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Efficacy I: a new method for estimating relative efficacy of full agonists via a newly defined efficacy related parameter

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Abstract

A new method for estimating relative efficacies and relative intrinsic efficacies of agonists is described. Relative efficacy is estimated by employing a newly defined efficacy related parameter (e^{ES}) and it may be estimated without prior knowledge of efficacy values or the value of the equilibrium dissociation constants, K_A, of agonist-receptor complexes. The parameter e^{ES} is directly related to efficacy (e) and is defined as the ratio of maximal stimulus to maximal effect of an agonist. The value of e^{ES} indicates whether or not spare receptors are present for a particular agonist–effector system. The e^{ES} values of agonists are estimated by utilizing submaximal concentration–effect curves determined with fixed agonist-competitive antagonist concentration combinations and choosing a suitable reference (height of an agonistic concentration–effect curve) to which the height of the stimulus concentration–effect curves of the agonist may be compared. In addition to e^{ES}, other new agonist–effector parameters, namely S_{Em}/S_m and ϕ_{min}, were also defined.

Keywords: Efficacy, agonist; Efficacy, relative; Agonist-antagonist combination; Spare receptor; (New parameter)

1. Introduction

Studies of relative intrinsic efficacy had shown this to be a useful scale for the classification of agonists and the prediction of pharmacological effects. There are definite advantages to quantifying relative agonist efficacy which relate to the power of in vitro and in vivo experimentation to predict agonist effects across animal species and in man (Kenakin, 1985). The reason for this is the fact that the dependence of agonist activity upon receptor density and tissue stimulus–effect capability is quite different for agonists of different efficacy. The pharmacological effects produced by agonists of greater efficacy would be less affected by differences in organ receptor density and efficiency of stimulus–effect coupling than are effects to agonists of low efficacy. A high efficacy agonist would produce a full tissue effect by activation of only a small fraction of the receptor population, while an agonist of lower efficacy would require a correspondingly larger fraction of the receptors to produce a comparable effect. An intervention that reduces tissue stimulus–effect capability, or the density of drug receptors, would decrease the effect of the lower efficacy agonist more than that of the greater efficacy agonist.

There is no independent method of estimating agonist efficacy (e), since it is simply a scaling factor in classical occupational theory (Kenakin, 1985, 1990). Therefore, the general tendency in pharmacology is to estimate relative efficacy or relative intrinsic efficacy. The relationship between efficacy (e) and intrinsic efficacy ( ) is given by $= e/[R]_T$, where $[R]_T$ refers to the tissue concentration of receptors (Furchgott, 1966). The estimation of relative efficacy and/or relative intrinsic efficacy for full agonists are, however, problematic when spare receptors (receptor reserve) are present in the particular system. Spare receptors are defined as the fraction of the total receptor pool not required for maximal tissue response. Therefore, when spare receptors are present a submaximal stimulus will correspond to a maximal effect, and if the stimulus increases further the effect will remain the same (Furchgott, 1955; Nickerson, 1956; Stephenson, 1956; Ariëns et al., 1960, 1964b; Furchgott, 1966; Venter, 1979). For full agonists such a nonlinear relationship between receptor occupancy and effect appear to be the rule rather than the exception in experimental pharmacology (Ruffolo, 1982; Kenakin, 1984). Since efficacy is actually a function of maximal stimulus, it is evident that difficulties would arise in the estimation of efficacy if the maximal stimulus

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and/or equilibrium dissociation constants of the agonist-receptor complex (K_A) is unknown.

Several procedures were devised for the estimation and quantification of the relative efficacies and relative intrinsic efficacies of agonists. All these methods require accurate measurements of the equilibrium dissociation constants of the agonist-receptor complexes (K_A) as a prerequisite. Unfortunately, accurate estimation of K_A values is complicated by nonlinear stimulus–effect relationships which may be present in isolated organs (Ruffolo, 1982; Kenakin, 1984). To circumvent the use of K_A, Mackay (1966a,b) described a method to determine the relative order of efficacy of agonists that does not require prior knowledge of one or more of the agonist equilibrium dissociation constants. Mackay used a double-reciprocal equation to relate equiactive concentrations of two agonists, $[A_1]$ and $[A_2]$. A double-reciprocal plot of $1/[A_1]$ against $1/[A_2]$ should yield a straight line, in which the intercept with the ordinate is given by $_1(1 - _2/_1)/_2K_A$, where $_1$ and $_2$ represent the intrinsic efficacies of agonists A_1 and A_2 respectively. The arithmetic sign of the intercept will be zero if $_1 = _2$, negative if $_1 < _2$ and positive when $_1 > _2$. Although the strength of this approach lies in its independence from K_A values, a numerical estimate of $_2/_1$ cannot be made if the value of K_A is unknown. In practice concentration–effect curves for full agonists usually are parallel, causing the intercept of such a regression to approach zero and thereby making this procedure difficult to use. Also, the double-reciprocal regressions for two concentration–effect curves giving maximal effects do not yield accurate estimates of intercepts because of the variance in the regression line (Kenakin, 1987c).

A widely used method to measure relative intrinsic efficacy is by comparison of measures of respective receptor occupancy by two agonists at equiactive concentrations (Furchgott, 1966; Furchgott and Bursztyn, 1967). By employing this method a numerical estimate of relative intrinsic efficacy can be made as long as the receptor occupancy can be calculated. Unfortunately, to do this, the exact K_A for each agonist is required. Attempts have also been made to calculate values of intrinsic efficacy in terms of the assumption that stimulus (S) equals unity at 50% receptor occupancy (Stephenson, 1956).

To date there is no independent experimental method to verify estimates of stimulus or relative efficacy (Kenakin, 1985) and none of the methods currently employed are really satisfactory for the practical estimation of efficacy (for an overview see: Kenakin, 1985; Kenakin, 1987d). This paper will, however, forward a method for estimating relative intrinsic efficacies, relative efficacies and an efficacy related parameter of full agonists when spare receptors are present in the system. In contrast to currently employed methods, estimates with this method are made without any knowledge of the value of the dissociation constant, K_A, of the agonist-receptor complex.

