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Efficacy II. Estimation of a newly defined efficacy related parameter

Daniel P. Venter *

Department of Pharmacology, Potchefstroom University for Christian Higher Education, Potchefstroom 2520, South Africa

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Abstract

A new method for estimation of agonist-affinity (K_A) and relative efficacy was introduced. This method afforded a procedure by which relative efficacy may be estimated while the actual K_A values of agonist-receptor complexes are unknown. The relative efficacy may be estimated by employing a newly defined drug parameter, namely the e^{ES} value. The e^{ES} value is related to drug efficacy and is defined in such a manner that an isolated e^{ES} is a meaningful quantity which may indicate whether or not spare receptors are present in an agonist–effector system. The estimation of e^{ES} was based on the fact that fixed agonist-competitive antagonist combinations mimic partial agonists and mediate submaximal concentration–effect curves. However, for the practical estimation of e^{ES} one may employ data acquired from agonistic concentration–effect curves determined in the absence and presence of increasing concentrations of a competitive antagonist. This procedure was illustrated by utilizing theoretical concentration–effect curves and applied practically by estimating e^{ES} and K_A values acquired from sets of carbachol and salbutamol curves. The sets of carbachol and salbutamol concentration–effect curves were determined in the absence and presence of their respective competitive antagonists, namely tripitramine and pindolol.

Keywords: Pharmacological method, new; Efficacy estimation; Agonist affinity; Carbachol; Salbutamol

1. Introduction

Agonists belong to a specific group of drugs possessing two fundamental molecular characteristics which dictate the receptor binding ability of the agonist and activate (stimulate) the receptor. The former process is of a physico-chemical nature and can be described on a molecular level by the equilibrium dissociation constant of the agonist-receptor complex (K_A). The capacity of an agonist to activate a receptor is generally referred to as efficacy. Efficacy is a dimensionless proportionality factor denoting the power of an agonist to produce a stimulus, and eventually an effect, in a tissue (Kenakin, 1987). In general the true value of e is unimportant, since the meaning of an isolated e is meaningless. In contrast to affinity, efficacy (e) has no molecular definition and was defined by Stephenson (1956) as a constant of proportionality: $S = e \cdot \rho$, where S and ρ signify stimulus and receptor occupancy respectively.

It is important to note that efficacy is a tissue- and not receptor-dependent parameter. However, measurements of relative efficacy of two agonists in a single tissue would be receptor dependent since tissue factors, such as receptor density, would cancel (Kenakin, 1985). Therefore, relative efficacy is considered to be a receptor-specific parameter that theoretically is useful for the classification of drugs and drug receptors. To date there is no independent experimental method to verify estimates of efficacy or relative efficacy. Presently, known methods to quantify the relative efficacy of agonists require an accurate estimate of one or both of the equilibrium dissociation constants of the agonist-receptor complexes (K_A).

Venter (1996), however, described a method to determine an efficacy related parameter, namely e^{ES}, which is related to efficacy. The quantity e^{ES} was defined as an effect-stimulus parameter which is given by the ratio of the stimulus curve height (h_{AB}) to the effect curve height (H_{AB}), i.e., $e^{ES} = h_{AB}/H_{AB}$. It followed that spare receptors for maximal effect is present in a drug–effector system if $e^{ES} > 1$ and absent if $e^{ES} = 1$. It was also reported that the relative efficacy may be determined directly from e^{ES} values of two agonists. In contrast to previously described methods (null methods) the e^{ES} value of an agonist as well as relative efficacy may be estimated without any knowledge of the equilibrium dissociation constant K_A. Based on the theory described by Venter

* Corresponding author. Tel.: (27-148) 299-2247; Fax: (27-148) 299-2225; e-mail: fkldpv@puknet.puk.ac.za

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(1996, 1997), this paper forwards experimental procedures whereby e^{ES} values may be determined.

2. Materials and methods

2.1. Theory

The method for estimating e^{ES} values of agonists was 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 (Venter, 1996, 1997). An example of such submaximal concentration–effect curves determined with different fixed agonist-competitive antagonist ratios is shown in Fig. 1. Venter (1996) reported that the efficacy related e^{ES} value of an agonist A may be estimated by utilizing the various submaximal effect curve heights acquired from Fig. 1. Estimation of e^{ES} values was based on the following linear equation:

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

where ϕ represents a fixed agonist-antagonist concentration ratio, i.e., $\phi = [B]/[A]$, and $\boldsymbol{H}$ represents the relative heights of submaximal concentration–effect curves determined for different ϕ values. The relative effect curve height $\boldsymbol{H}$ was defined as: $\boldsymbol{H} = H_{AB}/H_m$, where H_{AB} and H_m respectively represent the experimentally measured submaximal effect curve height (in arbitrary units) and the maximal curve height. H_m therefore represents the effect curve height obtained with agonist A in the absence of the competitive antagonist B. The equilibrium dissociation constants K_A and K_B respectively denote the affinities of agonist A and competitive antagonist B for the receptors in question.

