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THEORETICAL HYPERBOLIC MODEL OF A PARTIAL AGONISM: EXPLICIT FORMULAS FOR AFFINITY, EFFICACY AND AMPLIFICATION*

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Abstract. The quantitative analysis of receptor-mediated effect is based on experimental concentration-response data in which the independent variable, the concentration of a receptor ligand, is linked with a dependent variable, the biological response. The steps between the drug–receptor interaction and the subsequent biological effect are to some extent unknown.

The shape of the fitting curve of the experimental data may give some insights into the nature of the concentration–receptor–response (C-R-R) mechanism. It can be evaluated by non-linear regression analysis of the experimental data points of the independent and dependent variables, which could be considered as a history of the interaction between the drug and receptors. However, this information is not enough to evaluate such important parameters of the mechanism as the dissociation constant (affinity) and efficacy. There are two ways to provide more detailed information about the C-R-R mechanism: (i) an experimental way for obtaining data with new or

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selective or inactive compounds; and (ii) a theoretical way by making additional assumptions and experimental observations about some elements of the C-R-R mechanism.

Using the second way and basic postulates of the so-called occupation theory, a Theoretical Hyperbolic Model (THM) was developed in this study, in order to justify the nature of partial agonism in *in-vitro bioassay* studies. The model could be used for sensitive analysis of the partial agonist's behavior from the experimental dose-response data. The explicit formulas derived from the model describe the affinity and relative Stephenson's efficacy.

Moreover, THM allows estimation of the receptor reserve of the almost full agonists under the assumption presented. When the design of the *in-vitro bioassay* allows assessing the maximal possible effect of a given isolated tissue, the affinity and relative efficacy of the respective partial agonists could be calculated from the experimental dose-response data. It was proved theoretically that the partial agonists have no receptor reserve. This finding confirms experimental results for partial agonists with varying potency. The THM is used further to explain the C-R-R mechanism and to understand more deeply the character of the affinity and efficacy of the agonists by introduction of a new agonist feature called amplification and the parameter amplifier. The THM is compared with other models related with the occupation theory of agonism. The differences and limitations of the application of THM are discussed.

Introduction. In-vitro bioassays have the benefit of providing more precise, quantitative information about relative potency, receptor affinity and relative agonist efficacy within a given series of chemical compounds [16, 41, 44, 45, 67]. They are used to transform the physiological response measured in chemical quantity, which we further use to characterize the chemical structure and eventually its property to be a potential drug [60, 62]. In the case of analgesics for example, agonist efficacy as a function of the maximal response a drug may produce could not be determined by in-vivo models of nociception, because it requires noxious stimuli of damaging intensity [45]. Thus the advantages of in-vitro bioassays in isolated tissue allow appreciating the properties of a chemical compound to evoke effect on both the drug–receptor and the receptor–response part of the receptor interaction. In the case of agonist action, the compound exerts effects on both parts of this process, while the antagonist has only a binding parameter. Both of them interact with the receptors, which are the common component of the tissue. However, only the agonist reveals a physiological response. The agonist–receptor–response interaction could be described quantitatively by mathematical models, the parameters , which should have a biological meaning.

For the purposes of modeling, the following generally accepted notations

are used: $[A]$—concentration of agonist; k_A—dissociation constant with dimensions of $[A]$; R—concentration of receptors of a given type; X—concentration of a complex between receptors of a given type and agonist (AR complex); E_m^T—potential maximum response of the tissue; E_m^A—maximum response produced by agonist A; E^A—measured response that is produced by concentration $[A]$; S—stimulus, which is dimensionless; e_A—efficacy of the agonist A, which is dimensionless; ε_A—intrinsic efficacy, which has the dimensions of the reciprocal value of the receptor concentration R; EC_{50}^A—concentration of an agonist, which produces $0.5E_m^T$; $[A_{50}]$—concentration of an agonist, which produces $0.5E_m^A$.

To obtain the parameters with relevant biological interpretation, some of the traditional models pass through the following main steps: (i) incorporation of the law of mass action into a receptor theory for agonist action [3, 17, 18, 31]; (ii) introduction of the term "intrinsic activity" [4], to characterize more fully the receptor–response part of the agonist action and to scale the effect; (iii) introduction of the term "stimulus" [66] as a product of fractional occupancy of the receptors and the parameter "efficacy", to characterize the response-eliciting power of the agonist; and (iv) incorporation of the concentration of free receptors of the given type, as an explicit parameter, and of the hybrid term "intrinsic efficacy" [30]. Thus, according to these basic assumptions, the agonist action could be measured by: (i) the affinity constant $\left(\frac{1}{k_A}\right)$, which reflects agonist–receptor interaction or binding; and (ii) the efficacy e_A or the intrinsic efficacyε_A, which characterizes the agonist-dependent part of the receptor–response interaction.

The tissue-dependent part of the receptor–response interaction, which does not concern the chemical substance, is defined by the receptor concentration R and by the stimulus-effect relation. So the tissue response possesses its own, drug-independent mechanism. As pointed out by Black and Leff [14], the abstract nature of the stimulus concept and the lack of a chemical identity for e_A are limitations of the comparative multiple agonist assays. At the same time, according to Stephenson [66] the function describing the agonist action toward the stimulus is unknown.

The majority of mathematical approaches used to analyze the concentration–response data obtained by in-vitro bioassays in isolated tissues eliminate Stephenson's concept of pharmacological stimulus [52–55]. The Operational model of Black and Leff [14] also avoids this concept. Moreover, authors assume a particular mathematical form and relation between the response and the concentration of the agonist–receptor complex. They further combine this relation with the occupancy hypothesis to obtain the next relation between the response and the concentration of the agonist. This relation is used by the same authors

to define operational affinity and operational efficacy and to obtain them numerically out of concentration–response data. Details about the limitations of these models are well documented [46, 48–50, 55].

Various mathematical approaches which analyze quantitatively the interaction between ligand and receptors are presented in numerous articles and reviews [1, 24–26, 28, 34, 35]. Analyses of the dose-response curves and the problem of the so-called "spare" receptors have a special place in these investigations.