2. Materials and methods

2.1. Theory

The new method for estimating relative efficacy of full agonists in the presence of spare receptors is based on the idea that a combination consisting of a fixed concentration ratio of an agonist A and a competitive antagonist B may mimic a partial agonist and therefore give rise to a submaximal effect (Feuerstein et al., 1994; Venter, 1996). The theoretical background of this method was developed from the occupancy theory of agonist action. It follows from the occupancy theory that the fraction of the maximal stimulus (S_A/S_m) may be calculated by means of the following equation (Stephenson, 1956; Ariëns et al., 1964a):

$$\frac{S_A}{S_m} = \frac{e}{1 + \frac{K_A}{[A]}} \quad (1)$$

where K_A represents the equilibrium dissociation constant of the agonist-receptor complex. [A] and e respectively represent the concentration and efficaccy of agonist A. Efficacy e is a dimensionless proportionality factor denoting the power of an agonist to produce a stimulus and eventually a pharmacological effect in a tissue (Kenakin, 1987a). It follows from Eq. (1) that the maximal stimulus value will be produced when e possesses maximal value. In general the true value of e is unimportant, since the meaning of an isolated e is meaningless.

Eq. (1) also serves as a basis for establishing the conditions which permit analysis of simple competitive antagonism. Receptor theory (Arunlakshana and Schild, 1959; Ariëns et al., 1964a) predicts that a competitive antagonist B will affect Eq. (1) as follows:

$$\frac{S_{AB}}{S_m} = \frac{e}{1 + \left(1 + \frac{[B]}{K_B}\right)\frac{K_A}{[A]}} \quad (2)$$

in which S_{AB}/S_m signifies the fraction of the maximal stimulus of agonist A in the presence of competitive antagonist B, [B] is the concentration of competitive antagonist B, and K_B is the equilibrium dissociation constant of the competitive antagonist-receptor complex. If [A] and [B] are combined in a fixed ratio (ϕ), then it follows that:

$$[B]/[A] = \phi \quad (3)$$

Venter (1996) had shown that the combination of Eq. (2) and Eq. (3) gives rise to:

$$\frac{S_{AB}}{S_m} = \frac{\left(\frac{e}{1 + \phi K_A/K_B}\right)}{1 + \left(\frac{1}{1 + \phi K_A/K_B}\right)\frac{K_A}{[A]}} \quad (4)$$

which may be written as:

$$\frac{S_{AB}}{S_m} = \frac{h}{1 + \frac{[A]_{1/2}}{[A]}} \quad (5)$$

Eq. (5) is basically analogous to Eq. (1). In Eq. (5) h represents the relative height of the concentration-stimulus curve obtained with a fixed agonist-competitive antagonist combination, while $[A]_{1/2}$ is the concentration at which half of the curve height $(h/2)$ is obtained. It is important to note that height h should be dimensionless (S_{AB}/S_m and $[A]_{1/2}/[A]$ are dimensionless), and therefore, the height h should be expressed as a fraction of the maximal height: $h = h_{AB}/h_m$, where h_{AB} represents the height of the submaximal concentration-stimulus curves of agonist A obtained with fixed agonist-competitive antagonist combinations, and h_m is the maximal height of the concentration-stimulus curve of agonist A. The quantity h_m actually represents the height of the concentration-stimulus curve of A in the absence of the competitive antagonist B. By comparing Eq. (4) and Eq. (5) it can be seen that:

$$h = \frac{h_{AB}}{h_m} = \frac{e}{(1 + \phi K_A/K_B)} \quad (6)$$

and

$$[A]_{1/2} = \frac{K_A}{(1 + \phi K_A/K_B)} \quad (7)$$

The estimation of K_A by employing Eq. (7) was described by Venter (1996), and therefore, this study concentrates only on applications based on Eq. (6). The latter equation affords the possibility to estimate agonist affinity and a parameter which is related to agonist efficacy. Eq. (6) modifies to:

$$e = h(1 + \phi K_A/K_B) \quad (8)$$

and,

$$h = -\frac{K_A}{K_B}\phi h + e \quad (9)$$

in which the relationship between h and ϕh will be linear if the law of mass action is operative and the interaction between A and B is competitive in nature. Under these circumstances Eq. (9) would yield a straight line when h is plotted against ϕh. The value of e is obtained from the intercept of the straight line with the ordinate (h-axis), since $h = e$ if $\phi h = 0$. Note that the value of e is obtained in the absence of B, because if $[B] = 0$, then $\phi = 0$ and it follows that $\phi h = 0$. It should be stressed, however, that absolute efficacy cannot be estimated by employing Eq. (8) or Eq. (9), because the value of e, as is described by these equations, is a function of h which is an arbitrary chosen value (Venter, 1996, 1997). Therefore, what is needed now is a practical parameter relating e, which may be determined by employing practically usable equations based on Eq. (8) and Eq. (9).

2.1.1. Conversion of Eq. (8) and Eq. (9) to practically applicable equations

In Eq. (8) and Eq. (9) the relationship $h = h_{AB}/h_m$ represents relative heights of concentration-stimulus curves, while response is all the experimenter has to work with in experimental pharmacology. It is therefore necessary that Eq. (8) and Eq. (9) should be transformed into practical equations which relates heights of experimental concentration–effect curves rather than concentration-stimulus curves.

The conversion of Eq. (8) and Eq. (9) are based on the assumption that when a submaximal effect is obtained with a fixed agonist-competitive antagonist combination, then, as illustrated in Fig. 1 and Fig. 2, this submaximal concentration–effect curve and its accompanying concentration-stimulus curve should coincide (Venter, 1996). If it is supposed that H_m represents the maximal height of the concentration–effect curve of the full agonist A in the absence of B, and H_{AB} represents the height of a submaximal concentration–effect curve obtained with a fixed agonist-antagonist combination, then the relative height ($\boldsymbol{H}$) of the submaximal concentration–effect curve heights (H_{AB}) versus maximal effect curve height (H_m) is given as $\boldsymbol{H} = H_{AB}/H_m$.

