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Have many estimates of efficacy and affinity been misled? Revisiting the operational model of agonism

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The operational model of agonism offers a general equation to account for steep or flat functional curves by including a slope parameter different from 1. However, because this equation is not a Hill equation, those steep or flat experimental curves that follow the Hill model are excluded from the operational framework. This conceptual omission could have significant consequences in the estimation of affinity and efficacy – the operational model tends to overestimate agonist–receptor dissociation constants and operational efficacy parameters to accommodate the shape of theoretical curves to steep or flat experimental Hill curves. To avoid misled parameter estimates for an ample space of pharmacological data a new version of the operational model has been developed.

Introduction

The operational model of agonism [1] is based on a two-step process that follows the following premises. First, the initial step in the production of an effect by a ligand is a bimolecular ligand–receptor binding interaction that obeys the law of mass action. Second, if the agonist concentration–effect ($E/[A]$) curve follows a rectangular hyperbolic function then the relationship between E and the concentration of agonist–receptor complex ($E/[AR]$) is also rectangular hyperbolic. A rectangular hyperbola is equivalent to a Hill equation (Eqn 1) with a Hill coefficient of 1.

$$E([A]) = \frac{a[A]^n}{b^n + [A]^n} \quad (1)$$

where a is the asymptotic maximum E value (Top); n the Hill coefficient; and b the value of $[A]$ for half-maximum E ($[A_{50}]$). This function yields symmetric sigmoid curves when agonist concentration is expressed in a logarithmic (base 10) scale with a slope of $0.576{\cdot}n$ at the midpoint when they are normalised (divided by Top) [2–4]. We will use the term Gradient (G) for these normalised slopes. Eqn 2–4 explicitly express the geometric determinants of Eqn 1.

$$\mathrm{Top} = \lim_{[A]\to\infty} E = a \quad (2)$$

$$[A_{50}] = [A] \quad \text{for} \quad (E = \mathrm{Top}/2) = b \quad (3)$$

$$G = \frac{\left(\frac{\mathrm{d}E}{\mathrm{d}x}\right)_{x=x_{50}}}{\mathrm{Top}} = \frac{n{\cdot}\ln 10}{4} = 0.576{\cdot}n; \quad \text{with } x = \log([A]) \text{ and } x_{50} = \log([A_{50}]) \quad (4)$$

Because there are experimental $E/[A]$ curves that need to be described by mathematical relationships other than a rectangular hyperbola, Black and Leff [1] proposed a variation of the earlier rectangular hyperbolic relationship between receptor occupation and response. The new proposal enabled a mathematical model that can describe experimental $E/[A]$ curves steeper or flatter than a rectangular hyperbola. However, when following this proposal the resulting $E/[A]$ curves are asymmetric. Thus, there is a need to expand the operational model of agonism to encompass those curves that follow symmetric Hill equations with a Hill coefficient different from 1. This constitutes the aim of the present article. With this in mind, we will first revisit the principles on which the operational model of agonism is based.

The operational model of agonism

The operational model of agonism [1] assumes that the signal transduction process consists of

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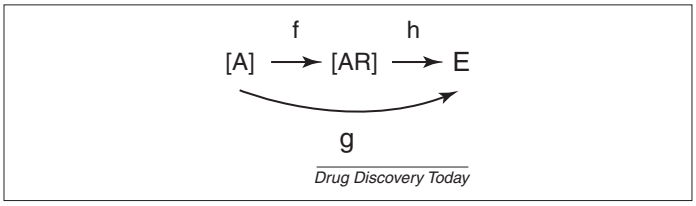

FIGURE 1

The pharmacologic effect (*E*) and the concentrations of agonist ([*A*]) and agonist–receptor complex ([*AR*]) are connected by three functions.

two steps. First, the binding of the agonist (*A*) to the receptor (*R*) to form the agonist–receptor complex (*AR*); and, second, the transduction of receptor occupancy into effect (*E*) (Fig. 1).

Three functions govern the process (with only two being independent): *f*, for ligand–receptor binding; *h*, for the transduction of receptor occupancy into effect; and *g*, for representing *E*/[*A*] curve data. *f* displays a rectangular hyperbola if we assume that the binding equilibrium follows a bimolecular reversible reaction that obeys the law of mass action [1]. That is to say: if $A + R \leftrightarrow AR$, $K_A = ([A][R])/[AR]$, and $[R_0] = [R] + [AR]$, with $[R_0]$ being the total receptor concentration then:

$$[AR] = f([A]) = \frac{[R_0][A]}{K_A + [A]} \quad (5)$$

which is a Hill equation with *n* equal to 1.

The definition of *f* is fixed because of the postulated mechanistic conditions. By contrast, different expressions for *h* and *g* can be obtained depending on the empirical assumptions made. The following cases have been considered.