The existence of partial agonists was described by Ariens [4, 5] and Stephenson [66]. Stephenson's modification of receptor theory arose from the need to explain the phenomena of partial agonism and "spare" receptors. His article [66] was very influential among pharmacologists, but some theoretical proposals in it were slightly wrong [19, 23]. The reason is that Stephenson's theory assumes that the two free parameters – e_A (measuring efficacy) and k_A (measure of affinity) – must be independent, whereas in fact affinity depends on efficacy [27]. As a consequence, the methods that have been proposed for measurement of affinity and efficacy, including that of Fuchgott's intrinsic efficacy [30, 66], do not work [19]. This problem was also propagated into the Operational model of Black and Leff and related approaches of Kenakin [21, 44]. All this stimulated us to develop a new model of partial agonist action, developing Stephenson's ideas.

Theoretical hyperbolic model (THM). Our main purposes were: (i) to express the different parts of the agonist–receptor–response mechanism as explicit functions based on the appropriate assumptions; (ii) to obtain mathematical evidence of the nature of the physiological response E^A as a function of the stimulus S in a form of explicit expression, where E_m^T and C_2 (measure unit of the stimulus S) are parameters; (iii) to obtain explicit formulas for determining the main parameters of agonist action k_A and e_A, respectively ε_A. During the course of our study it also became important to: (i) specify the necessary and sufficient parameters which describe agonist action; (ii) characterize the almost full agonist more specifically to estimate its receptor reserve (RR) if it exists; and (iii) introduce a new parameter, named "amplifier", for more precise quantitative description of the C-R-R mechanism and giving the real interpretation of the term stimulus introduced by Stephenson [66].

In order to achieve all these purposes, we developed a novel model of pharmacological agonism, named Theoretical hyperbolic model (THM), which aims at estimating the agonist parameters and their interpretation, according to the following main criteria: (1) to reflect the biological mechanism as closely as possible: it is desirable that the mathematical relationships between the independent and the dependent variables be based on physical, chemical or biological laws and observations; and (2) to be as simple as possible: it is preferable for the other,

more complex model if it describes the biological process and its parameters with the same accuracy.

The basis of our investigation is mainly Stephenson's [66] model of a concentration-occupation-stimulus-response mechanism and some approaches in the papers by Agneter et al. [1, 2, 3], Barlow et al., [9, 10, 11], Black and Leff [14, 15], Colquhoun [19–25], Kenakin [43], Mackay [52–55], Furchgott [30], etc.

In general the presented THM follows the classical work of Stephenson, but with some more profound considerations which allow us to: (1) determine Stephenson's unknown function of the biological response as an explicit function of stimulus; (2) give a proper interpretation of the stimulus character; (3) determine the dissociation constant k_A from the experimental data and show its inherent dependence on the efficacy; (4) determine the efficacy e_A from the experimental data and clear up its biological sense; (5) estimate RR of almost full agonists, if it exists.

THM-1. Axiomatic. The theory of the partial and almost-full agonism, which will be postulated here, is based on the axiomatic approach and on the following well-known suggestions and facts in quantitative pharmacology:

(a) the interaction between a drug and a receptor is bimolecular and obeys the law of mass action, i.e., $Y = \dfrac{X}{R} = \dfrac{[A]}{[A] + k_A}$;

(b) the occupied receptors X generate stimulus S which is quantitatively defined as: $S = e_A \dfrac{X}{R} = \dfrac{e_A[A]}{[A] + k_A} = \varepsilon_A \dfrac{R[A]}{[A] + k_A} = \varepsilon_A X$; this stimulus leads to some observed (measured) effect;

(c) the best approximation function of the experimental concentration-response data is a function of the type $E^A = \dfrac{L_1^A[A]}{[A] + L_2^A}$, where L_1^A and L_2^A are numerical constants that have a particular significance;

(d) E_m^T depends only on the given tissue; there are drugs which produce this maximum response (full agonist) or one close to it;

(e) the relation between stimulus and response is a property of the tissue and not of the drug (drug-independent property);

(f) equal stimuli lead to equal tissue responses.

The assumption (a) is a generally accepted relation and follows from the law of mass action. It describes the equilibrium stage of the process of binding and gives a quantitative characteristic of the number X of the AR complex. It does not explain what is going on in the process itself—how many receptors and molecules are involved to supply the equilibrium state number X. Assumption (a) is valid only under suggestion $R \ll [A]$, i.e., X is very small compared to $[A]$.

Assumption (b) is Stephenson's parameter S [66], named stimulus, which can be defined by Stephenson's efficacy e_A or by Furchgott's intrinsic efficacy ε_A [30]. Efficacy e_A is a dimensionless proportionality factor denoting the power of a drug A to produce a response in a tissue. The intrinsic efficacy $\varepsilon_A = \dfrac{e_A}{R}$ is a quantum unit for the capacity of a drug to initiate a stimulus from a receptor. The biological sense of S may be different for the different tissues and depending on the kind of effect that is measured. A deep consideration of Stefenson's theory and its history is given by Colquhoun [19–23].

Assumption (c) originates from the empirical observation that the best-fitting curves of the experimental data points (most of them, if not all), are hyperbolas or semi logarithmic hyperbolas (in a logarithmic scale) [8–14, 30, 32, 43, 68, 70].

In assumption (d) E_m^T is a very important tissue characteristic and depends on the pattern of the tissue response. Assumptions (e) and (f) follow Stephenson's definition of the stimulus–response mechanism [66] and are generally accepted in quantitative pharmacology.

Assumptions (a–f) and the relations deriving from them constitute the THM suggested here.

