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The Hill equation and the origin of quantitative pharmacology

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Abstract This review addresses the 100-year-old Hill equation (published in January 22, 1910), the first formula relating the result of a reversible association (e.g., concentration of a complex, magnitude of an effect) to the variable concentration of one of the associating substances (the other being present in a constant and relatively low concentration). In addition, the Hill equation was the first (and is the simplest) quantitative receptor model in pharmacology. Although the Hill equation is an empirical receptor model (its parameters have only physico-chemical meaning for a simple ligand binding reaction), it requires only minor a priori knowledge about the mechanism of action for the investigated agonist to reliably fit concentration-response curve data and to yield useful results (in contrast to most of the advanced receptor models). Thus, the Hill equation has remained an important tool for physiological and pharmacological investigations including drug discovery, moreover it serves as a theoretical basis for the development of new pharmacological models.

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1 Introduction

> As to methods there may be a million and then some, but principles are few. The man who grasps principles can successfully select his own methods. The man who tries methods, ignoring principles, is sure to have trouble.
>
> Ralph Waldo Emerson (1803–1882)

This review addresses the Hill equation, published first in its presently known form on January 22 1910. This equation possesses three of the most important features of scientific models, i.e.,: it offers a didactical and mnemotechnic aid for understanding and memorizing knowledge and/or information; it can be used to resolve practical problems; and it can serve as a starting point for the development of more detailed and particular models. The Hill equation is the first milestone in quantitative pharmacology, being the first formula that relates a consequence of a reversible association (as an effect) to the variable concentration of one of two associating substances, as long as the other substance is present in a constant and relatively low concentration. Therefore, the Hill equation was the first exact (quantitative) receptor model in pharmacology. Beyond its historical role, the Hill model has theoretical and practical significance even today. In this paper we will trace how the Hill equation was first introduced, how it was modified and how it is currently used within pharmacology. The more than 100 year history of this equation is a good illustration of a rather common feature of theoretical models; the fact that they undergo multiple transformations in light of expanding knowledge of the underlying phenomenon. These transformations are generally of two kinds (1) a refinement of the original model/equation and (2) a more precise understanding of its applicability in specific situations. The history of the Hill equation shows both of those trends and is therefore a good case study for understanding the development of quantitative models in the life sciences.

2 Historical background

The history of pharmacology, as a separate scientific discipline, goes back to the mid- and late 19th century, to the works of Rudolf Buchheim (1820–1879) and his student Oswald Schmiedeberg (1838–1921), generally recognized as the founders of modern pharmacology (Scheindlin 2001; Rang 2006). Similarly to the evolution of physiology from anatomy, pharmacology emancipated from physiology when it developed its own methods to examine the living organism. Although the subject of investigation is nearly the same for physiology and pharmacology, they are guided by different principles (leitmotifs), as they address different questions: while physiology asks: *how*, pharmacology questions: *how to influence with chemical agents* (Table 1)?

In the late 19th century, inspired by the spectacular advances in chemistry and physics, some scientists turned to apply mathematical approaches to phenomena in the life sciences. In physiology, Carl Ludwig (1816–1895) and his followers, the "organic physicists," rejected the concepts of vitalism, and wanted to describe living nature using mathematical formalism (Zimmer 1996). The mathematical approach in pharmacology (more precisely: in a topic that is thought to be pharmacological nowadays)

Table 1 Principles and leitmotifs of some disciplines in life sciences

Discipline	Principle
Anatomy	The body is a machine (or like a machine, this question is of course out of the scope of anatomy)
Physiology	Every function of a living organism aims the maintenance of an equilibrium (complex of equilibria is homeostasis)
Biochemistry	The same laws apply to molecules in inanimate nature as those in living organisms (metabolism is based on chemical reactions)
Pharmacology	Functions of a living organism can be affected by certain exogenous agents (called pharmacons if the influence is beneficial), with special regard to those that can evoke or inhibit effects of endogenous regulatory molecules)
Quantitative pharmacology	Interaction between a pharmacon and a living organism can be described in a mathematical manner