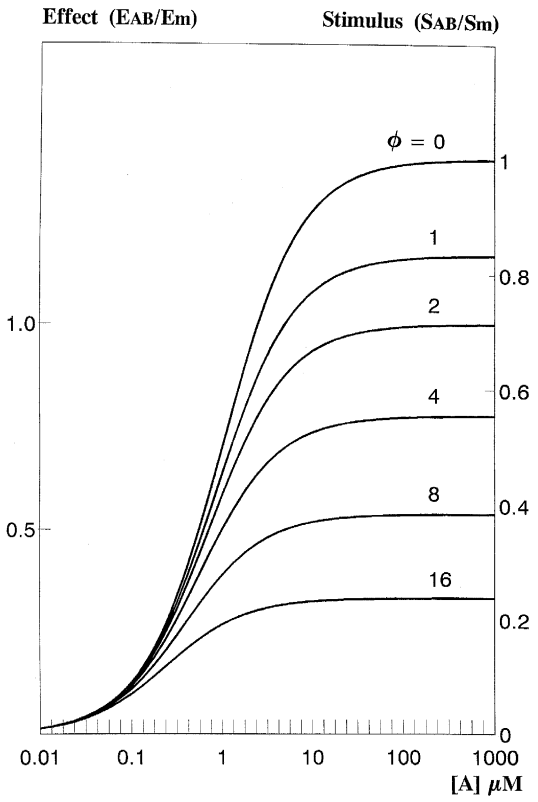

Fig. 1. Linear stimulus–effect relationship. Theoretical concentration–effect curves of fixed agonist-competitive antagonist combinations. Curves were calculated according to Eq. 4 in Venter (1997). $K_A = 1$ μM, $K_B = 5$ μM, $e = 1$, $\phi = [B]/[A] = 0$–16).

According to Eq. (1) a straight line should be obtained when $\boldsymbol{H}$ is plotted against $\phi\boldsymbol{H}$. It follows from this equation that the value of e^{ES} is given by the intercept of the straight line with the ordinate ($\boldsymbol{H}$-axis) (see insets: Fig. 2 and Fig. 3), while the dissociation constant K_A may be estimated from the slope of the straight line if K_B is known: slope $= -K_A/K_B$.

Since e^{ES} is directly related to efficacy (e) (Venter, 1997), it follows that the relative efficacy e^R of two agonists may be calculated according to:

$$e^R = \frac{e_1}{e_2} = \frac{e_1^{ES}}{e_2^{ES}}$$

where e_1^{ES} and e_2^{ES} represent the e^{ES} values of agonists A_1 and A_2 respectively and e_1 and e_2 signify the respective efficacies of A_1 and A_2.

It is important to note that the absolute value of e^{ES} depends on the units in which the effect curve heights were measured, therefore, different units will lead to different e^{ES} values for any particular agonist. To be meaningful the scale for e^{ES} values should be standardized by setting it as a prerequisite that only relative heights should be employed when applying Eq. (1). The maximal height of the agonist curve obtained in the absence of a competitive antagonist should be set equal to unity and the submaximal effect curve heights should then be expressed in relation to the maximal height. By adhering to this prerequisite the e^{ES} values of various agonists may be compared. In contrast to efficacy an isolated e^{ES} in itself is a meaningful quantity since it would indicate the absence or presence of spare receptors for maximal effect. As mentioned, spare receptors will be present when $e^{ES} > 1.0$ and absent when $e^{ES} = 1.0$. Since the actual value of e^{ES} relates to the quantity of spare receptors, it follows that the larger e^{ES} the more spare receptors will be present in the agonist–effector system in question.

2.2. Practical estimation of e^{ES} and K_A

The practical application of Eq. (1) and the estimation of e^{ES} and K_A values may be illustrated by employing theoretical and experimentally determined concentration–effect curves. The relative effect curve heights $\boldsymbol{H}$ may be

obtained directly from sets of agonist concentration–effect curves determined with fixed agonist-competitive antagonist mixtures (Fig. 1) (Venter, 1997), or indirectly from agonist effect curves determined in the absence and presence of increasing concentrations of a competitive antagonist (see Section 2.2.1 and Section 2.2.2). Because of the ease at which reliable sets of agonistic concentration–effect curves can be determined in the presence of increasing concentrations of a competitive antagonist, this study concentrated mainly on the estimation of e^{ES} and K_A values by utilizing these readily available sets of concentration–effect curves.