After a mathematical combination of assumptions (a), (b) and (c) (appendix, point 1), the relation between E^A and S in the stimulus-response mechanism is the following formula:

$$E^A = \frac{C_1^A S}{S + C_2^A}, \tag{1}$$

where

$$C_1^A = \frac{L_1^A k_A}{k_A - L_2^A} \quad \text{and} \quad C_2^A = \frac{e_A L_2^A}{k_A - L_2^A}. \tag{2}$$

The relations (1) and (2) are true for any agonist A that acts on a given tissue and the same type of receptors (assumption (a)) having in mind that the best fit of the experimental data obeys assumption (c). On the other hand the stimulus-effect relationship (1) is a drug-independent property (assumption (e)). That is why there are two constants C_1 and C_2 such that:

$$C_1^A = C_1,\ C_2^A = C_2 \ \text{ for any agonist } A \text{ and } \ E^A = \frac{C_1 S}{S + C_2}. \tag{3}$$

The constants C_1 and C_2 are one and the same for a given type of tissue and receptors and do not depend on the agonist A acting on this tissue.

Consequently, from (2) and (3) it follows:

$$C_1 = \frac{L_1^A k_A}{k_A - L_2^A} \quad \text{and} \quad C_2 = \frac{e_A L_2^A}{k_A - L_2^A}. \tag{4}$$

Solving the system (4), the parameters k_A and e_A are expressed in the following way:

$$k_A = \frac{C_1 L_2^A}{C_1 - L_1^A} \quad \text{and} \quad e_A = \frac{C_2 L_1^A}{C_1 - L_1^A} \quad \text{for any agonist } A. \tag{5}$$

The expressions (5) allow us to calculate or to estimate the dissociation constant k_A, the affinity $1/k_A$ and the efficacy e_A, if the parameters L_1^A, L_2^A, C_1 and C_2 are known. Stephenson [66] suggests, in his extension of the occupation theory, that there is an unknown, monotonic and continuous function $f(S)$ of the stimulus S such that the response E^A could be presented as:

$$E^A = E_m^T f(S), \quad \text{where } f(S) \text{ is an unknown function.} \tag{6}$$

In the presented THM, according to our findings (3), the response E^A is a hyperbolic function of S, which is monotonic and continuous by nature, since:

$$E^A = \frac{C_1 S}{S + C_2}. \tag{7}$$

THM-2. A pharmacological interpretation of the parameters and their calculation. The explicit formulas for L_1^A and L_2^A, as functions of the experimental data, are presented in the appendix (item 2). From the assumption (c) it follows that $L_1^A \approx E_m^A$ and $L_2^A = [A_{50}]$. The values of E_m^A and $[A_{50}]$ could be calculated after the best fitting of the experimental data. The pharmacological meaning of E_m^A and $[A_{50}]$ is a result of their definitions. From the assumption (d) and (7) it follows that $C_1 \approx E_m^T$ (maximum possible effect of the tissue) and the effectE^A could be presented as a function of S as follows:

$$E^A = \frac{E_m^T S}{S + C_2} \text{ and Stephenson's "unknown" function } f(S) = \frac{S}{S + C_2}, \tag{8}$$

as a function of the variable S.

The maximum effect of the tissue E_m^T can be determined (assumption (d)). There are cases when E_m^T could be derived from the control responses of the test system. For example the commonly used in-vitro bioassays for testing the

potency of opioid compounds are guinea pig ileum-longitudinal muscle and mouse vas deferens. Using a standard experimental procedure, where the parameters of field electrical stimulation, size of isolated preparations, etc., are unchangeable, we evoke control contractile responses which have been partially or fully inhibited by the opioids. In this case these initial contractions in fact could be considered operationally as E_m^T [61].

Following (8), the parameter C_2 could be considered as stimulus S , which elicits $0.5E_m^T$, because if $S = C_2$ then $E^A = \dfrac{E_m^T C_2}{2C_2} = 0.5E_m^T$. So C_2 could be used as a measure (unit) of stimulus S in a given tissue. Thus any stimulus S could be presented by this measure C_2 and a numerical parameter t as follows:

$$S = tC_2,\ t \in [0, +\infty)\,, \quad \text{i.e., the response } \ E^A = \frac{E_m^T t}{t+1}. \tag{9}$$

After replacing the coefficients L_1^A, L_2^A and C_1 with E_m^A, $[A_{50}]$ and E_m^T respectively in formulas (5), we obtain the following explicit expressions:

$$k_A = \frac{[A_{50}]\, E_m^T}{E_m^T - E_m^A} \ \text{ and } \ \frac{e_A}{C_2} = \frac{E_m^A}{E_m^T - E_m^A}.$$

These formulas are correct only when $E_m^A < E_m^T$ or $E_m^A \approx E_m^T$. This means that A is a partial agonist or almost full agonist.

Using notations $\lambda_A = \dfrac{E_m^A}{E_m^T}$ ($\lambda_A < 1$, because A is a partial or almost full agonist) and $\mu_A = \dfrac{\lambda_A}{1-\lambda_A}$, the dissociation constant k_A and the relative efficacy $\dfrac{e_A}{C_2}$ are expressed as follows:

$$k_A = \frac{[A_{50}]}{1-\lambda_A}, \quad \frac{e_A}{C_2} = \frac{\lambda_A}{1-\lambda_A} = \mu_A, \tag{10}$$

$$k_A = \frac{\mu_A}{\lambda_A}\,[A_{50}]\,, \quad \lambda_A = \frac{\mu_A}{\mu_A + 1} \quad \text{and} \quad k_A = (\mu_A + 1)\,[A_{50}]\,.$$

The parameters E_m^A and $[A_{50}]$ could be calculated from the experimental data; E_m^T (respectively λ_A and μ_A), by using the experimental data of some full agonist or apriority. The parameters λ_A and μ_A are important for explaining the C-R-R mechanism and allow a sensitive analysis of the agonist behavior in a given tissue.

The parameter μ_A does not depend on the measure unit C_2 and gives an absolute quantitative characteristic of the ability of agonist A to produce a

biological effect. The type of biological effect (the tissue's answer to the agonist action on the receptors) measured depends on the character of the experiment and the tissue. This defines the biological content of E_m^A and E_m^T.