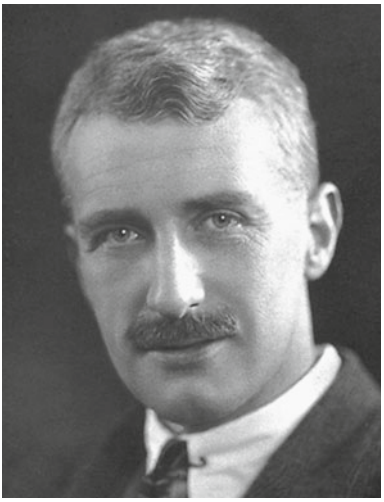

Fig. 1 Archibald Vivian Hill, British physiologist and pharmacologist (1886–1977)

only emerged at the beginning of the 20th century. This breakthrough occurred in the context of the receptor theory, a hypothesis that had emerged just before the birth of quantitative pharmacology.

The suggestion that both exogenous and endogenous agents must be specifically bound to structures inherent to the living organisms before exerting an effect was first proposed by John Newport Langley (1852–1925) in 1878, based on his experiments on salivary secretion in the dog (Maehle 2004). Paul Ehrlich (1854–1915) developed a similar hypothesis starting from the results of his bacteriological investigations in 1897 (Maehle 2009). They refined these ideas in close competition, which is also reflected in the terms they used to describe the specific chemical structure on the cell receiving the agent evoking a cellular response: "side chain" (Ehrlich in 1897), "receptor" (Ehrlich in 1900; the term proved to be enduring), and "receptive substance" (Langley in 1905) (Maehle 2004, 2009). The receptor concept can be summarized by the classic maxim of Ehrlich: "Corpora non agunt nisi fixata" (agents do not act unless they are bound) (Pelner 1972).

At the beginning of the 20th century, Archibald Vivian Hill (1886–1977; Fig. 1) was the first who quantified drug induced biological responses by introducing an equation (Hill 1910) that has since been named after him. This relationship was then incorporated by Alfred Joseph Clark (1885–1941) into the first extensive model of receptor function (Kenakin 2004; Colquhoun 2006; Rang 2006).

3 The Hill equation described for ligand binding (Hill–Langmuir equation)

Although the relationship, revealed by the Hill equation, is already implicit in Hill's first paper dealing with actions of nicotine and curare (or curari) on skeletal muscle (Hill 1909), the equation, in the form known today, was first introduced in his second publication describing the interaction of hemoglobin with oxygen (Hill 1910). In the form it later became widely accepted, Hill's formulation is a good approximation for every specific and saturable binding. In this context the term "specific," in its broadest sense, means that binding occurs between those particular substances that are of interest for the examiner. In a narrower sense, a binding is specific when there is a considerable affinity between the particular substances relative to the single binding ability of both of these substances to other materials. In turn, the "saturability" stems from the fact that one of the particular substances is present in a significantly lower amount than the other(s). Thus, above a certain level, increasing concentrations of this/these later can only slightly increase the amount of resulting complexes. This formal relationship between binding substances was also published, independently from Hill, a few years later by Irving Langmuir in relation to the adsorption of gases on plain surfaces (Langmuir 1918), therefore the Eq. 2 expressing this link (see below) is referred to as Hill, Langmuir, Langmuir–Hill, and Hill–Langmuir equation, respectively. In a recent proposal, the relationship between concentration and effect has been defined as "Hill equation," while the term "Hill–Langmuir equation" has been suggested for quantifying the ligand–receptor interaction (Neubig et al. 2003). For the sake of simplicity, we will use the term "Hill equation" throughout this paper.