The practically equivalents of Eq. (8) and Eq. (9) may be derived by applying the relationship $\boldsymbol{H} = H_{AB}/H_m$, i.e., by replacing h in these equations by $\boldsymbol{H}$. Eq. (8) now changes to:

$$e^{ES} = \boldsymbol{H}(1 + \phi K_A/K_B) \quad (10)$$

while Eq. (9) changes to:

$$\boldsymbol{H} = -\frac{K_A}{K_B}\phi \boldsymbol{H} + e^{ES} \quad (11)$$

Note that $\boldsymbol{H}$ represents the relative height of experimentally determined concentration–effect curves, whereas h, in Eq. (8) and Eq. (9), represents the relative height of concentration-stimulus curves. According to Eq. (11) a straight line should be obtained when $\boldsymbol{H}$ is plotted against $\phi \boldsymbol{H}$. It should be noted that such a straight line will only be obtained if a linear relationship exists between $\boldsymbol{H}$ and $\phi \boldsymbol{H}$, while the latter would realize if the submaximal concentration–effect curves have the same heights as do their corresponding concentration-stimulus curves, i.e., $H_{AB} = h_{AB}$. The equilibrium dissociation constant, K_A, of the agonist-receptor complex may be estimated from the slope of the straight line if K_B is known: slope $= -K_A/K_B$.

The quantity e^{ES} represents an effect-stimulus parameter which may be estimated directly by employing Eq. (10) if K_A and K_B are known, or graphically by employing Eq. (11). It follows from Eq. (11) that the value of e^{ES} is

given by the intercept of the straight line with the ordinate ($\boldsymbol{H}$-axis), since $\boldsymbol{H} = e^{ES}$ if $\phi\boldsymbol{H} = 0$. The value of $\phi\boldsymbol{H}$ will be zero in the absence of a competitive antagonist B, thus, if [B] = 0, then $\phi = [B]/[A] = 0$ and hence $\phi\boldsymbol{H} = 0$.

In practice, however, the e^{ES} value of an agonist may be defined as: $e^{ES} = h_m/H_m$, where h_m represents the maximal height of the stimulus curve of A in the absence of B (see Fig. 3). As illustrated in Fig. 3, the concentration–effect curves of different full agonists will have the same height. Therefore, for full agonists acting on the same effector the quantity H_m (height of an agonistic effect curve) will be a constant. It follows from $e^{ES} = h_m/H_m$ that the parameter e^{ES} is directly related to the height h_m of the stimulus curve of A. Since efficacy e denotes the power of an agonist to produce a stimulus (Kenakin, 1987a), the height of the agonistic concentration-stimulus curve will be directly related to efficacy e. It is thus obvious that e^{ES} should also be directly related to e, therefore, an e^{ES} value would indicate whether or not spare receptors are present for an agonist–effector system. It follows further from the definition of an e^{ES} value that spare receptors would be present when $e^{ES} > 1.0$, while $e^{ES} = 1.0$ indicates the absence of spare receptors.

Although it was assumed that the concentration–effect and concentration-stimulus curves should coincide (Venter, 1996), it should be noted at this stage that this assumption is only important for the estimation of exact K_A values, because, if these curves do not coincide one would only be able to estimate apparent K_A values (Venter, 1996). For the estimation of e^{ES} values the only prerequisite is that the heights of the submaximal concentration–effect curves and their concentration-stimulus curves should be equal, i.e., $H_{AB} = h_{AB}$.

It should be noted that if $H_{AB} = h_{AB}$ then a straight line should also be obtained if H_{AB} is plotted against ϕH_{AB}, because it follows from Eq. (11) that:

$$H_{AB} = -\frac{K_A}{K_B}\phi H_{AB} + E \qquad (12)$$

Note that the intersect with the ordinate is denoted as E and that the absolute value of this ordinate intersect depends on the units in which effect curve height H_{AB} were measured. If one keep the latter important fact in mind, then it is obvious that one should always utilize relative effect curve heights ($\boldsymbol{H}$) when estimating e^{ES} values. By adhering to this prerequisite a standardized and useful scale for e^{ES} values would result.

2.2. Practical estimation of the e^{ES} value

The parameter e^{ES} may be estimated when a submaximal effect is obtained with a fixed agonist-competitive antagonist combination. For the practical estimation of e^{ES} the height (H_{AB}) of various submaximal concentration–effect curves, obtained with different agonist-antagonist combinations (different ϕ values), are measured relative to the maximal height H_m of the agonistic concentration–effect curve determined in the absence of a competitive antagonist. The value of $\boldsymbol{H}$ will be maximal, i.e., $\boldsymbol{H} = 1$, if $H_{AB} = H_m$, and it follows that the relative height $\boldsymbol{H}$ of the concentration–effect curve reflecting maximal curve height, also reflects maximal effect, namely $E_{AB}/E_m = 1$ (see: Fig. 1 and Fig. 2, Tables 1 and 2). Therefore, the effect curve heights (H_{AB}) may be measured as fractions of the maximal effect (see Tables 1 and 2), and the relative curve heights $\boldsymbol{H} = H_{AB}/H_m$ are then plotted against $\phi\boldsymbol{H}$ (see insets: Fig. 1 and Fig. 2).