2.2.1. Theoretical concentration–effect curves: utilizing sets of agonist curves determined in the presence of a competitive antagonist

Theoretical concentration-stimulus curves of a full agonist A (Fig. 2 and Fig. 3), in the absence and presence of increasing concentrations of a competitive antagonist B, were calculated according to the following equation

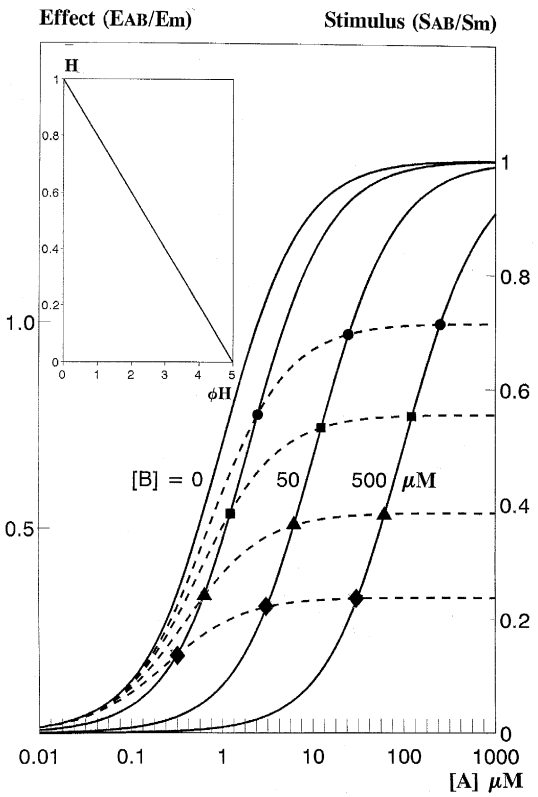

Fig. 2. Linear stimulus–effect relationship. Theoretical concentration-stimulus curves of a full agonist A, in the absence and presence of increasing concentrations of a competitive antagonist B, calculated according to Eq. (2): $K_A = 1$ μM, $K_B = 5$ μM, $e = 1$, [B] = 0, 5, 50 and 500 μM. Concentration–effect curves are represented as continues lines and coincide exactly with the calculated concentration-stimulus curves. The broken lines represent concentration–effect curves of fixed [agonist]/[antagonist] ratios (ϕ). The various markers represent positions of constant ϕ and were calculated according to ϕ = [B]/[A] (●, ϕ = 2; ■, ϕ = 4; ▲, ϕ = 8; ◆, ϕ = 16). Inset: plot of $\boldsymbol{H}$ against ϕ$\boldsymbol{H}$ (Table 1). Ordinate intercept: $\boldsymbol{H} = e^{ES} = 1.0$; slope: $K_A / K_B = 0.2$.

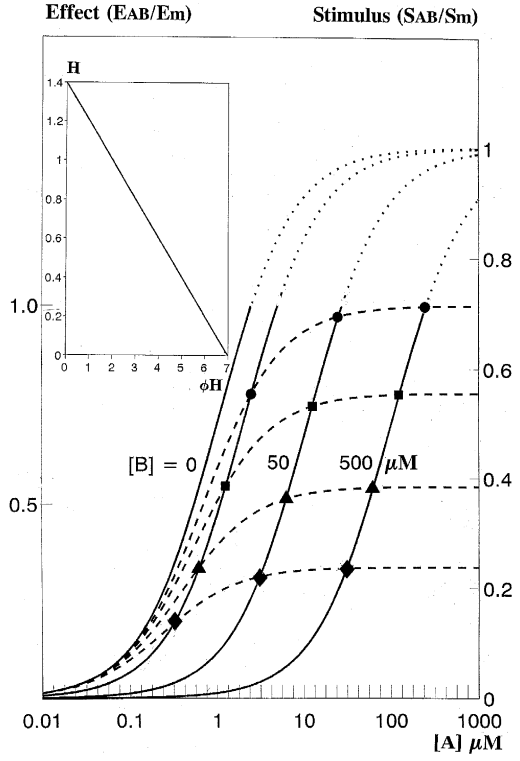

Fig. 3. Nonlinear stimulus–effect relationship. Theoretical concentration-stimulus curves (dotted lines) of a full agonist A in the absence and presence of increasing concentrations of a competitive antagonist B, calculated according to Eq. (2): $K_A = 1$ μM, $K_B = 5$ μM, $e = 1$, [B] = 0, 5, 50 and 500 μM. Concentration–effect curves are represented as continues lines and partially coincide with the concentration-stimulus curves. The broken lines represent concentration–effect curves of fixed [agonist]/[antagonist] ratios (ϕ). The various markers represent positions of constant ϕ and were calculated according to ϕ = [B]/[A] (●, ϕ = 2; ■, ϕ = 4; ▲, ϕ = 8; ◆, ϕ = 16). Inset: plot of $\boldsymbol{H}$ against ϕ$\boldsymbol{H}$ (Table 2). Ordinate intercept: $\boldsymbol{H} = e^{ES} = 1.4$; slope: $K_A / K_B = 0.2$.

(Stephenson, 1956; Ariëns et al., 1964; Venter, 1996, 1997):

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

S_{AB}/S_m signifies the fraction of the maximal stimulus of agonist A in the presence of competitive antagonist B, while [A] and [B] represent the concentration of the agonist A and competitive antagonist B respectively.