The Hill equation can be derived from the law of mass action (more precisely: from the mass action model for equilibrium):

$$nL + R \underset{k_2}{\overset{k_1}{\rightleftarrows}} L_n R \tag{1}$$

$$[L_n R] = [R_0] \cdot \frac{[L]^n}{[L]^n + K_\mathrm{d}} = [R_0] \cdot \frac{[L]^n}{[L]^n + (K_\mathrm{A})^n} \tag{2}$$

where L—the ligand that can be present in variable concentration; R—the receptor, amount of which is constant and is significantly exceeded by the amount of the ligand; $[L_n R]$—the concentration of the ligand–receptor complex; $[R_0]$—the total receptor concentration (receptor number); $[L]$—the concentration of the free ligand (in practice, however, it is treated as the total ligand concentration); k_1 and k_2—the rate constants of association and dissociation, respectively; $K_\mathrm{d} = k_2/k_1$—the equilibrium dissociation

constant of the ligand–receptor complex; K_A—the ligand concentration, at which half the receptors are ligand-bound (if $n = 1$, it equals the K_d); n—originally, the number of binding sites for the given ligand in one receptor (as indicated in the Eq. 1), but its interpretation is more complex than it would seem at first glance (it is commonly referred to as the Hill coefficient or Hill slope factor, see below). K_A serves as a measure for affinity of the ligand to the receptor: the smaller the K_A, the greater the affinity is. The $[L]^n/([L]^n + (K_A)^n)$ quotient (on the right side of the Eq. 2) is referred to as fractional receptor occupancy.

There are some assumptions concerning Eq. 2. As mentioned above, an absolute requirement is that the binding reaction reaches equilibrium, otherwise the rate of association and dissociation affects the result (Kenakin 2009). Furthermore, the free ligand concentration is assumed not to differ significantly from the total ligand concentration (this is fulfilled if total ligand concentration significantly exceeds the total receptor concentration, which is common in biological systems). In addition, it is important to point out that the reaction described by the Eq. 1 requires that, during ligand binding, all binding sites of a receptor should be simultaneously occupied; in other words, only the free L, the free R, and the fully bound L_nR forms are permitted. So, if incompletely ligand-bound receptors accumulate to a significant degree, Eq. 2 fails to provide proper (=physico-chemically correct) equilibrium concentrations of the permitted forms and/or constants characterizing the interaction (Weiss 1997). Consistently, although the Hill coefficient stems from the equation of the binding reaction (Eq. 1), it has been observed for a long time that the Hill equation only yields an accurate estimate of the binding sites in one receptor, if there is excessive positive cooperativity among these binding sites. This is due to the fact that an increasing affinity of the receptor after the binding of the first ligand accelerates the binding of the further ligands and thereby reduces the amount of incompletely ligand-bound receptors (Weiss 1997). If a receptor has more than one binding sites and there is no extreme positive cooperativity among them, the Hill coefficient underestimates the number of binding sites and, in parallel, it overestimates the K_d (or K_A). So, when fitting the Hill equation to the data of a binding reaction, the Hill coefficient should be considered as a compound measure of molecularity and cooperativity. Furthermore, if the Hill coefficient significantly differs from unity, K_A values should be treated with caution (Weiss 1997; Goutelle et al. 2008). In conclusion, because of the complex nature of the Hill coefficient, if its value is other than unity, fitted parameters provided by the Hill equation for ligand binding cannot considered to be physico-chemically meaningful without reservation. This fact that adds an empirical dimension to the use of this equation.

4 The receptor theory and the Hill equation

The receptor theory is widely thought to be the concept most characteristic for pharmacology (Kenakin 2004; Colquhoun 2006; Rang 2006; Maehle 2009). Following preliminary suggestions by Langley and Ehrlich, Joseph Clark established the first comprehensive receptor theory, the so-called occupation (or occupancy, or traditional receptor-stimulus) theory (Clark 1926). Clark incorporated the Hill equation into the

occupation theory (referred to in this context as Langmuir's adsorption isotherm). As a result of Clark's theoretical work, the Hill equation became the first quantitative receptor model linking concentration of an agonist (a ligand capable of exerting a cellular response) to the effect evoked.

In the context of the occupation theory, the Hill equation was used to evaluate concentration-response (E/c) curves in a form, in which $[L_n R]$ and $[R_0]$ were replaced by the effect of the agonist concentration and by the maximal effect achievable with the given agonist in the given system, respectively. However, as it turned out, features of a ligand–receptor binding cannot be simply extrapolated to the agonist-effect relationship, i.e., the whole process of the receptor function. As a consequence, the early version of the occupation theory often conflicted with experimental observations.