2.2.1. Theoretical concentration–effect curves: utilizing effect curves determined with fixed agonist-antagonist combinations

The theoretical concentration-stimulus curves in Fig. 1 and Fig. 2 were calculated according to Eq. (4). The values of K_A, K_B and e were kept constant ($K_A = 1$ μM, $K_B = 5$ μM, $e = 1$) while the value for ϕ were increased gradually from zero to 16. The theoretical concentration–effect curves shown in Fig. 1 were determined for an agonist–effector system supposed to be void of spare receptors and it was therefore assumed that the maximal effect ($E_{AB}/E_m = 1$) occurs at a stimulus value of $S_{AB}/S_m = 1.0$ (Fig. 1). In this case the concentration-stimulus curves and concentration–effect curves coincided completely.

The concentration–effect curves shown in Fig. 2 were obtained by assuming that spare receptors are present in the agonist–effector system in question and the theoretical concentration–effect curves shown in Fig. 2 were constructed as described by Venter (1994, 1996). The presence of spare receptors in the agonist–effector system were simulated by arbitrarily assuming that the maximal effect ($E_{AB}/E_m = 1$) is obtained at a stimulus value of $S_{AB}/S_m = 0.714286$. Note, that the value $S_{AB}/S_m = 0.714286$ was simply chosen for convenience, and, as can be seen in Fig. 2 this particular stimulus value corresponds exactly to the maximal effect ($E_{AB}/E_m = 1$) of a concentration–effect curve obtained when $\phi = 2$. The value $\phi = 2$ represents a special ϕ value, namely ϕ_{min}, for which the maximal effect is obtained when $H_m = h_{AB}$. The value ϕ_{min} can be defined as the minimum value of ϕ that mediates a concentration–effect curve which produces the maximal possible effect on the effector, namely $E_{AB}/E_m = 1$, while the height of the effect curve equals the height of its corresponding stimulus curve ($H_m = h_{AB}$). Since the concentration–effect curve in question depicts the maximal possible effect on an effector it follows that $\boldsymbol{H} = 1$ for this particular effect curve, and thus, the parameter ϕ_{min} may be calculated by employing the following equation which is derived by substituting $\boldsymbol{H} = 1$ in Eq. (11):

$$\phi_{min} = (e^{ES} - 1)/\text{slope} \qquad (13)$$

If the notation S_{Em}/S_m is assigned to the specific stimulus value which corresponds to maximal effect, then it follows that $S_{AB}/S_m = S_{Em}/S_m$ when $E_{AB}/E_m = 1$. The value of S_{Em}/S_m may be calculated from the e^{ES} value if the maximal stimulus is taken as unity ($S_{AB}/S_m = 1$). It now follows that:

$$S_{E_m}/S_m = 1/e^{ES} \quad (14)$$

2.3. Estimation of relative efficacy

For the estimation of relative efficacy one needs to compare the efficacies of two agonists. If the efficacies (e) of various members of an agonistic family are all expressed in relation to the efficacy of one reference compound from the same family, then the relative efficacy thus obtained would be a suitable basis for comparing the efficacies within the family in question. In principle it is immaterial which drug serves as a reference; the choice only determines the scale on which the relative efficacies of the members of a family are expressed and has no influence on their mutual ratio. Theoretically, however, it seems preferable to express the efficacy of an agonist in relation to the greatest efficacy in the family. If the relative efficacy expressed in this manner is indicated as e^R, then relative efficacy may be defined as: $e^R = e/e_m$, in which e is the (absolute) efficacy of an agonist A and e_m the maximal (absolute) efficacy in the family.

A full agonist with maximal efficacy e_m is able to cause the maximal stimulus S_m that can be brought about via the receptor system in question. Since e is directly related to e^{ES}, the full agonist which possesses maximal e^{ES}, namely e_m^{ES}, should also possess maximal efficacy (e_m). It follows from the definition of relative efficacy that:

$$e^R = \frac{e}{e_m} = \frac{e^{ES}}{e_m^{ES}} \quad (15)$$

Eq. (15) affords the possibility to estimate e^R without any knowledge of the agonistic affinity values (K_A). The e^{ES} values of various agonists may be estimated on the same effector and their relative efficacies e^R would then be expressed in relation to e_m^{ES}, the maximal e^{ES} value in the agonistic family.

The relative efficacy e^R of the two full agonists A_1 and A_2 may also be estimated by employing single submaximal curves determined by employing fixed agonist-antagonist combinations (Fig. 3). The heights of these submaximal concentration–effect curves relative to each other are represented by $\boldsymbol{H}_{1sub}$ and $\boldsymbol{H}_{2sub}$. It follows, analogous to Eq. (10), that the following equation is valid for a fixed combination of agonist A_1 and a competitive antagonist B:

$$e_1^{ES} = \boldsymbol{H}_{1sub}\left(1 + \phi_1 K_{A_1}/K_B\right) \quad (16)$$

For a fixed combination of agonist A_2 and the same competitive antagonist B it follows that:

$$e_2^{ES} = \boldsymbol{H}_{2sub}\left(1 + \phi_2 K_{A_2}/K_B\right) \quad (17)$$

Combination of Eq. (16) and Eq. (17) with Eq. (15) gives:

$$e_R = \frac{e_1}{e_2} = \frac{\boldsymbol{H}_{1sub}\left(1 + \phi_1 K_{A_1}/K_B\right)}{\boldsymbol{H}_{2sub}\left(1 + \phi_2 K_{A_2}/K_B\right)} \quad (18)$$

from which relative efficacy e^R of agonists A_1 and A_2 can be estimated if K_{A_1} and K_{A_2} and K_B are known. Ideally, as indicated previously, e_2 should be the maximal efficacy, namely e_m, in the family of agonists. Combination of Eq. (7) and Eq. (18) give rise to:

$$e_R = \frac{e_1}{e_2} = \frac{\boldsymbol{H}_{1sub}[A_2]_{1/2} K_{A_1}}{\boldsymbol{H}_{2sub}[A_1]_{1/2} K_{A_2}} \quad (19)$$