The stimulus generated by different concentrations of agonist A where calculated by keeping the values of K_A, K_B and e constant while the value of [B] was increased. The theoretical concentration–effect curves shown in Fig. 2 and Fig. 3 simulate experimental concentration–effect curves and were constructed as described by Venter (1994, 1996, 1997). The theoretical concentration–effect curves shown in Fig. 2 were determined on an agonist–effector system assumed to be void of spare receptors and therefore the maximal effect ($E_{AB}/E_m = 1$) and zero effect where

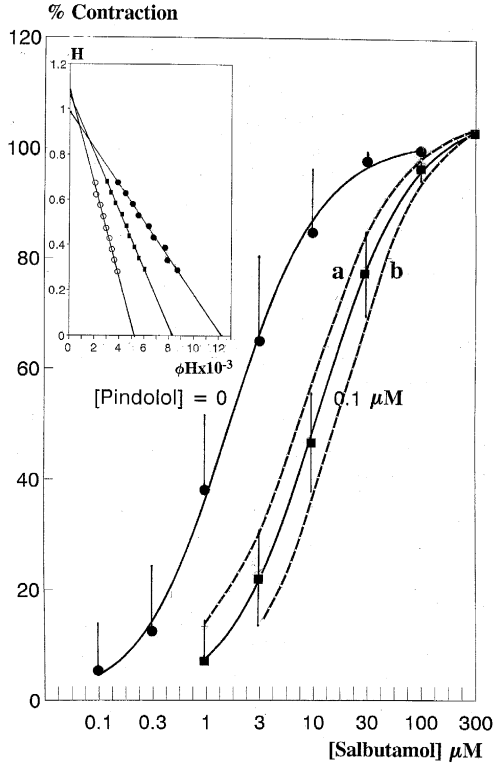

Fig. 4. Salbutamol-pindolol. Antagonism of salbutamol-induced positive chronotropic effect on cardiac β_1-adrenoceptors of guinea-pig atrium. Concentration–effect curves for salbutamol were obtained before (●) and after exposure to 0.1 μM (■) pindolol. Each point on the continues lines is the mean ± S.E. (E_{mean} ± S.E.) of six observations. Inset: plots of relative curve heights $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ for a fixed salbutamol-pindolol combination (Table 3). (■) E_{mean} ± S.E. – Eq. (3a), ordinate intercept: 1.06 ± 0.01; slope: $K_A/K_B = 128.7 \pm 3.5$; correlation coefficient, R: 0.997. (○) E_{mean} + S.E. – Eq. (3b), ordinate intercept: 1.08 ± 0.01; slope: $K_A/K_B = 205.9 \pm 5.5$; correlation coefficient, R: 0.999. (●) E_{mean}-S.E. – Eq. (3c), ordinate intercept: 0.99 ± 0.01; slope: $K_A/K_B = 80.8 \pm 1.7$; correlation coefficient, R: 0.998. All the data points were fitted to their respective straight lines by the method of least squares.

obtained at stimulus values of $S_{AB}/S_m = 1.0$ and $S_{AB}/S_m = 0$ respectively. On the other hand Fig. 3 shows theoretical concentration–effect curves when spare receptors are assumed to be present for an agonist–effector system. In this case the maximal effect ($E_{AB}/E_m = 1$) was assumed to be generated by a stimulus value of $S_{AB}/S_m = 0.714286$, while zero effect was obtained when $S_{AB}/S_m = 0$. The value of $S_{AB}/S_m = 0.714286$ was chosen because it corresponds to ϕ_{min}, where $\phi_{min} = (e^{ES} - 1)/\text{slope}$ and slope $= K_A/K_B$ in Eq. (1). Venter (1997) defined the quantity ϕ_{min} as the minimum value of ϕ that causes a concentration–effect curve possessing the maximal possible effect on the effector in question.

By utilizing these sets of concentration–effect curves and applying the method described by Venter (1996), one could easily calculate ϕ (= [B]/[A]) at various curve heights of choice. In Fig. 2 and Fig. 3 the heights representing equal ϕ values were marked with identical markers on the concentration–effect curves of agonist A. The heights of the submaximal concentration–effect curves (broken lines) that would have been mediated by fixed agonist-antagonist combinations, were now obtained by linking the various calculated positions (see Fig. 2 and Fig. 3). As can be seen in these figures the curves mediated by fixed agonist-antagonist ratios ascend from zero effect to their maximal effect over a relatively short concentration span. Therefore, when employing agonistic curves determined in the presence of increasing concentrations of a competitive antagonist one may usually accept that the maximal effect for a fixed agonist-antagonist combination would have been reached in the presence of a certain antagonist concentration causing the agonistic curve to shift more or less by a factor ten (ten concentration units) to the right. It is plausible, however, to calculate ϕ at the same height for two or more competitive antagonist concentrations as was done for Fig. 2 and Fig. 3. If the ϕ values calculated at the same height for different concentration–effect curves are the same, or differ within acceptable experimental errors, then it should be safe to assume that one is working with the true maximal heights of the agonist-antagonist combinations. The most appealing aspect of the latter procedure is the possibility that it can be performed at any curve height(s) (H_{AB}) of choice.