To avoid these conflicts, an advanced form of the occupation theory was developed assuming that receptor function consists of two major components: signal-recognition (via formation of an agonist–receptor complex serving as a stimulus for the subsequent structures), and signal-transduction (by means of changes produced by the stimulus) (Ruffolo 1982; Black and Leff 1983; Kenakin 2004). The Hill equation (more precisely, Eq. 2 with $n = 1$) was used to describe the stimulus; thereby this part remained the exact (parametric) element of the modified occupation theory. To describe the signal-transduction, a nonparametric function was introduced that related the stimulus to the biological response (Stephenson 1956). Because this nonparametric function could hardly be determined, the major use of this semiparametric occupation theory was to study comparative assays, in which the mutual signal-transduction characterized by the nonparametric function can be canceled out by generating ratios. This procedure forms the basis for the so-called null method in pharmacology (Ruffolo 1982; Black and Leff 1983).

To overcome the limitations of the occupation theory, attempts were made to develop parametric receptor models more sophisticated than the Hill equation. The first of these was the operational model of agonism that was derived from the following general reaction equation (Black and Leff 1983):

$$mA + mR + \varepsilon \underset{k_2}{\overset{k_1}{\rightleftarrows}} mAR + \varepsilon \underset{k_2'}{\overset{k_1'}{\rightleftarrows}} (AR)_m\varepsilon \tag{3}$$

where A—the agonist; R—the corresponding receptor; ε—the effector having low affinity for the free receptor but high affinity for the agonist–receptor complex (ε represents all postreceptorial elements playing a role in the generation of an effect); m—the number of the agonist–receptor complexes that is necessary to bind to the effector to evoke an effect (the so-called operational slope factor, see Eqs. 5 and 6 below); k_1 and k_2—the rate constants of association and dissociation for the agonist–receptor complex, respectively; k_1' and k_2'—the rate constants of association and dissociation for the agonist–receptor–effector complex, respectively; AR—the agonist–receptor complex; $(AR)_m\varepsilon$—the agonist–receptor–effector complex.

It should be noted that equations containing the equilibrium concentrations for the first and the second part of the Eq. 3 are none other than the Hill equation described for

ligand binding and an analogue of the Hill equation described for signal-transduction, respectively:

$$[AR] = [R_0] \cdot \frac{[A]}{[A] + K_{\mathrm{A}}} \tag{4}$$

$$E(\sim [(AR)_m \varepsilon]) = E_m \cdot \frac{[AR]^m}{[AR]^m + (K_E)^m} \tag{5}$$

Substituting Eq. 4 into the Eq. 5 and then rearranging it slightly yields the following equation:

$$E = E_m \cdot \frac{([A] \cdot [R_0])^m}{(K_{\mathrm{E}} \cdot (K_{\mathrm{A}} + [A]))^m + ([R_0] \cdot [A])^m} \tag{6}$$

where $[A]$—the agonist concentration; $[R_0]$—the total receptor concentration; $[AR]$—the concentration of the agonist–receptor complex; $[(AR)_m \varepsilon]$—the concentration of the agonist–receptor–effector complex (assumed directly proportional to the effect); K_{A}—the agonist concentration producing half-maximal receptor occupancy; E_m—the maximal effect achievable by stimulating the given receptor in the given system; K_{E}—the agonist–receptor complex concentration producing half-maximal E_m; E—the effect elicited by the agonist at $[A]$.

In Eqs. 5 and 6, the m operational slope factor characterizes the signal-transduction as a whole rather than purely the molecularity of this process (similarly to the Hill coefficient concerning the molecularity of the binding reaction). Because of this general approach, the operational model preserves the comprehensive nature of the earlier receptor models (Black and Leff 1983).