The parameter k_A characterizes not only the process of binding, but the conformation stage as well. The parameter $[A_{50}]$ describes what we see but does not tell us what is going on underneath. The parameter k_A through μ_A tells us what is going on. The value of X strongly depends on k_A (assumption (a)). The dissociation constant is defined as $k_A = \dfrac{k_2}{k_1}$, where k_1 and k_2 characterize association and dissociation parts of the drug–receptor interaction. A high k_A means that the dissociation dominates the association and consequently a large part of the total number of receptors and molecules are involved in the process of binding, supplying the necessary level of occupancy X at any moment of the equilibrium state. The formulas (10) concerning k_A show its strong dependence on the efficacy e_A since $\dfrac{e_A}{C_2} = \dfrac{\lambda_A}{1-\lambda_A} = \mu_A$ and according to the last part of (10) $k_A = (\mu_A + 1)\,[A_{50}]$.

In some cases under investigation it is interesting to compare the action of agonists in a given tissue through their numerical parameters of agonist activity. For any two partial agonists A_1 and A_2 their ratio efficacy $\dfrac{e_{A_1}}{e_{A_2}}$ and ratio affinity $\dfrac{k_{A_1}}{k_{A_2}}$ could also be determined by (10) as functions of λ_A and μ_A as follows:

$$\frac{e_{A_1}}{e_{A_2}} = \frac{\lambda_{A_1}\,(1-\lambda_{A_2})}{\lambda_{A_2}\,(1-\lambda_{A_1})} = \frac{\mu_{A_1}}{\mu_2}, \quad \frac{k_{A_1}}{k_{A_2}} = \frac{[A_{50}^1].(1-\lambda_{A_2})}{[A_{50}^2].(1-\lambda_{A_1})} = \frac{\lambda_{A_1}}{\lambda_{A_2}}\frac{\mu_{A_1}}{\mu_{A_2}}\frac{\left[A_{50}^1\right]}{\left[A_{50}^2\right]}$$

These and formulas (10) allow a sensitive analysis of the behavior of agonists with respect to λ_A and μ_A.

THM-3. The C-R-R mechanism. The stimulus–response (S-R) relation in the tissue which is described by equation (8) depends on the parameters E_m^T and C_2 and on the variable S. The specific feature of the C-R-R mechanism needs additional parameters to analyze a stimulus–response process. Combining (8) and (10) with the assumption (b) $\left(S = e_A\dfrac{X}{R}\right)$, the stimulus S and the response E^A are connected as follows:

$$S = \frac{C_2}{R}\mu_A X \quad \text{and} \quad E^A = \frac{E_m^T \mu_A X}{\mu_A X + R}. \tag{11}$$

Let us introduce the term "intrinsic stimulus" c_2 (this measure is condi-

tional), defined as:

$$c_2 = \frac{C_2}{R}. \tag{12}$$

It is a quantum unit for the stimulus initiated by one receptor of a given type in the tissue. The ratio $\mu_A = \dfrac{\lambda_A}{1-\lambda_A}$ noted in (10) should be termed "amplifier". It needs to be pointed out that μ_A is just the relative efficacy e_A/C_2 (relative, because the measure unit is C_2, described in THM-2). In these terms the first equation in (11) can be rewritten as:

$$S = (\mu_A X)c_2 = \left(\mu_A \frac{X}{R}\right) Rc_2. \tag{13}$$

From (13) it follows that μ_A is the number of bindings of one receptor with molecules of drug A for any equilibrium state X. Therefore, the stimulus $S = \mu_A X c_2$ imported to the tissue by a given concentration of an agonist A is the sum of the quantum stimuli c_2 of all responding units $\mu_A X$. It could be suggested that $\mu_A X$ reveals the "number of activations" of all receptors of the tissue, because: (a) X is the number of receptors which are involved in the equilibrium state of the process of binding (at any moment of the equilibrium state the receptors supplying the quantity X may be different); and (b) amplifier μ_A shows how many times one receptor is "activated" at any equilibrium state of the process.

Note to THM-3. The processes at the equilibrium stage of the binding can be described mathematically precisely by using a special type of a partial differential equation, but this is a subject of another consideration.

The tissue processes the total stimulus into a tissue response $E^A = \dfrac{E_m^T \mu_A X}{\mu_A X + R}$ (the second part of (11)) and this response does not depend on the measures C_2 and c_2 of the stimulus S. From (13) it follows that the stimulus S depends on two parameters—c_2 and μ_A, and the variable X. The parameter c_2 together with parameters E_m^T and R (see (12)) define exactly the S–R relations of the tissue and appear to be a receptor property. It depends on the type of the receptor and on how it acts. The variable X depends on R, $[A]$ and k_A and therefore is a drug-tissue dependent. The parameter μ_A depends on E_m^T and E_m^A and reflects drug and tissue properties. The following relation between μ_A and the intrinsic efficacy ε_A is also valid: $\varepsilon_A = \mu_A c_2$. This is a precise expression of intrinsic efficacy as a classical parameter which depends on parameters c_2 and μ_A. Since c_2 is a constant measure unit for a given tissue (it depends on the

tissue and the type of receptors), μ_A defines the power of the agonist to produce a tissue response by one receptor. All this allows us to give some insight into the nature of the C-R-R mechanism.

The first step of this mechanism (C-R or concentration–receptor) is the interaction between the receptors and the molecules of the agonist, according to assumption (a). The process is dynamic and it involves many more receptors (some of them many times) as compared with X on the condition that $R \ll [A]$. All this allows a sufficient number of molecules of the drug A to supply the equilibrium stage X which depends on the period of interaction between the molecule of the agonist and the receptor. The number of occupied receptors X at any moment of the equilibrium state strongly depends on k_A. The bigger k_A, the shorter this period is (lower affinity) and therefore many receptors, more than X, react with the molecules many times. According to (13) ($k_A = (\mu_A + 1)\,[A_{50}]$), the affinity may be presented as a function of k_A and $[A_{50}]$ (appendix, item 5).

The second step R-R (receptor–response) has two stages:

– receptors process a stimulus $S = \mu_A X c_2$;

– this stimulus is transformed into an effect $E^A = \dfrac{E_m^T \mu_A X}{\mu_A X + R}$, which is a function of the variable X and parameters E_m^T, R and μ_A.