2.2.2. Experimental concentration–effect curves: utilizing sets of agonist curves determined in the presence of a competitive antagonist

To illustrate the practicability and potential of the new method various agonist parameters were estimated by utilizing experimentally determined sets of carbachol and salbutamol concentration–effect curves. The set of salbutamol curves was determined in the absence and presence of the competitive antagonist pindolol on β-receptors of

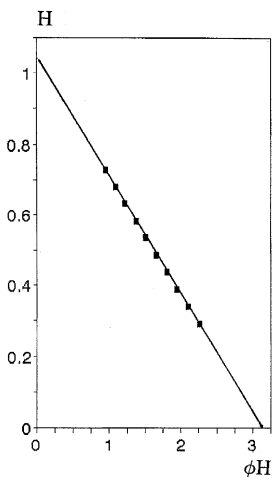

Fig. 5. Carbachol-tripitramine. A plot of relative curve heights $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ for carbachol-tripitramine combinations on M_3-receptors of guinea-pig ileum (Table 4) (Chiarini et al., 1995). Ordinate intercept: 1.046 ± 0.004; slope: $K_A/K_B = 0.336 \pm 0.003$; correlation coefficient, R: 0.999. The data points were fitted to a straight line by the method of least squares.

guinea-pig atrium (Section 2.3) (Fig. 4), while the set of carbachol curves shown in Fig. 5 were determined by Chiarini et al. (1995). The latter set of curves were determined in the absence and presence of increasing concentrations of the competitive antagonist tripitramine on muscarinic M_3-receptors of guinea-pig ileum.

From the set of salbutamol concentration–effect curves shown in Fig. 4 one could determine the ϕ values (= [pindolol]/[salbutamol]) at predetermined heights. These heights (H_{AB}) represent the various maximal heights of submaximal concentration–effect curves that should have been reached by fixed salbutamol-pindolol combinations (see Section 2.2.1). The different values of ϕ were only determined from the 'linear' section of the sigmoidal shaped concentration–effect curves (between 30% and 70% effect), because in a system with a possible receptor reserve the upper section of the concentration–effect curves reflecting a maximal effect should be 'linear' as can be seen in Fig. 2 and Fig. 3. The ϕ values, curve heights H_{AB} and relative curve heights $\boldsymbol{H}$ were tabulated in Table 3. In this case the curve heights were measured in centimeters.

The same procedure as described above was applied to the set of carbachol concentration–effect curves obtained from the literature. The ϕ values, submaximal curve heights H_{AB} and relative curve heights $\boldsymbol{H}$ were tabulated in Table 4. As in the previous case submaximal curve heights H_{AB} were measured in centimeters.

2.3. Determination of experimental concentration–effect curves

Adult guinea-pigs of both sexes, with an average mass of 400 g, were killed by a sharp blow on the skull, their hearts were rapidly removed and immersed in Tyrode solution of the following composition (mM): NaCl 136.9, KCl 5.4, $MgCl_2.6H_2O$ 0.5, $CaCl_2.2H_2O$ 1.4, $NaHCO_3$ 11.9 and glucose 5.5. The atria were carefully dissected from the ventricles and suspended in an organ bath containing Tyrode solution at 37°C at a tension of 1 g and oxygenated with carbogen (95% O_2 and 5% CO_2). The spontaneously beating atria were attached to a force displacement transducer (Grass model FTO,03). The amplified output from the force displacement transducer was used to drive a tachograph (Grass model 7 P4 AB). This arrangement allowed continuous monitoring of rate. Before drugs were investigated, the preparation was washed several times and allowed to stabilize until the spontaneous rate did not change by more than ±3% during a 10-min observation period. This usually required about 60 min. By employing the technique described by Van Rossum (1963) cumulative concentration–effect curves were obtained for salbutamol alone and in the presence of a 0.1 μM pindolol concentration.

2.3.1. Data analysis

The apparent dissociation constant (K_B) for pindolol was derived from the equation K_B = [pindolol]/(dose ratio − 1) (Furchgott, 1972). Data are represented as means ± S.E. of 6 experiments.

2.3.2. Drugs

Pindolol and salbutamol were purchased from Sigma.

3. Results

Fig. 2 and Fig. 3 show theoretical concentration–effect curves and concentration-stimulus curves of a full agonist A in the absence and presence of increasing concentrations of a competitive antagonist B calculated according to Eq. (2). The broken lines in Fig. 2 and Fig. 3 represent concentration–effect curves of fixed agonist-antagonist combinations (for which ϕ = 2, 4, 8 and 16), deduced by employing data obtained from simulated concentration–effect curves determined with A in the presence of various [B]. The curve heights (H_{AB}) were measured as fractions of the maximal effect ($E_{AB}/E_m = 1.0$) (Tables 1 and 2), and the relative curve heights $\boldsymbol{H} = H_{AB}/H_m$ were plotted against $\phi\boldsymbol{H}$ yielding the respective straight lines (insets: Fig. 2 and Fig. 3). Although not shown, it is obvious that a plot of H_{AB} against ϕH_{AB} would also produce a straight line.