Several important methods in pharmacology are based on the occupation theory (e.g., Furchgott's method to determine K_{A} as a measure of affinity: Furchgott and Bursztyn 1967) as well as the operational model (to assess K_{A} and parameters characterizing efficacy of the agonist and effectiveness of the receptor-holding system, e.g.,: Van der Graaf and Danhof 1997). Since the operational model contains both physicochemically meaningful (K_{A}) and arbitrary (m, K_{E}, E_{m}) constants, some authors treat it as a hybrid model that possesses features of both the so-called mechanistic (mechanism-based) and the empirical (phenomenological) models (Giraldo et al. 2002).

However, these models of agonist action have been challenged on theoretical grounds (Colquhoun 1998, 2006). The essence of this objection is that the fundamental reaction equation of these models (e.g., the Eq. 3 in case of the operational model) does not contain the following step already described in 1957 (Del Castillo and Katz 1957):

$$AR \underset{k_2''}{\overset{k_1''}{\rightleftarrows}} AR^* \tag{7}$$

where: AR and AR^*—the inactive and active agonist–receptor complex (before and after a conformational change that enables the receptor to generate an effect),

respectively; k_1'' and k_2''—the rate constants of the conformational change activating and inactivating the receptor, respectively.

It should be noted that the above-mentioned objection does not apply to the use of the Hill equation per se, because the Hill model does not divide the signal-transduction process, i.e., it does not provide separate constants for the different parts of the signaling.

Subsequently, more recent receptor models have been developed on the basis of particular rather than general reaction equations. These mechanism-based models are far more specific for a given sort of receptors, e.g., ternary complex models for G-protein coupled receptors (Giraldo et al. 2002; Kenakin 2004). Despite being more accurate in describing particular processes and molecular interactions, these specific models are not the most suitable approaches for some practical purposes (e.g., curve fitting) due to their high complexity. Thus, the simpler models did not lose their significance in current pharmacology.

5 The Hill equation described for E/c curves

The Hill equation, described for E/c (agonist concentration) curves, is generally the first choice to phenomenologically characterize the link between agonist concentration and effect. The most frequent way to do this is fitting the Hill equation to E/c curve data using non-linear regression (Motulsky and Christopoulos 2003).

In agreement with the considerations discussed above, when fitting the Hill equation to E/c curves representing both the "cognitive" and the "transducing" functions of the receptors, the provided parameters should be treated as best-fit values that geometrically describe the curve. A classic form of the Hill equation defined for E/c curves is:

$$E = E_{\max} \cdot \frac{c^n}{c^n + (EC_{50})^n} \tag{8}$$

where c—the concentration of an agonist; E—the effect elicited by the agonist at c; E_{max}—the maximal effect that can be evoked by the given agonist in the given system (geometrically: upper asymptote); EC_{50}—the concentration producing half-maximum effect (geometrically: midpoint location); n—the Hill coefficient (geometrically: a factor characterizing the slope of the E/c curve at the midpoint).

In this three-parametric (E_{max}, EC_{50}, n) form of the Hill equation, the lower asymptote is implicitly zero and therefore is not present in the equation. For simplicity, this form is presented throughout this review. If the system has a basal activity ($E \neq 0$ at $c = 0$), the four-parametric form (containing a lower asymptote) can be used to determine precisely the baseline effect. On the other hand, subtraction of the effect measured at $c = 0$ from every particular effect value enables the proper fitting of the three-parametric Hill equation as well (Motulsky and Christopoulos 2003).