The stimulus S has a clear meaning, because μ_A and X are well defined, and $\mu_A X$ is the number of "activated" receptors of X in the equilibrium state. The measure c_2 is conventional and depends only on the tissue and the type of receptors.

The biological effect $E^A = \dfrac{E_m^T \mu_A X}{\mu_A X + R}$ depends on two parameters of the tissue, R and E_m^T. They define the following two constraints on X:

– when $E_m^A < E_m^T$ ($\mu_A$is not too large) the equilibrium level $X = R$ processes the maximum response E_m^A;

– when $E_m^A \approx E_m^T$, the equilibrium level $X < R$ may process the maximum response E_m^A.

The second possibility depends on the dissociation constant k_A and assumes the existence of a receptor reserve (RR). The influence of k_A on the existence of RR is the matter of a separate investigation.

The amplifier μ_A (respectivelyλ_A) could be considered as a quantitative measure of a drug biological activity. It allows a precise classification of drugs as antagonists, partial agonists and full agonists.

For $\mu_A \geq 1$ ($\lambda_A \geq 0.5$) the parameter EC_{50}^A is well defined and the following equation holds:

$$k_A = (\mu_A - 1)\, EC_{50}^A. \tag{14}$$

Combining (13) and (14), the two location parameters EC_{50}^{A} and $[A_{50}]$ are connected by the relation:

$$EC_{50}^{A} = \left(\frac{\mu_A + 1}{\mu_A - 1}\right).[A_{50}]. \tag{15}$$

This is true only for the cases when $\mu_A > 1$ ($E_m^A > 0.5E_m^T$).

THM-4. Receptor reserve (spare receptors). Substituting (13) in (8), the response E^A is described as a function of the receptors R, occupied receptors X and the receptor reserve $\mathrm{RR} = (R - X)$ as follows:

$$E^A = \frac{\lambda_A X}{R - \lambda_A (R - X)} E_m^T = \frac{\mu_A X}{\mu_A X + R} E_m^T. \tag{16}$$

Let X_m^A be the value of the AR complex that elicits E_m^A. Using (16), the following equation holds:

$$(R - X_m^A) = \lambda_A (R - X_m^A). \tag{17}$$

When $\lambda_A < 1$, the equation (17) is possible, if and only if:

$$X_m^A = R.$$

This means that all partial agonists do not have a receptor reserve, which confirms the experimental observations [5, 6] that partial agonists have no RR. When $\lambda_A \approx 1$, equation (17) may be true for $R - X_m^A > 0$. This means that those agonists which are close to full agonists as biological activity behavior may or may not have a receptor reserve. The main problem is: is it possible to recognize the existence of RR from the experimental dose-response data? The answer of this question shall be given in our further investigations.

If RR exists ($(R - X_m^A) > 0$), byintroducing the variable $\lambda = \dfrac{E^A}{E_m^T}$ ($0 \leq \lambda \leq \lambda_A$), the RR and any equilibrium state X of the AR complex can be estimated as functions of λ:

$$(R - X) = \left(1 - \frac{\lambda}{\mu_A (1 - \lambda)}\right) R \quad \text{and} \quad X = \frac{1}{\mu_A} \frac{\lambda}{(1 - \lambda)} R. \tag{18}$$

The relations (18) are interesting only for the case when $\lambda_A \approx 1$ (agonist A is an almost full agonist). For $\lambda = \dfrac{n}{n+1}$, $n = 1, 2, \ldots$, let X_n^A denote the

concentration of AR complex supplying $\frac{E^A}{E_m^T} = \frac{n}{n+1}$ response. Then the RR in this case is $\left(1 - \frac{n}{\mu_A}\right) R$ and $X_n^A = \frac{n}{\mu_A} R$.

THM-5. The case of non-classical hyperbolic E/[A] curves. In the case of Tallarida and Jacobs [68] when n molecules of agonist A react with one molecule of R:

$$\frac{X}{R} = \frac{[A]^n}{[A]^n + k_A}.$$

The assumption that E^A is directly proportional to X leads to

$$E^A = \frac{E_m^A\,[A]^n}{[A]^n + k_A}.$$

Having this in mind this and the observation that in many cases the experimental $E/[A]$ curves are steeper or flatter than the classical hyperbola, the experimental data could be fitted by the following function:

$$E^A = \frac{E_m^A [A]^P}{[A]^P + [A_{50}]^P}, \tag{19}$$

which have three parameters E_m^A, $[A_{50}]$ and p, where the parameter p denotes the steepness of the curve [70]. We developed THM for pharmacological agonism on the basis of axioms (a–f). This theory can be extended for the experimental curves formulated as in (19). Since the classical hyperbola is a special case of (19), when $p = 1$, we introduced the following steps to comprise the general case:

(i) Let us choose n points $p_1, p_2, \ldots, p_n$ from the interval $[a, b]$;

(ii) Let us fit the experimental data by the following family of curves:

$$E^A = \frac{E_m^A [A]^{P_i}}{[A]^{P_i} + [A_{50}]^{P_i}} \; i = 1, \ldots, n;$$

(iii) Choose the curve giving the best fit of the experimental data. Let i_0 be the index of the optimal fitting curve:

$$E^A = \frac{E_m^A [A]^{P_{i_0}}}{[A]^{p_{i_0}} + [A_{50}]^{p_{i_0}}}. \tag{20}$$

Using (20) in assumption (c), it is not difficult to show that there exists a function $f(S, p_{i_0}, e_A)$ such that

$$(k_A)^{p_{i_0}} = \frac{f(S, p_{i_0}, e_A).\,[A_{50}]}{1 - \lambda_A} \quad \text{and} \quad \left(\frac{e_A}{C_2}\right)^{p_{i_0}} = \frac{\lambda_A}{1 - \lambda_A} = \mu_A.$$

The function $f(S, p_{i_0}, e_A)$ is continuous and decreasing with respect to S and $\lim f(S, p_{i_0}, e_A) = 1$ when $S \to e_A$.