As can be expected, it follows from Eq. (19) that the value of relative efficacy e^R does not depend on K_B or the ratio $\phi = [B]/[A]$. Note that K_{A_1} and K_{A_2} may be actual affinity values or apparent affinity values, since:

$$\frac{K_{A_1}(\text{actual})}{K_{A_2}(\text{actual})} = \frac{K_{A_1}(\text{apparent})}{K_{A_2}(\text{apparent})} \quad (20)$$

The estimation of relative efficacy by utilizing Eq. (19) is illustrated in Fig. 3 which shows the concentration-stimulus curves and simulated concentration–effect curves of two full agonists, A_1 and A_2, in the absence and presence of a competitive antagonist B. The concentration-stimulus curves of A_1 and A_2 were calculated according to Eq. (4). For agonist A_1 the following values were used: $K_{A_1} = 1$ μM, $e_1 = 1$, $\phi = 4$. The values used for agonist A_2 were: $K_{A_2} = 10$ μM, $e_2 = 0.8$, $\phi = 0.1$.

2.4. Estimation of relative intrinsic efficacy

Efficacy (e) is a drug- and tissue-related term, whereas intrinsic efficacy is supposed to be a drug-receptor parameter (Kenakin, 1985). The importance of the latter parameter lies in the fact that it is unique for each drug-receptor interaction and does not depend on species, type of tissue, or type of effect. Therefore, it can be used to classify drugs and drug receptors. Accurate measurements of relative intrinsic efficacy and K_A values form the basis of receptor pharmacology (Kenakin, 1987a).

It follows from the definition of intrinsic efficacy, namely $= e/[R]_T$, and Eq. (15) that:

$$\text{Relative intrinsic efficacy} = \frac{1}{2} = \frac{e_1^{ES}[R_2]_T}{e_2^{ES}[R_1]_T} \quad (21)$$

where $[R_1]_T$ and $[R_2]_T$ refer to the tissue concentrations of receptors. Analogous to the derivation of Eq. (19), it

follows from the combination of Eq. (16), Eq. (17) and Eq. (7) with Eq. (20) that:

$$\frac{1}{2} = \frac{\boldsymbol{H}_{1\text{sub}}[\mathrm{A}_2]_{1/2}[\mathrm{R}_2]_{\mathrm{T}}K_{\mathrm{A}_1}}{\boldsymbol{H}_{2\text{sub}}[\mathrm{A}_1]_{1/2}[\mathrm{R}_1]_{\mathrm{T}}K_{\mathrm{A}_2}} \qquad (22)$$

Eq. (21) is applicable when $_1/_2$ is determined on different effectors. As mentioned in Section 2.3, K_{A_1} and K_{A_2} may be actual or apparent affinity values. If relative intrinsic efficacies for different agonists are determined on the same effector, then $[\mathrm{R}_1]_{\mathrm{T}} = [\mathrm{R}_2]_{\mathrm{T}}$ and Eq. (21) simplifies to:

$$\frac{1}{2} = \frac{H_{1\text{sub}}[\mathrm{A}_2]_{1/2}K_{\mathrm{A}_1}}{H_{2\text{sub}}[\mathrm{A}_1]_{1/2}K_{\mathrm{A}_2}} \qquad (23)$$

which is the equivalent of Eq. (19).

3. Results

The simulated concentration–effect curves in Fig. 1 and Fig. 2 illustrate the influence of a competitive antagonist B on an agonist A for different values of $\phi = [\mathrm{B}]/[\mathrm{A}]$ (ϕ = 1, 2, 4, 8 and 16). In Fig. 2 submaximal effects were obtained for ϕ = 4, 8 and 16. The EC_{50} of the full agonist A in Fig. 1 was obtained at 1.0 μM, while the EC_{50} of the full agonist A in Fig. 2 was obtained at 5.5556×10^{-1} μM. the curve heights (H_{AB}) and the relative curve heights ($\boldsymbol{H}$) were acquired from Fig. 1 and Fig. 2 and are shown in Tables 1 and 2, respectively. By plotting $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ straight lines were obtained (insets: Fig. 1 and Fig. 2). Data from Fig. 1 produced the following linear equation:

$$\boldsymbol{H} = -0.2\phi\boldsymbol{H} + 1.0. \qquad (24)$$

It followed from the intercept of the straight line with the ordinate that $e^{\mathrm{ES}} = 1.0$. The latter value indicates the absence of spare receptors for maximal effect and that the maximal stimulus curve height is equal to the maximal effect curve height, i.e., $h_{\mathrm{m}}/H_{\mathrm{m}} = 1.0$. From the slope of the straight line ($K_{\mathrm{A}}/K_{\mathrm{B}} = 0.2$) it followed that $K_{\mathrm{A}} = 1$ μM if $K_{\mathrm{B}} = 5$ μM.