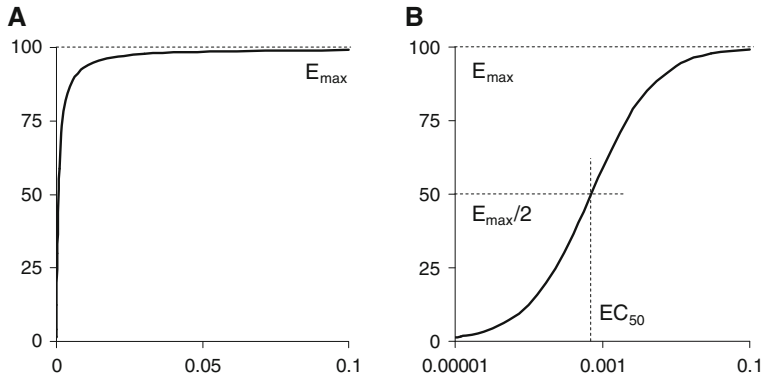

Fig. 2 The Hill equation in linear and semilogarithmic plotting. Parameters for the plotted Hill equation were chosen to represent a typical E/c curve ($E_{max} = 100\%$; $EC_{50} = 700\mu M$; $n = 1$). The x axis shows the molar concentration (**A** using a linear scale; **B** using a logarithmic scale), while the y axis denotes the effect in percentage of its maximal value. For this function (and for typical E/c curves), the midpoint of the curve is the same as the point of symmetry (the so-called point of inflection) in the case of semilogarithmic plotting (**B**)

6 Curve fitting with use of the Hill equation

When analyzing ligand binding or E/c curves, Eqs. 2 or 8 can be fitted to the curve data, respectively. This procedure will supply regression parameters (best-fit values) that define the shape and location (along the x axis) for the curve. When plotting the concentration on a linear scale, the fitted Hill equation will generally follow a hyperboloid shape (Fig.2a). Geometrically, if $n > 1$, the fitted function will be sigmoid, but this is only conspicuous at very great Hill coefficients. At the same time, when plotting the logarithm of the concentration (or, equivalently, depicting the concentration on a logarithmic scale), the fitted Hill equation will follow a sigmoid shape at any value of n (Fig.2b) (Motulsky and Christopoulos 2003; Goutelle et al. 2008).

In order to get symmetrical confidence intervals and correct standard errors for the obtained parameters (that allow proper comparison between different ligand binding or E/c curves), it is recommended to plot the $[L_nR]$ or E versus the logarithm of ligand concentration, and to fit a rearranged form of the Hill equation, in which the agonist concentration and the concentration-like constant (K_A or EC_{50}) are expressed as logarithm (Motulsky and Christopoulos 2003):

$$[L_nR] = \frac{[R_0]}{1 + 10^{n\cdot(\log K_A - \log c)}} \tag{9}$$

$$E = \frac{E_{max}}{1 + 10^{n\cdot(\log EC_{50} - \log c)}} \tag{10}$$

The reason for this is the fact that distribution of concentration-like constants is asymmetric, but the logarithm of these constants follows a symmetric (Gaussian) distribution (Motulsky and Christopoulos 2003).

7 Features of the Hill equation

The only formal difference between the Hill equation and the Michaelis–Menten equation published three years later (Michaelis and Menten 1913) is the Hill coefficient, i.e., the variable nature of the slope factor in the Hill equation. This suggests that the equation of Leonor Michaelis (1875–1949) and Maud Leonora Menten (1879–1960), relating velocity of an enzyme mediated reaction to the substrate concentration, is equivalent with the Hill equation if $n = 1$. Indeed, both ligand–receptor binding and substrate-enzyme association are specific, reversible and saturable. However, the equation of an enzymatic reaction is more complex than the Eq. 1, so the Michaelis-Menten constant (K_M) is only apparently equivalent with K_d in the Eq. 2, as K_M depends on the rate constant of product formation in addition to affinity of the substrate to the enzyme.

An important feature of the Hill equation is that it exclusively determines symmetric functions, for which the midpoint and the point of inflection is the same (Giraldo et al. 2002) (when plotting semilogarithmically (see Fig.2b), point of inflection of an E/c curve is the point having the greatest steepness, while the midpoint is associated with half the maximal effect). Accordingly, use of the Hill model to evaluate markedly asymmetric E/c curves may lead to incorrect estimates (Van der Graaf and Schoemaker 1999). To resolve this problem, it is worth comparing the fit of a model defining symmetric functions with the fit of a corresponding model accounting for asymmetry (such a counterpart of the Hill equation is the Richards equation: Richards 1959). The simplest way for model comparison is to perform an F test. If the asymmetric model fits the E/c data better, that should be used (Giraldo et al. 2002; Motulsky and Christopoulos 2003).