In terms of μ_A

$$(k_A)^{p_{i_0}} = (\mu_A + 1)\,[A_{50}]\,f(\cdot) \quad \text{and} \quad \left(\frac{e_A}{C_2}\right)^{p_{i_0}} = \mu_A. \tag{21}$$

From (21) it follows that only k_A depends on $f(\cdot)$.

For the full calculations leading to (21), see the appendix (item 3).

Discussion. The roots of the presented THM lie at the assumptions (a–f). The questions related with (a–f) concern the identity and accessibility of the parameters contained in the model used to describe partial and almost-full agonism. The basic idea in this study was to develop Stephenson's model and extend its application.

Assumption (a) is well known and follows the law of mass action. Under the condition $R \ll [A]$ the equilibrium concentration of the agonist equals the initial applied concentration. This condition is not necessary but leads to a simple and realistic expression of the number of AR complexes. It is true that (a) is used in almost all theoretical papers [4, 13, 14, 28, 30, 42, 52, 54, 65, 66, 68] concerning drug interaction and is widely applied in all methods discussed in the introduction.

Assumption (c) defines the character of the fitting curves of the experimental data. This can be considered as a law of dose–response action. The shape of concentration–response curves may give some insights into the nature of the relation between receptor occupancy and ensuing response. Hyperbolic curves and curves indistinguishable from them are an important subclass of the curves used in the best approximation of the concentration–response data. They cover a wide range of C-R-R interaction with different receptors in different tissues [14, 15]. Many experimental results prove that around 70% of the fitting curves in the considered cases are hyperbolic or close to them [1, 11, 28]. Let us mention that the various forms of the logistic curves have been used for analysis of concentration-response data [26, 32, 36–41]. Finally, it is necessary to note that the hyperbolic curves in the case of a logarithmic scale are named sigmoid (appendix, point 4), semi logarithmic or S-shape functions.

Assumptions (b), (d) and (e) are commented together because they concern the stimulus–response mechanism on the whole. These assumptions are introduced by Stephenson [66] in his basic model. They are accepted in their entirety in the THM, because they allow us to explain the behaviour of the full agonists. According to them the maximal response might be obtained with

only a very small fraction of the receptors occupied [4, 66], which underlines the differences between full and partial agonism. However, many authors avoid Stephenson's model, because the nature of the stimulus S introduced in his model is ill-defined. In the THM (especially THN-3), the nature of stimulus S obtains a clear interpretation, which is in accordance with the suggestions of Barlow et al. [13].

Assumption (d) is discussed by many authors [14, 30, 42, 49, 52, 54, 64, 66]. This assumption (the definition of E_m^T is given by Furchgott [30]) raises the following question: when might the response be measured? In the case of muscle contraction the response might be the isometric tension produced, or the change in length of the tissue under isotonic conditions. Alternatively, changes in the electrical properties or membrane permeability of the cells might be used as a measure of the response. It seems very unlikely that all these various types of response would be related in the same way to the fraction of receptors occupied by the agonist [23, 54]. In quantitative pharmacology there is no clear answer when a drug is a full agonist, and this is because the definition of full agonist uses E_m^T. On the other hand E_m^T is defined as a maximum potential effect produced by a full agonist [14, 42, 53, 68, 69]. From this it follows that E_m^T could be defined only by using the properties of the tissue (muscle contraction, electric properties and so on). Experimental confirmations of the assumption (d) are: the method of competitive bioassay [7] and the irreversible elimination method [30].

Assumption (e) concerns the stimulus–response mechanism in a given tissue which is fully determined by Stephenson's so-called "unknown" function $f(S)$ and this function is the same for different agonists acting on the same tissue. This is mentioned on many occasions in the literature [14, 23, 30, 42, 48, 52–55, 66, 68]. The use of special forms of $f(S)$ [14, 30, 42] leads to different models of stimulus–response relationships. Formula (8) shows that in the considered THM the function $f(S)$ appears to be hyperbolic. The measured biological effect E^A as a function of X is presented by (11). This is quite different from such functions considered until now [14, 42]. These functions are usually postulated and there are no arguments why they are considered. The skepticism with respect to the stimulus-response mechanism is formulated by Kenakin [42]: "However, the clear indication that a tissue response cannot be used as a direct measure of receptor events (binding and quantal stimulation) necessitate the use of null techniques in receptor pharmacology". Mackay shares the same view [54, 55].

Assumption (f) is generally accepted in quantitative pharmacology and will not be commented upon. Let us only mention that the null methods are based on the idea: equal stimuli should produce equal responses. The same is used in the methods for determining the dissociation constant of competitive

antagonists [68].

Finally, according to our view, concerning assumptions (a–f), assumptions (b), (c), (d) and (e) are the most important and lead to obtaining the explicit formulas (10) and (11). Indeed, (c) defines the law of dose-response relation and together with (b), (d) and (e) supplies an explicit expression of affinity, efficacy and effect of partial agonists through the parameters E_m^A, $[A_{50}]$ and E_m^T, calculated from the experimental data for a given tissue.

Maybe the most detailed mathematical model for describing and quantifying pharmacological agonism is the pre-eminent operational model of Black and Leff [14], which according to Kenakin [43] avoids the inclusion of *ad hoc* terms for efficacy. Different sides of this model are discussed, compared and criticized with another models by many authors [1, 2, 33, 55]. The operational model is based on the observation that the relationship between agonist concentration and tissue response is most often hyperbolic. The main differences between the operational model and the THM, presented in this paper, are as follows:

– in the operational model the main postulate is that E^A is a hyperbolic function of the receptors occupied, while in the THM E^A is a hyperbolic function of the concentration of the compound applied; so assumption (c) in the THM could be verified by fitting the experimental data, which allow us to realize the applicability of the model.