Data from Fig. 2 produced the following linear equation:

$$\boldsymbol{H} = -0.2\phi\boldsymbol{H} + 1.0.$$

It followed from the intercept of the straight line with the ordinate that $e^{ES} = 1.0$. This result is to be expected since an e^{ES} value of one points to the absence of spare receptors for maximal effect (Venter, 1997). If the maximal stimulus is taken as unity ($S_{AB}/S_m = 1.0$), then the maximal effect ($E_{AB}/E_m = 1.0$) would be obtained at a stimulus value of $S_{Em}/S_m = 1.0$ (where $S_m/S_m = 1/e^{ES}$). Venter (1997) assigned the notation S_{Em}/S_m to the specific stimulus value which corresponds to maximal effect. From the slope of the straight line ($K_A/K_B = 0.2$) it followed that $K_A = 1.0$ μM if $K_B = 5.0$ μM while the EC_{50} of the agonist A was obtained at 1.0 μM.

Table 1
Data obtained from theoretical concentration–effect curves determined with a full agonist A in the absence and presence of increasing concentrations of a competitive antagonist B (Fig. 2)

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. $\boldsymbol{H} = H_{AB}/H$, where H_m = maximal curve height of agonist concentration–effect curve in absence of the competitive antagonist ($H_m = 1.0$).

Table 2
Data obtained from theoretical concentration–effect curves determined with a full agonist A in the absence and presence of increasing concentrations of a competitive antagonist B (Fig. 3)

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 ($H_m = 1.0$).

Data from Fig. 3 produced a straight line which was described by the following linear equation:

$$\boldsymbol{H} = -0.2\phi\boldsymbol{H} + 1.4.$$

It followed from the intercept of the straight line with the ordinate that $e^{ES} = 1.4$ which is indicative of spare receptors for maximal effect. Venter (1997) had shown that spare receptors for maximal effect are present in an agonist–effector system if $e^{ES} > 1.0$. From the slope of the straight line ($K_A/K_B = 0.2$) it followed that $K_A = 1.0$ μM if $K_B = 5.0$ μM while the EC_{50} of the agonist A was obtained at 5.5556×10^{-1} μM. If the maximal stimulus is taken as unity ($S_{AB}/S_m = 1.0$), then the maximal effect ($E_{AB}/E_m = 1.0$) would be obtained at a stimulus value of $S_{Em}/S_m = 0.714286$ (where $S_{Em}/S_m = 1/e^{ES}$) while $\phi_{min} = 2.0$.

The e^{ES}, S_{Em}/S_m and ϕ_{min} values for the agonists salbutamol and carbachol as well as the apparent K_A values for the salbutamol-receptor complex and the carbachol-receptor complex were estimated from the experimental sets of salbutamol and carbachol concentration–effect curves. As can be seen in Fig. 4, pindolol (0.1 μM) caused a dose-dependent inhibition of salbutamol-induced positive chronotropy of the spontaneous beating guinea-pig right atria. In the presence of pindolol the concentration–effect curve of salbutamol was displaced in a parallel fashion to the right (Fig. 4). From the shift of the agonist concentration–effect curve a K_B value of 1.914×10^{-2} μM was found for pindolol at cardiac β-adrenoceptors while the EC_{50} value for salbutamol was found at 1.9 μM. The ϕ values of different effect curve heights for the ratio [pindolol]/[salbutamol] shown in Table 3 were calculated for [pindolol] = 0.1 μM. Note that $H_m = 20.6$ cm in Table 3 represents the curve height of salbutamol in the presence of 0.1 μM pindolol, whereas the curve height of salbutamol in the absence of pindolol is 20.0 cm.

The inset in Fig. 4 shows a plot of relative curve heights $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ for experimental salbutamol-pindolol combinations (Table 3). As predicted by receptor theory, i.e., Eq. (1), the plot of $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ for fixed ratios of salbutamol-pindolol curves resulted in a straight line. Linear regression analyses of the salbutamol-pindolol results yielded the following linear equation:

$$\boldsymbol{H} = -128.7\phi\boldsymbol{H} + 1.06 \quad (3a)$$

Eq. (3a) was obtained by utilizing data acquired from the concentration–effect curve drawn through the mean of the pharmacological effects (E_{mean}) mediated by different agonist concentrations. From the intercept of the straight line with the ordinate it was estimated that $e^{ES} = 1.06 \pm 0.01$ for salbutamol, and from the slope of this linear relationship was estimated that the apparent $K_A = 2.49 \pm 0.07$ μM for the β-adrenoceptor-salbutamol complex. For all practical purposes it may be assumed that $e^{ES} = 1.0$ and it follows therefore that spare receptors for maximal effect are absent for the salbutamol–effector system (Venter, 1997). The stimulus value corresponding to 100% effect was obtained at $S_{Em}/S_m = 0.943 \approx 1.0$ which underlie the notion that no spare receptors are present. This result was to be expected since it is generally accepted that partial

Table 3
Data obtained from concentration–effect curves determined with salbutamol on cardiac β-adrenoceptors of guinea-pig. The salbutamol curves were determined in the absence and presence of pindolol (Fig. 4)