Today, the Hill equation is classified as an empirical model (for E/c curves), similarly to several other relatively simple equations (such as the so-called modified Hill equation, Gompertz equation, Hodgkin equation, Douglas equation and Richards equation). Parameters yielded by empirical models may lack physico-chemical meaning, and in this case they only geometrically characterize the E/c curve (Giraldo et al. 2002; Keller et al. 2002). In contrast, the mechanistic models contain physico-chemical constants allowing deeper insight to the signal amplification cascade and more proper extrapolation from a test system (investigated experimentally) to the target system (set up to be modeled by the test system) (Mager et al. 2003; Danhof et al. 2007). To offer these benefits, however, mechanism-based models require detailed a priori knowledge about particular mechanisms of the signal-transduction (Kenakin 2006). In addition, too many parameters in the equation to be fitted can yield highly correlated estimates with large standard errors (Van der Graaf and Stam 1999; Motulsky and Christopoulos 2003). These considerations indicate that, under certain circumstances, it is reasonable to use empirical models, especially the prototype Hill equation, which is simple, flexible, and requires only minor previous knowledge about the properties of the agonist and the pharmacological system investigated. Therefore, when screening the biological activity of pharmacological agents, the first step is to take E/c curves and then to fit them to the Hill equation (Kenakin 2006).

8 Conclusions

The Hill equation was the first quantitative (parametric) model that related the response of a system to the concentration of a pharmacological agonist. Subsequently developed receptor models generally incorporated the Hill equation, typically in more and more complex formulae. Further research revealed that the parameters of the Hill equation (or its simple derivatives) only have a precise physico-chemical meaning when applied to ligand binding reactions (especially when the Hill coefficient is unity). However, in contrast to most of advanced models developed since, the Hill equation requires only minor a priori knowledge or assumptions (e.g., the symmetry of the E/c relationship) about the mechanism of action for the investigated agonist in the given system to reliably fit the E/c data and to provide useful results. In summary, simplicity, flexibility and reliability of the Hill equation make it an essential tool for the first step of E/c data analysis in physiological and pharmacological investigations, furthermore a theoretical starting point for the development of new pharmacological models.

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References

Black, J.W., and P. Leff. 1983. Operational models of pharmacological agonism. *Proceedings of the Royal Society B: Biological Sciences* 220: 141–162.

Clark, A.J. 1926. The antagonism of acetylcholine by atropine. *Journal of Physiology (London)* 61: 547–556.

Colquhoun, D. 1998. Binding, gating, affinity and efficacy: The interpretation of structure-activity relationships for agonists and of the effects of mutating receptors. *British Journal of Pharmacology* 125: 924–947.

Colquhoun, D. 2006. The quantitative analysis of drug-receptor interactions: A short history. *Trends in Pharmacological Sciences* 27: 149–157.

Danhof, M., J. de Jongh, E.C. De Lange, O. Della Pasqua, B.A. Ploeger, and R.A. Voskuyl. 2007. Mechanism-based pharmacokinetic-pharmacodynamic modeling: Biophase distribution, receptor theory, and dynamical systems analysis. *Annual Review of Pharmacology and Toxicology* 47: 357–400.

Del Castillo, J., and B. Katz. 1957. Interaction at end-plate receptors between different choline derivatives. *Proceedings of the Royal Society B: Biological Sciences* 146: 369–381.

Furchgott, R.F., and P. Bursztyn. 1967. Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors. *Annals of the New York Academy of Sciences* 144: 882–899.

Giraldo, J., N.M. Vivas, E. Vila, and A. Badia. 2002. Assessing the (a)symmetry of concentration-effect curves: Empirical versus mechanistic models. *Pharmacol Ther* 95: 21–45.

Goutelle, S., M. Maurin, F. Rougier, X. Barbaut, L. Bourguignon, M., Ducher, and P. Maire. 2008. The Hill equation: A review of its capabilities in pharmacological modelling. *Fundamental and Clinical Pharmacology* 22: 633–648.