– in THM the affinity and the efficacy have a classical meaning; in an operational model it is necessary to define the so-called "transducer ratio" τ as a measure of efficacy. The connection between parameters $[A_{50}]$, k_A and τ according to Black and Leff [14] is:

$$[A_{50}] = \frac{k_A}{\tau + 1} \quad \text{or} \quad k_A = (\tau + 1)\,[A_{50}]\,. \tag{22}$$

The correct connection between the above parameters, however, is:

$$[A_{50}] = \frac{k_A}{\tau - 1} \quad \text{or} \quad k_A = (\tau - 1)\,[A_{50}]\,. \tag{23}$$

The relation (23) holds if and only if $\tau > 1$. This is a strong restriction on drugs acting on a given tissue. (23) is derived in the appendix (item 6).

Relation (22) and other questions arising around the operational model are discussed by Agneter [3], Geraldo [33] and others [59]. Equation (23) limits the application of operational model only for partial agonists and similar suggestions are presented in various papers [47, 48, 51]. From the definition of τ it follows that the operational model is applicable if τ is greater than one, i.e., it is incorrect to analyze partial agonists with low maximal effect by using the operational model.

The reason is the suggestion that there is a value of AR complex (named K_E in the paper) that elicits half-maximal effect.

The lack of an operational model is a result of an unclear definition of E_m. If E_m equals E_m^A, then $k_A = (\tau - 1)\,[A_{50}]$; if E_m equals E_m^T, then $k_A = (\tau - 1)\,[EC_{50}]$. In the first case, the operational model does not allow us to compare the agonist's action of different drugs in a given tissue.

Such faults, or similar ones, raise the skepticism of Agneter et al., [2], Colquhoun [23] and others concerning the models of Furchgott [30] and of Black and Leff [14].

An approach different from Stephenson's was proposed by Del Castillo and Katz [27] to explain partial agonism. They wrote down a simple explicit reaction scheme known as the "Del Castillo-Katz mechanism". This scheme predicts two steps: (1) ability to bind and (2) isomerisation between inactive and active receptors. The concentration–stimulus-response mechanism presented in the THM (according to THM-3) could also be considered in two steps: (1) occupation of receptors and (2) activation and amplification of receptors to stimulus S which produces a response $E^A = \dfrac{E_m^T \mu_A X}{\mu_A X + R}$, presented as a function of the number of occupied receptors X.

The second formula in (11) illustrates another essential difference between THM and the operational model of Black and Leff [14].

A very important fact in quantitative pharmacology is the observation that E_m^T may be achieved when only a relative small fraction of receptors is occupied, i.e., when a full or (almost) full agonist interacts with receptors. In several papers [1–3, 28, 29] the concept of "spare" receptors is considered in detail. There a receptor reserve is quantified from experimental data using "mechanistic general response functions" (nonsymmetrical sigmoid) developed to reflect the existence of "spare" receptors. This model recognizes the existence of RR using a slope parameter [28]. Thus they avoid the faults of the previous models of Black and Leff [14] and Furchgott [30]. The THM presented in this study gives quantitative details (THM-4.) for RR and its estimation (formula (18)), if RR exists. Moreover, THM proposes that RR exists only for almost full and full agonists. For partial agonists RR does not exist (THM-4.).

Agneter [1] mentions that previous theories usually consider only functions transforming binding into response. Therefore, they theoretically exclude congruence of a concentration-binding curve and the corresponding concentration-response curve, except in the case of direct proportionality. Formulas (1–7) and (10–11) and their explanation show that the initial binding reaction and resulting response are not independent processes, i.e., they unite these processes. This

dependence reflects into affinity and efficacy of drugs. The agonist affinity (formula (10)) measures dependence on the characteristics of both, not only on the initial binding. This confirms the suggestion of Colquhoun that affinity strongly depends on the efficacy of the drug, which is discussed many times [19–23].

On the other hand, THM allows interpretation of the relationship between occupancy of receptors and induced response, discussed in (THM-3). According to formula (11) the response depends on the tissue parameters E_m^T and R, the drug-tissue parameter μ_A and the variable X, but does not depend on the measure c_2. This statement is correct, because assumption (c) is observed to be true for different tissues and type of receptors [14] and the law (11) serves all these cases.

Conclusion. After the first suggestions of the THM [56], some further results [60, 62] showed that $[A_{50}]$ or EC_{50} cannot characterize completely agonist behaviour of compounds with various potency in different tissues. This stimulated us to undertake experiments with isolated tissues where E_m^T could be well defined [57, 58, 61, 63]. Together with the fitting curves of the experimental data, affinity and efficacy of the tested compounds were calculated, applying formulas similar to (10). Numerical results for k_A and e_A confirm the biological and pharmacological properties of the drugs investigated in these papers. So all these steps stimulate us to develop THM as a whole as an evolution of Stephenson's idea and this complete model is the object of the present study. Let us mention that formulas (10) were incorporated into a scheme of an artificial neural network architecture, proposed for investigation of quantitative structure–activity relationship [5].

Appendix.

1. Using the assumption (b), after simple mathematical operations, $[A]$ can be expressed by S as follows

$$(*) \qquad [A] = \frac{k_A S}{e_A - S}.$$

Combining assumption (c) with $(*)$ the response E^A is presented as a function of S:

$$E^A = \frac{L_1^A [A]}{[A] + L_2^A} = \frac{L_1^A \left(\dfrac{k_A S}{e_A - S} \right)}{\dfrac{k_A S}{e_A - S} + L_2^A}$$

$$= \frac{L_1^A k_A S}{S(k_A - L_2^A) + e_A L_2^A} = \frac{\dfrac{L_1^A k_A}{(k_A - L_2^A)} S}{S + \dfrac{e_A L_2^A}{(k_A - L_2^A)}} = \frac{C_1^A S}{S + C_2^A},$$

where $C_1^A = \dfrac{L_1^A k_A}{(k_A - L_2^A)}$ and $C_2^A = \dfrac{L_2^A e_A}{(k_A - L_2^A)}$.

2. The experimental data points $(E_i, [A_i])$, $i = 1, \ldots, N$, are fitted by the hyperbola

$$E^A = \frac{L_1^A [A]}{[A] + L_2^A} \text{ (assumption(c))}.$$

The constants L_1^A and L_2^A are obtained by using the least square method in the following form: let $E_i = \varepsilon_i \dfrac{L_1^A [A_i]}{[A_i] + L_2^A}$, $i = 1, \ldots, N$.