[sal] [a], μM	ϕ [b] $\times 10^{-3}$	H_{AB} [c], cm	$\boldsymbol{H}$ [d]	$\phi\boldsymbol{H} \times 10^{-3}$	$[sal]_{(+)}$ [e], μM	$\phi_{(+)}$ [f] $\times 10^{-3}$	$[sal]_{(-)}$ [g], μM	$\phi_{(-)}$ [h] $\times 10^{-3}$
		20.6	1.0					
22.62	4.422	14	0.680	3.005	34.01	2.940	17.91	5.583
18.99	5.267	13	0.631	3.324	30.27	3.304	14.27	7.008
15.94	6.273	12	0.583	3.654	23.97	4.171	11.57	8.644
12.63	7.920	11	0.534	4.229	20.13	4.968	9.71	10.296
10.60	9.434	10	0.485	4.580	16.90	5.918	7.69	12.999
9.16	10.914	9	0.437	4.768	13.78	7.257	6.46	15.484
7.47	13.384	8	0.388	5.198	11.57	8.644	5.12	19.550
6.09	16.413	7	0.340	5.577	9.43	10.6	4.29	23.286
4.83	20.072	6	0.291	6.036	7.69	13.0	3.40	29.401

[a] [salbutamol] used to calculate ϕ; [sal] was obtained from the mean effect curve in Fig. 4. [b] ϕ = [pindolol]/[sal], where [pindolol] = 0.1 μM. [c] Heights of submaximal effect curves. H_{AB} is expressed in cm. [d] Relative curve heights. $\boldsymbol{H} = H_{AB}/H_m$, where H_m = maximal curve height ($H_m = 20.6$ cm). [e] [salbutamol] used to calculate $\phi_{(+)}$; $[sal]_{(+)}$ was obtained from curve (a) in Fig. 4. [f] $\phi_{(+)} = [pindolol]/[sal]_{(+)}$. [g] [salbutamol] used to calculate $\phi_{(-)}$; $[sal]_{(-)}$ was obtained from curve (b) in Fig. 4. [h] $\phi_{(-)} = [pindolol]/[sal]_{(-)}$. Note: the values of $\phi_{(+)}\boldsymbol{H}$ and $\phi_{(-)}\boldsymbol{H}$ are note shown.

agonists, such as salbutamol on cardiac β_1-receptors, possess no spare receptors for maximal effect. Since spare receptors are absent, the ϕ_{min} value for salbutamol will be zero.

The following equation was obtained by utilizing data acquired from the concentration–effect curve drawn through the pharmacological effects obtained when adding standard errors (S.E.) to the mean pharmacological effects, i.e., E_{mean} + S.E. (see curve a in Fig. 4).

$$\boldsymbol{H} = -205.9\phi\boldsymbol{H} + 1.08 \quad (3b)$$

Eq. (3c), on the other hand, was obtained by utilizing data acquired from the concentration–effect curve drawn through the pharmacological effects E_{mean} − S.E. In this case the standard error (s.e.) was subtracted from the mean pharmacological effect (see curve b in Fig. 4).

$$\boldsymbol{H} = -80.8\phi\boldsymbol{H} + 0.99 \quad (3c)$$

From Eq. (3b) it followed that $K_A = 1.54 \pm 0.03$ μM, while Eq. (3c) yielded $K_A = 3.94 \pm 0.11$ μM. The mean of the K_A values obtained by utilizing Eq. (3a), Eq. (3b) and Eq. (3c) (1.533, 2.494 and 3.941 μM) is 2.66 ± 0.99 μM, while the mean e^{ES} value is equal to 1.04 ± 0.01.

Carbachol curves determined in the absence and presence of increasing tripitramine concentrations (5, 10, 50 and 100 μM) (see Fig. 5 in Chiarini et al., 1995), were utilized to determine the various ϕ values tabulated in Table 4. These ϕ values are the mean of ϕ values calculated for tripitramine concentrations of 10, 50 and 100 μM. Linear regression analyses of the carbachol-tripitramine results yielded the following linear equation:

$$\boldsymbol{H} = -0.336\phi\boldsymbol{H} + 1.046.$$

A plot of $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ resulted in the straight line shown in Fig. 5. From the intercept of the straight line with the ordinate it was estimated that $e^{ES} = 1.046 \pm 0.004$ for carbachol while $S_{Em}/S_m = 0.956$. It may be assumed that spare receptors for maximal effect are absent for the interaction of carbachol on the muscarinic receptors, because $e^{ES} \approx 1.0$ and $S_{Em}/S_m S \approx 1.0$. The EC_{50} value of carbachol was obtained at 8.9×10^{-1} μM, and from the slope of the obtained straight line was estimated the apparent $K_A = 1.1 \times 10^{-1}$ μM for the carbachol-muscarinic M_3-receptor complex. In calculating the apparent K_A (carbachol) a K_B value (tripitramine) of 3.16×10^{-1} μM was used (Chiarini et al., 1995).