Hill, A.V. 1909. The mode of action of nicotine and curari, determined by the form of the contraction curve and the method of temperature coefficients. *Journal of Physiology (London)* 39: 361–373.

Hill, A.V. 1910. The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *Journal of Physiology (London)* 40: Proceedings iv–vii.

Keller, F., M. Giehl, D. Czock, and D. Zellner. 2002. PK-PD curve-fitting problems with the Hill equation? Try one of the 1-exp functions derived from Hodgkin, Douglas or Gompertz. *International Journal of Clinical Pharmacology and Therapeutics* 40: 23–29.

Kenakin, T. 2006. Data-driven analysis in drug discovery. *Journal of Receptors and Signal Transduction Research* 26: 299–327.

Kenakin, T. 2004. Principles: Receptor theory in pharmacology. *Trends in Pharmacological Sciences* 25: 186–192.
Kenakin, T. 2009. Quantifying biological activity in chemical terms: A pharmacology primer to describe drug effect. *ACS Chemical Biology* 4: 249–260.
Langmuir, I. 1918. The adsorption of gases on plane surfaces of glass, mica and platinum. *Journal of the American Chemical Society* 40: 1361.
Maehle, A.H. 2004. Receptive substances": John Newport Langley (1852–1925) and his path to a receptor theory of drug action. *Medical History* 48: 153–174.
Maehle, A.H. 2009. A binding question: The evolution of the receptor concept. *Endeavour* 33: 135–140.
Mager, D.E., E. Wyska, and W.J. Jusko. 2003. Diversity of mechanism-based pharmacodynamic models. *Drug Metabolism and Disposition* 31: 510–518.
Michaelis, L., and M.L. Menten. 1913. Die Kinetik der Intertinwerkung. *Biochemische Zeitschrift* 49: 333–369.
Motulsky, H.J., and A. Christopoulos. 2003. *Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting.* Oxford: Oxford Press (Corrected online version: http://www.graphpad.com/manuals/Prism4/RegressionBook.pdf).
Neubig, R.R., M. Spedding, T. Kenakin, and A. Christopoulos. 2003. International union of pharmacology committee on receptor nomenclature and drug classification. XXXVIII. update on terms and symbols in quantitative pharmacology. *Pharmacological Reviews* 55: 597–606.
Pelner, L. 1972. Corpora non agunt nisi fixata. Maxim behind all of Ehrlich's great discoveries. *New York State Journal of Medicine* 72: 620–624.
Rang, H.P. 2006. The receptor concept: Pharmacology's big idea. *British Journal of Pharmacology* 147: S9–16.
Richards, F.J. 1959. A flexible growth function for empirical use *Journal of Experimental Botany* 10: 290–300.
Ruffolo, R.R. Jr. 1982. Review important concepts of receptor theory. *Journal of Autonomic Pharmacology* 2: 277–295.
Scheindlin, S. 2001. A brief history of pharmacology. *Modern Drug Discovery* 4: 87–88.
Stephenson, R.P. 1956. A modification of receptor theory. *British Journal of Pharmacology* 11: 379–393.
Van der Graaf, P.H., and M. Danhof. 1997. Analysis of drug-receptor interactions in vivo: A new approach in pharmacokinetic-pharmacodynamic modelling. *International Journal of Clinical Pharmacology and Therapeutics* 35: 442–446.
Van der Graaf, P.H., and Schoemaker, R.C. 1999. Analysis of asymmetry of agonist concentration-effect curves. *Journal of Pharmacological and Toxicological Methods* 41: 107–115.
Van der Graaf, P.H., and W.B. Stam. 1999. Analysis of receptor inactivation experiments with the operational model of agonism yields correlated estimates of agonist affinity and efficacy. *Journal of Pharmacological and Toxicological Methods* 41: 117–125.
Weiss, J.N. 1997. The Hill equation revisited: Uses and misuses. *FASEB Journal* 11: 835–841.
Zimmer, H.G. 1996. Carl Ludwig: the man, his time, his influence. *Pflugers Archiv* 432: R9–22.