Case 1: Hill E/[A] *curves with* n = *1*

If *g* is a Hill equation with *n* = 1

$$E = g([A]) = \frac{a'[A]}{b' + [A]} \quad (6)$$

Then as stated in [1] *h* is also a rectangular hyperbola (Eqn 8) as can be shown by substituting Eqn 7 into Eqn 6.

$$[A] = f^{-1}([AR]) = \frac{[AR]K_A}{[R_0] - [AR]} \quad (7)$$

$$E = h([AR]) = \frac{\frac{a'K_A}{K_A - b'}[AR]}{\frac{b'[R_0]}{K_A - b'} + [AR]} = \frac{a''[AR]}{b'' + [AR]} \quad (8)$$

Black and Leff [1] defined a'' as E_m and b'' as K_E and then:

$$E = h([AR]) = \frac{E_m[AR]}{K_E + [AR]} \quad (9)$$

By substituting Eqn 5 into Eqn 9 the *g* function for *E*/[*A*] curve data is obtained (Eqn 10), which is a Hill equation in agreement with the assumption made in Eqn 6.

$$E = g([A]) = \frac{\frac{E_m\tau}{1+\tau}[A]}{\frac{K_A}{1+\tau} + [A]} \quad (10)$$

where $\tau = [R_0]/K_E$ is the operational efficacy. The meaning of τ as efficacy can be seen by calculating two parameters: Top and $[A_{50}]$, as defined in Eqn 2 and 3, respectively.

$$\text{Top} = \lim_{[A]\to\infty} E = \frac{E_m\tau}{1+\tau} \quad (11)$$

$$[A_{50}] = [A] \quad \text{for} \quad (E = \text{Top}/2) = \frac{K_A}{1+\tau} \quad (12)$$

High values of τ led to two pharmacologic results: (i) Top values close to E_m and (ii) $[A_{50}]$ values much smaller than K_A. These conditions are typical of full agonism. Also because *E*/[*A*] is a rectangular hyperbola the gradient of *E* in a semilogarithmic scale is:

$$G = \frac{\left(\frac{dE}{dx}\right)_{x=x_{50}}}{\text{Top}} = \frac{\ln 10}{4} = 0.576 \quad (13)$$

Case 2: steep or flat non-Hill E/[A] *curves*

To account for experimental *E*/[*A*] curves steeper or flatter than the rectangular hyperbola, Black and Leff [1] proposed a Hill equation with $n \neq 1$ for the *h* function (Eqn 14).

$$E = h([AR]) = \frac{E_m[AR]^n}{K_E^n + [AR]^n} \quad (14)$$

It is worth noting that the proposal of Eqn 14 for the *h* function is an arbitrary decision, which was made to generate steep or flat $E = g([A])$ curves. However, the choice of the shape (symmetric) of the *h* function determines the shape (asymmetric) of the *g* function.

The substitution of Eqn 5 into Eqn 14 leads to the *g* function, being represented by Eqn 15, which is not a Hill equation.

$$E = g([A]) = \frac{E_m[A]^n}{\left(\frac{K_A + [A]}{\tau}\right)^n + [A]^n} \quad (15)$$

In this case, the geometric Top, $[A_{50}]$ and *G* parameters are [1,5]:

$$\text{Top} = \frac{E_m\tau^n}{1+\tau^n} \quad (16)$$

$$[A_{50}] = \frac{K_A}{(2+\tau^n)^{1/n} - 1} \quad (17)$$

$$G = \frac{0.576 \cdot n(2+\tau^n)((2+\tau^n)^{1/n} - 1)}{(2+\tau^n)^{1/n}(1+\tau^n)} \quad (18)$$

As noted by the authors of the operational model of agonism [1], Top and $[A_{50}]$ depend on *n* in addition to τ and E_m, in the case of Top, and to τ and K_A, in the case of $[A_{50}]$. However, as also noted [1], for a particular system in which *n* is fixed, τ conserves its definition as operational efficacy. Comparison between Eqn 18 and 4 shows the differences in the Gradient between the operational model and the Hill equation. Importantly, the Gradient of the operational model tends towards that of the Hill equation for large τ values (full agonism) [5]. Finally, it can be seen that consistency is preserved between Cases 1 and 2 because for *n* = 1 all equations developed for Case 2 coincide with the corresponding ones for Case 1.

Thus, the question arises of how to obtain an operational model of agonism representing experimental steep or flat Hill *E*/[*A*] curves and identifying the corresponding *h* function translating receptor occupancy into effect.

Case 3: steep or flat Hill E/[A] *curves*

Let us propose Eqn 19 as a Hill equation for *E*/[*A*] curves.

$$E = g([A]) = \frac{a'[A]^n}{b'^n + [A]^n} \quad (19)$$

To put Eqn 19 in terms of [*AR*] we will make use of Eqn 7:

$$E = h([AR]) = \frac{a'K_A^n[AR]^n}{b'^n([R_0] - [AR])^n + K_A^n[AR]^n} \quad (20)$$

which is not a Hill equation, in contrast to Eqn 14.

To achieve parameters that fit within the context of the operational model we must provide, in analogy with Case 1 (see Eqn 10), expressions of $(E_m\tau/(1+\tau))$ and $(K_A/(1+\tau))$, for a' and b', respectively, which make Eqn 19 equal to Eqn 10 when *n* = 1. Different possibilities appear because there is not a single combination of parameter values. Thus, in the same way as in the operational model of agonism it appeared a conceptual arbitrariness in the choice of a Hill equation for the transducer $E = h([AR])$ function, here an arbitrariness appears for the operational expressions of the Hill parameters in the $E = g([A])$ function. The solution that most resembles the Hill equation