Minimize the function

$$F(L_1^A, L_2^A) = \sum_{i=1}^{N} (\varepsilon_i - 1)^2 = \sum_{i=1}^{N} \left(\left(\frac{E_i}{L_1^A} + \frac{E_i}{[A_i]} \frac{L_2^A}{L_1^A} \right) - 1 \right)^2,$$

with respect to L_1^A and L_2^A.

From the necessary and sufficient conditions $\dfrac{\partial F}{\partial L_1^A} = 0$, $\dfrac{\partial F}{\partial L_2^A} = 0$ it follows the explicit formulas for L_1^A and L_2^A from the experimental data:

$$L_1^A = \frac{\left(\sum_{i=1}^{N} E_i \right) \left(\sum_{i=1}^{N} \left(\frac{E_i}{[A_i]} \right)^2 \right) - \left(\sum_{i=1}^{N} \frac{E_i^2}{[A_i]} \right)^2}{\left(\sum_{i=1}^{N} E_i \right) \left(\sum_{i=1}^{N} \left(\frac{E_i}{[A_i]} \right)^2 \right) - \left(\sum_{i=1}^{N} \frac{E_i}{[A_i]} \right) \left(\sum_{i=1}^{N} \frac{E_i^2}{[A_i]} \right)}$$

$$L_2^A = \frac{\left(\sum_{i=1}^{N} E_i \right) \left(\sum_{i=1}^{N} \frac{E_i^2}{[A_i]} \right) - \left(\sum_{i=1}^{N} \frac{E_i}{[A_i]} \right) \left(\sum_{i=1}^{N} E_i \right)}{\left(\sum_{i=1}^{N} E_i \right) \left(\sum_{i=1}^{N} \left(\frac{E_i}{[A_i]} \right)^2 \right) - \left(\sum_{i=1}^{N} \frac{E_i}{[A_i]} \right) \left(\sum_{i=1}^{N} \frac{E_i^2}{[A_i]} \right)}.$$

3. Let the best-fitting function be

$$(**) \qquad E^A = \frac{E_m^A [A]^{P_{i_0}}}{[A]^{p_{i_0}} + [A_{50}]^{p_{i_0}}}.$$

Substituting $(*)$ in $(**)$, E^A is expressed as a function of S as follows

$$(***) \qquad E^A = \frac{L_1^A (k_A S)^{p_{i_0}}}{(k_A S)^{p_{i_0}} + L_2^A (e_A - S)^{p_{i_0}}}.$$

If $f(S,p_{i_0},e_A) = \left(\frac{e_A}{S}\right)^{p_{i_0}} - \left(\frac{e_A}{S}-1\right)^{p_{i_0}}$ then $(e_A - S)^{p_{i_0}} = e_A^{p_{i_0}} - f(S,p_{i_0},e_A)S^{p_{i_0}}$. Using this, $(***)$ can be rearranged in the form $E^A = \frac{C_1^A S^{p_{i_0}}}{S^{p_{i_0}} + C_2^A}$, where

$$C_1^A = \frac{L_1^A k_A^{p_{i_0}}}{k_A^{p_{i_0}} - f(.)L_2^A} \text{ and } C_2^A = \frac{L_2^A e_A^{p_{i_0}}}{k_A^{p_{i_0}} - f(.)L_2^A}.$$

Solving the equations with respect to k_A and e_A, the following expressions are true

$$(k_A)^{p_{i_0}} = \frac{f(S,p_{i_0},e_A).[A_{50}]}{1-\lambda_A} \text{ and } \left(\frac{e_A}{C_2}\right)^{p_{i_0}} = \frac{\lambda_A}{1-\lambda_A} = \mu_A;$$

$$(k_A)^{p_{i_0}} = (\mu_A + 1)[A_{50}]\, f(\cdot) \text{ and } \left(\frac{e_A}{C_2}\right)^{p_{i_0}} = \mu_A$$

4. The hyperbola $(*)$ in the logarithmic scale has a sigmoidal shape because $E^A = \frac{L_1^A[A]}{[A]+L_2^A} = \frac{L_1^A 10^{\log[A]}}{10^{\log[A]} + 10^{\log L_2^A}}$ with a mean point $\log L_2^A = p[A_{50}]$. The parallel shift of the curve is equivalent to the irreversible blockade, which is equivalent to the increasing of L_2^A (which is equal to $[A_{50}]$). Irreversible blockade produces parallel rightward displacement of the curve of the full agonist and this reduces the efficiency of the stimulus-response mechanism.

5. The ratio $1/k_A$ as a measure of the affinity of drugs. Let $1/k_A < 1/k_B$ for two drugs A and B acting on the same receptor on the same tissue. For equal concentrations $[A]$ and $[B]$ $X_A = \frac{R[A]}{[A]+k_A} = \frac{R[B]}{[B]+k_A} < \frac{R[B]}{[B]+k_B} = X_B$, because $1/k_A < 1/k_B$ implies $k_B < k_A$. The inequality $X_A < X_B$ means: the drug A occupies a smaller number of receptors than drug$B$, acting on the receptors with the same concentration. If $X_A = X_B$ $(X_A = \frac{R[A]}{[A]+k_A} = \frac{R[B]}{[B]+k_B} = X_B)$ and $k_B < k_A$, then $[B] < [A]$. This means that drug A needs higher concentration $[A]$ than the concentration $[B]$ of drug B to supply the same level of occupancy.

6. Following Black and Leff [14] and using their notations, equation (5) from this article can be rewritten in the form:

$$\frac{E}{E_m} = \frac{\tau[A]}{(\tau+1)[A]+k_A}.$$

For $[A_{50}]$ $\frac{E}{E_m} = \frac{\tau[A_{50}]}{(\tau+1)[A_{50}]+k_A} = 0.5$ and $[A_{50}] = \frac{k_A}{\tau-1}$, or $k_A = (\tau-1)[A_{50}]$.

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