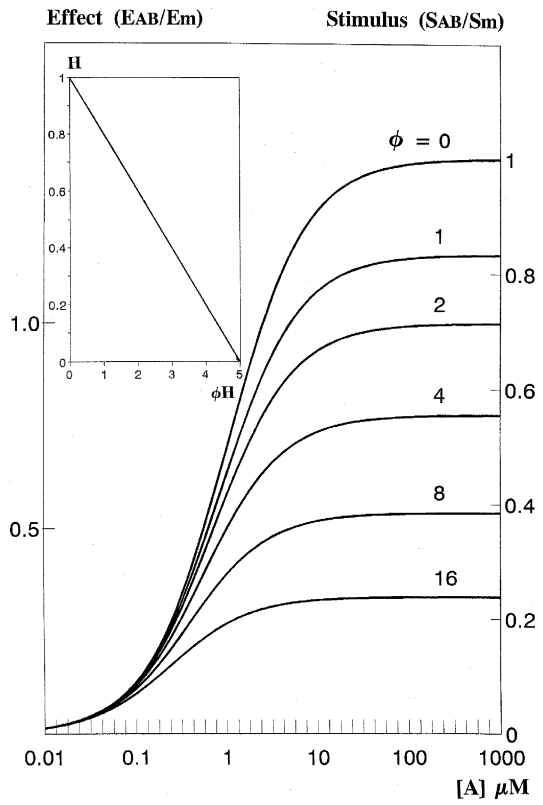

Fig. 1. Linear stimulus–effect relationship. Theoretical concentration-stimulus curves of a full agonist A combined in a fixed ratio with a competitive antagonist B. The curves were calculated according to Eq. (4): $K_{\mathrm{A}} = 1$ μM, $K_{\mathrm{B}} = 5$ μM, $e = 1$, $\phi = [\mathrm{B}]/[\mathrm{A}] = 0$–16. The simulated concentration–effect curves coincide completely with the concentration-stimulus curves and the heights of the effect curves are given in Table 1. The maximal curve height (obtained when $\phi = 0$, i.e. [B] = 0) was taken as unity. Inset: plot of $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ (Table 1). Ordinate intercept: $\boldsymbol{H} = e^{\mathrm{ES}} = 1.4$; slope: $K_{\mathrm{A}}/K_{\mathrm{B}} = 0.2$.

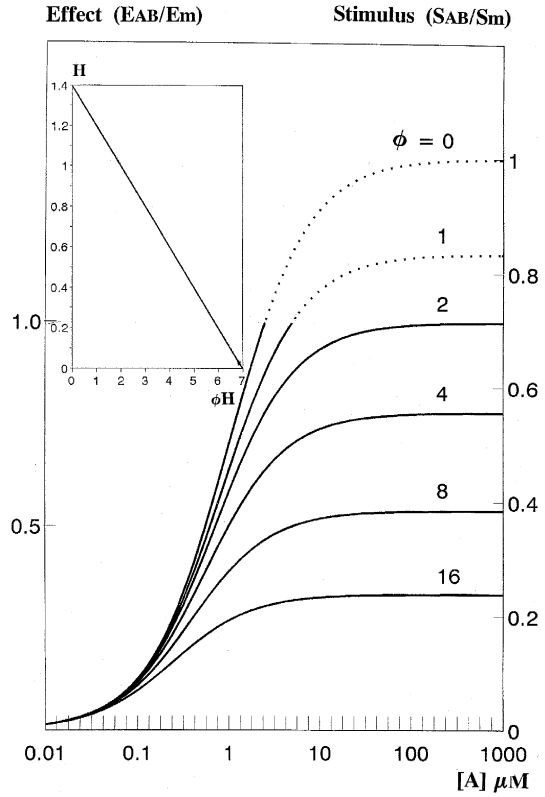

Fig. 2. Nonlinear stimulus–effect relationship. Theoretical concentration-stimulus curves (dotted lines) of a full agonist A combined in a fixed ratio with a competitive antagonist B. The curves were calculated according to Eq. (4): $K_{\mathrm{A}} = 1$ μM, $K_{\mathrm{B}} = 5$ μM, $e = 1$, $\phi = [\mathrm{B}]/[\mathrm{A}] = 0$–16. Simulated concentration–effect curves are represented as continues lines and coincide completely with the concentration-stimulus curves when submaximal effects are obtained, i.e. when the value of ϕ = 4, 8 and 16. The heights of these submaximal effect curves are given in (Table 2). The maximal curve height (obtained when $\phi = 0$, i.e. [B] = 0) was taken as unity. Inset: plot of $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ (Table 1). Ordinate intercept: $\boldsymbol{H} = e^{\mathrm{ES}} = 1.4$; slope: $K_{\mathrm{A}}/K_{\mathrm{B}} = 0.2$.

Table 1
Data obtained from theoretical concentration–effect curves determined with fixed agonist-competitive antagonist combinations (Fig. 1)

ϕ [a]	H_{AB} [b]	$\boldsymbol{H}$ [c]	$\phi\boldsymbol{H}$
2	1.0	1.0	2.0
4	0.555556	0.555556	2.222224
8	0.384615	0.384615	3.076920
16	0.238095	0.238095	3.809520

[a] ϕ = [competitive antagonist]/[agonist]. [b] Height of submaximal effect curves. H_{AB} is expressed as fraction of the maximal effect. [c] Relative curve heights. $\boldsymbol{H} = H_{AB}/H_m$, where H_m = maximal curve height of agonist concentration–effect curve in the absence of the competitive antagonist ($H_m = 1.0$).

By plotting $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ (Table 2) a straight line was obtained (inset: Fig. 2) which was described by the following linear equation:

$$\boldsymbol{H} = -0.2\phi\boldsymbol{H} + 1.4. \quad (25)$$

It followed from the intercept of the straight line with the ordinate that $e^{ES} = 1.4$. The latter value ($e^{ES} > 1.0$) is indicative of spare receptors for maximal effect and it follows that the ratio of maximal stimulus curve height to maximal effect curve height is 1.4 : 1.0, i.e., $h_m/H_m = 1.4$. If the maximal stimulus is taken as unity ($S_{AB}/S_m = 1$), then, according to Eq. (14) the maximal effect ($E_{AB}/E_m = 1.0$) would be obtained at a stimulus value of $S_{Em}/S_m = 0.714286$, while, according to Eq. (13) $\phi_{min} = 2.0$. From the slope of the straight line ($K_A/K_B = 0.2$) it followed that $K_A = 1$ μM if $K_B = 5$ μM.

Fig. 3 shows simulated concentration–effect curves of two full agonists (A_1 and A_2) determined in the absence and in combination of a competitive antagonist B. Relative efficacy of the two agonists, A_1 and A_2, were estimated by employing data obtained from Fig. 3. The height of the submaximal effect curve II_{sub} (obtained with a fixed [A_2] − [B] combination) was taken as unity, i.e., $\boldsymbol{H}_{2sub} = 1.0$, and the height of the submaximal effect curve I_{sub}, (obtained with an fixed [A_1] − [B] combination), relative to the height of curve II_{sub} was $\boldsymbol{H}_{1sub} = 0.5$. It followed from Fig. 3 that $[A_1]_{1/2} = 0.2$ μM and $[A_2]_{1/2} = 5.0$ μM. The relative efficacy of A_2 relative to A_1, namely $e^R = e_2/e_1 = 0.8$, was calculated according to Eq. (19). On the other hand, the same information could be obtained by determining the relative efficacy of A_1 relative to A_2. In the latter instance $e^R = e_1/e_2 = 1.25$ (= 1/0.8).

