denote negative cooperativity. As can be seen in Figure 3, positive cooperativity results in a steep curve and a decrease in the apparent value of K_A. On the other hand, negative cooperativity results in a flat curve and an increase in the apparent value of K_A.

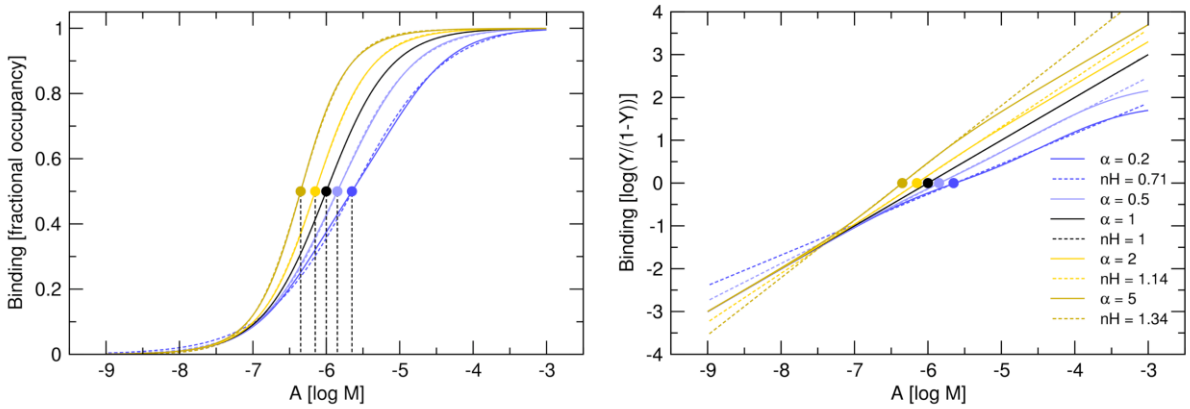

Figure 3 Homotropic cooperativity binding
Full lines, binding of an agonist to two sites with the same $K_A = 10^{-6}$ M exerting binding cooperativity α indicated in the legend, according to Eq. 10. Dashed lines, Hill equation (Eq. 8) fitted to the binding data. Estimates of Hill coefficients are indicated in the legends. Binding is expressed as fractional binding (left) and after logarithmic transformation (right).

For equipotent homotropic cooperativity, the value of apparent K_A (K'_A) is given by Eq. 11.

$$K'_A = \frac{K_A}{\sqrt{\alpha}} \qquad \text{Eq. 11}$$

As can be seen in Figure 3, fitting the Hill equation (Eq. 8) (dashed lines) is just an approximation. The differences are more overt after transformation (right). While the transformation of the Hill equation results in straight lines, the transformation of binding curves results in curves. In the case of a linear relationship between an agonist binding and functional response, the concentration-response curve has the same shape as the binding curve. Inflexion points (EC_{50} and K'_A values) and horizontal asymptotes (E'_{MAX} values) are the same. Thus, fitting the Hill equation to the concentration-response data gives a good estimate of K'_A and E'_{MAX} values that are usable in the fitting of the operational model of allosterically modulated agonism (OMAMA), using metanalysis of relationship between K'_A and E'_{MAX} values[8].

In contrast, the slope factor of Eq. 5 affects both E'_{MAX} and K'_A values (Figure 1). Moreover, the K'_A value of the steeper curve is greater than K'_A value of the flatter one. This is opposite to relationships in cooperative allosteric effects.

Conclusions

Analysis of the OMA with slope factor by Black et al. (Eq. 5) have shown that i) The slope factor **n** has a bidirectional effect on the relationship between the parameters E'_{MAX} and τ. ii) The slope factor **n** affects the relationship between the parameters EC_{50} and K_A that may result

in non-realistic $EC_{50}>K_A$ under certain conditions. Eq. 5 renders comparison of the relative intrinsic activities unusable for evaluation of signalling bias. Analysis of the OMA with Hill coefficient (Eq. 9) has shown that the Hill coefficient does not affect the relationship between the parameters E'_{MAX} and τ neither between the parameters EC_{50} and K_A. Fitting Hill equation (Eq. 8) to the concentration-response data gives good estimates of K'_A and E'_{MAX} values, which are suitable for further analysis of OMA, OMAMA and signalling bias.

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