Table 2
Data obtained from theoretical concentration–effect curves determined with fixed agonist-competitive antagonist combinations (Fig. 2)

ϕ [a]	H_{AB} [b]	$\boldsymbol{H}$ [c]	$\phi\boldsymbol{H}$
2	1.0	1.0	2.0
4	0.777778	0.777778	3.111112
8	0.538461	0.538461	4.307686
16	0.333333	0.333333	5.333326

[a] ϕ = [competitive antagonist]/[agonist]. [b] Height of submaximal effect curves. H_{AB} is expressed as fraction of the maximal effect. [c] Relative curve heights. $\boldsymbol{H} = H_{AB}/H_m$, where H_m = maximal curve height of agonist concentration–effect curve in the absence of the competitive antagonist ($H_m = 1.0$).

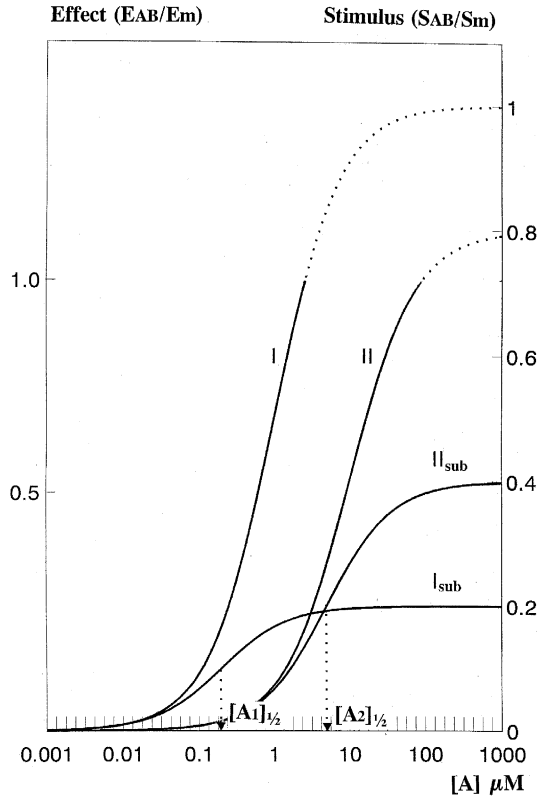

Fig. 3. Estimation of relative efficacy. Theoretical concentration-stimulus curves (dotted lines) of two full agonists A_1 (curve I) and A_2 (curve II) in the absence and presence of a competitive antagonist B. The curves were calculated according to Eq. (4): for A_1, $K_A = K_B = 1$ μM, $e = 1$, $\phi = 1.0$ and for A_2, $K_A = 10$ μM, $K_B = 1$ μM, $e = 0.1$, $\phi = 0.8$. The relative heights of the submaximal curves, I_{sub} and II_{sub}, are represented by $\boldsymbol{H}_{1sub}$ and $\boldsymbol{H}_{2sub}$ respectively, while the ratio $\boldsymbol{H}_{2sub} : \boldsymbol{H}_{1sub} = 1.0 : 0.5$. Half curve heights $\boldsymbol{H}_{1sub}/2$ and $\boldsymbol{H}_{2sub}/2$ are obtained at $[A_1]_{1/2} = 0.2$ μM and $[A_2]_{1/2} = 5.0$ μM, respectively.

The relative efficacy e^R was also calculated according to Eq. (15). The e^{ES} values of A_1 and A_2 were calculated by employing Eq. (10). The heights of the submaximal effect curves I_{sub} and II_{sub} (in Fig. 3) relative to the maximal curve height obtained when [B] = 0 were determined as $\boldsymbol{H}_1 = 0.2857142$ and $\boldsymbol{H}_2 = 0.5714285$ respectively. The e^{ES} values for A_1 and A_2 were foundto be: $e_1^{ES} = 1.4285714$ and $e_2^{ES} = 1.1428571$ respectively. It followed from Eq. (15) that $e^R = e_2^{ES}/e_1^{ES} = 0.8$.

4. Discussion

It was necessary to define a new experimentally determinable stimulus–effect parameter e^{ES}, because absolute efficacy (e) for full agonists cannot be determined at this

stage. The practical importance of such a new efficacy related parameter e^{ES} lies, firstly, in the fact that for a particular biological object and a particular type of agonist, e^{ES} gives the ratio of the concentration-stimulus curve height to the concentration–effect curve height obtainable with an agonist in the absence of a competitive antagonist, and secondly, efficacy e is directly related to height of the stimulus curve (i.e., the maximal stimulus S_m) and the effect-stimulus parameter e^{ES}. It follows that e^{ES}, analogous to e, is a dimensionless factor denoting the power of an agonist to produce a stimulus. From the definition of e^{ES} it is evident that the value of e^{ES} is a function of receptor density, which means e^{ES} is tissue dependent and that its value depends upon tissue type. The parameter e^{ES} has a number of advantages: (1) an isolated e^{ES} indeed has a practical meaning, which is in contrast to an isolated efficacy (e) which is really meaningless, (2) a given value of e^{ES} would hold direct information about the position of an agonist within the agonist family and it should therefore form a basis for comparing closely related compounds within the family. The e^{ES} values of all agonistic families would range from 1.0 upward, i.e., $e^{ES} \geq 1$, and (3), since e^{ES} reflects the height of the concentration-stimulus curve relative to the height of the concentration–effect curve of a full agonist, the value of e^{ES} would indicate directly whether or not spare receptors for maximal effect are present in a drug–effector system. The magnitude of the e^{ES} value is indicative of the amount spare receptors at maximal effect. When a nonlinear stimulus–effect relationship prevails in the system the value of e^{ES} will differ from unity, i.e., if $e^{ES} > 1$ then $E_m < S_m$ and spare receptors for maximal effect will be present in the system. If $e^{ES} = 1$, then $E_m = S_m = 1$ and no spare receptors for maximal effect will be present in the system. In the latter instance it would of course still possible that a nonlinear stimulus–effect relationship may exist for the specific agonist-receptor interaction, i.e., spare receptors at half maximal effect. The latter phenomena were reported for a number of cases when virtually all the receptors in an effector need to be occupied for maximal effect, but only a small percentage (much smaller than 50%) are required for half-maximal effect (Kenakin, 1987b). For example, in rabbit aorta (Besse and Furchgott, 1976) and canine aorta (Sastre et al., 1984), noradrenaline needs to occupy 6% and 10% respectively of a total receptor pool to elicit half-maximal effects, but no spare receptors could be demonstrated at maximal effects. Usually, in these instances the concentration-stimulus curve and concentration–effect curve of an agonist would not coincide, in fact, these curves would be positioned at totally different locations above the concentration axis (Kenakin, 1987b), while heights of the stimulus curves and their corresponding effect curves would be the same.

It is generally believed that spare receptors at maximal effect is absent for partial agonists, and it is thus expected that $e^{ES} = 1$ for all partial agonists. The latter is true because in the estimation of e^{ES} the maximal relative curve height $\boldsymbol{H}$ is taken as unity, regardless whether the agonist in question is a full or partial agonists. Therefore, an e^{ES} on its own would not indicate whether or not a particular agonist is a full or partial agonist. This distinction should be made by comparing the relative maximal effects (relative intrinsic activity) for different agonists according to the classical methods generally used in pharmacology.

Although it was assumed in this study that the stimulus and effect curves should coincide, one would not expect such an overlapping of the curves to be a general phenomenon (Kenakin, 1987b; Venter, 1996). Even so, this assumption conveniently simplifies the theory without nullifying the model. In the application of Eq. (10) or Eq. (11) it is expected that postreceptor events should play no role in e^{ES} estimations, because the e^{ES} value depends only on reserve receptors for maximal effect (i.e., height of the stimulus curve) and is independent of agonist affinity (K_A). For the estimation of e^{ES} it is thus irrelevant whether or not the stimulus and effect curves coincides. The only really important aspect of the assumption is the prerequisite that submaximal concentration–effect curves should have the same height than their respective concentration-stimulus curves. If this latter part is not true, then the estimation of e^{ES} will be in error.

Although Eq. (10) and Eq. (11) may be utilized in the estimation of K_A values, one should note at this point that the correct value of K_A can only be estimated if the stimulus curves and effect curves coincide. If the latter prerequisite does not hold, then estimated K_A values would be incorrect and it would thus only be possible to estimate apparent K_A values (Venter, 1996). Unfortunately, it is generally not known before hand whether or not the curves would coincide, and therefore it is advisable that one should rather determine relative K_A values which should be very useful parameters in structure activity relationship studies (Venter, 1996).

By comparing the new method described in this paper to the well known null methods, it is important to note that null methods employ a double reciprocal approach, i.e., $1/[A]_1$ of agonist A_1 is plotted against $1/[A]_2$ of agonist A_2. Unfortunately this double reciprocal approach confers a considerable degree of inaccuracy into the null method. In the new method, on the other hand, $\boldsymbol{H}$ is plotted against $\phi\boldsymbol{H}$. Although the relationship $\phi = [B]/[A]$ relates the reciprocal of [A], it should be remembered that $\boldsymbol{H}$ is independent from the position of the agonistic curve and that $\boldsymbol{H}$ is, depending on the method employed, a precisely measurable quantity (see Venter, 1997). It follows, therefore, that in this regard the new method offers the possibility that quite accurate apparent K_A values (and thus relative K_A values) and relative efficacies may be estimated.

The new method also affords the possibility that relative efficacies of full agonists may be estimated. It is usual to

assign relative efficacies within a series of agonists, using a full agonist as reference. An efficacy of 1.0 is arbitrarily assigned to this full agonist (Furchgott and Bursztyn, 1967). With the methods currently employed to estimate relative efficacy, it has as yet not been possible to ascertain whether the full agonist in question really possesses the maximal efficacy in the particular family of agonists. It was, therefore, impossible to choose a reference compound and set a scale for relative efficacies. However, by applying Eq. (11) it now seems possible to establish which agonist(s) possesses the greatest efficacy in the family and a reference compound can accordingly be identified. The agonist with the greatest e^{ES} value in the family should also have the greatest efficacy in the family, and it follows, therefore, that every family of drugs will have its own scale for relative efficacy values. The relative efficacy e^{R} may be estimated by arbitrarily setting $e = 1$ for the agonist possessing the greatest e^{ES} value in the particular family of agonists. It is important to note that only e^{ES} values of full agonists should be used for determination of e^{R}, because, as was previously mentioned, e^{ES} should be equal to unity for all partial agonists. The e^{R} value may be obtained by employing either Eq. (15) or Eq. (19). For calculation of e^{R} via Eq. (19) it is only necessary that the apparent K_A values, estimated according to the proposed method, should be known. On the other hand, only the e^{ES} value of the agonists should be known when Eq. (15) is employed.

In conclusion, this new method for estimating relative efficacies of full agonists in a particular family seems to be superior to methods currently employed. A distinct advantage of the method is its ability to quantify receptor reserve as well as maximal stimulus, and estimate which full agonist in the family possesses the greatest efficacy. This specific agonist may then be used as a reference compound to which the efficacies of the other full agonists may be compared.

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