Table 4
Data obtained from concentration–effect curves determined with carbachol on M_3-receptors of guinea-pig ileum (Chiarini et al., 1995). The carbachol curves were determined in the absence and presence of the competitive antagonist tripitramine (Fig. 5)

ϕ [a] $\times 10^{-2}$	H_{AB} [b], cm	$\boldsymbol{H}$ [c]	$\phi\boldsymbol{H}$ $\times 10^{-2}$
	10.3	1.0	
1.3166	7.5	0.7282	0.9587
1.6163	7.0	0.6796	1.0984
1.9441	6.5	0.6311	1.2268
2.3723	6.0	0.5825	1.3819
2.8396	5.5	0.5340	1.5163
3.4129	5.0	0.4854	1.6567
4.1329	4.5	0.4369	1.8056
5.0059	4.0	0.3883	1.9441
6.2067	3.5	0.3398	2.1091
7.7843	3.0	0.2913	2.2673

[a] ϕ = [tripitramine]/[carbachol]. [b] Heights of submaximal effect curves. H_{AB} is expressed in cm. [c] Relative curve heights. $\boldsymbol{H} = H_{AB}/H_m$, where H_m = maximal curve height (H_m = 10.3 cm).

4. Discussion

The utilization of experimental submaximal concentration–effect curves (such as is illustrated in Fig. 1) determined with fixed agonist-competitive antagonist combinations may yield relative inaccurate results (Venter, 1996). This is to be expected because in practice one is usually confronted with the fact that the determination of concentration–effect curves with fixed agonist-antagonist combinations generally do not allow enough time to ensure equilibrium between the competitive antagonist and its receptors. The logic solution to this practical problem would therefore involve the use of concentration–effect curves determined in the absence and presence of increasing concentrations of a competitive antagonist (Fig. 2 and Fig. 3 and Fig. 4). The utilization of such agonistic concentration–effect curves also has the advantage that these sets of agonistic concentration–effect curves are determined under controlled conditions yielding reliable pharmacological results which should render accurate drug parameters.

Sets of agonist concentration–effect curves determined in the absence and presence of increasing concentrations of a competitive antagonist furnishes an excellent possibility to illustrate the practical application of Eq. (1) for estimation of e^{ES} and K_A values. For this purpose use was made of carbachol curves determined in the absence and presence of tripitramine (Fig. 5, Table 4). The carbachol curves were determined by Chiarini et al. (1995) and analysis of these curves afforded data yielding a straight line when the relative curve heights $\boldsymbol{H}$ were plotted against $\phi\boldsymbol{H}$. The finding that $e^{ES} = 1.046 \approx 1.0$ while $S_{Em}/S_m = 0.956 \approx 1.0$, suggested the absence of spare receptors for carbachol on the M_3-receptors of the guinea-pig ileum.

The practical usefulness of the new procedure was further illustrated by analyzing the set of partial agonist (salbutamol) concentration–effect curves determined in the absence and presence of a competitive antagonist (pindolol) (Fig. 4, Table 3). Although the partial agonist salbutamol possesses greater affinity for β_2 than for β_1-adrenoceptors, it was chosen in this study to illustrate the absence of spare receptors for partial agonists at maximal effect. 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 (Venter, 1997). The absence of spare receptors in the latter system were illustrated by

the finding that $e^{ES} = 1.06 \approx 1.0$ and $S_{Em}/S_m = 0.943 \approx 1.0$. The nonselective competitive antagonist pindolol was used simply because it was available. Theoretically, however, the choice of competitive antagonist should not affect estimation of e^{ES} or K_A values (Venter, 1996).

It should be pointed out that the relevance of an e^{ES} values is not merely located in its ability to quantification spare receptors, but it may also be used for the estimation of relative efficacies (Venter, 1997). In contrast to presently employed methods the efficacy related e^{ES} value affords a method whereby relative efficacies of agonists may be estimated while the actual K_A values are unknown. By determining the e^{ES} values for various full agonists on the same receptor-type, one can estimate relative efficacies by comparing these e^{ES} values. In contrast to the inherently inaccurate null methods (Kenakin, 1987), this new method yields basically accurate relative efficacies and apparent K_A values. Of course, the accuracy of these parameters will be determined by the quality and reliability of the pharmacological data. The usefulness and basic accuracy of the method were amply illustrated by comparing the e^{ES} and K_A values obtained by applying Eq. (3a), Eq. (3b) and Eq. (3c). These equations were obtained from data acquired from the mean concentration–effect curve and concentration–effect curves drawn through the points of standard error (see Fig. 4). The e^{ES} values and apparent K_A values calculated by employing the various slope values of Eq. (3a), Eq. (3b) and Eq. (3c) differ within reasonable limits from each other. It is obvious, therefore, that normal experimental variations should not have an inordinately large influence on the attained results, this is in contrast to the null methods where small experimental deviations induce abnormally high inaccuracy levels (Kenakin, 1987).

Although plots of $\boldsymbol{H}$ against $\phi\boldsymbol{H}$ were shown to yield straight lines, it is obvious that plots of H_{AB} against ϕH_{AB} would also yield straight lines. Such straight lines would only be obtained when three important prerequisites are adhered to: (1), a linear relationship should exists between H_{AB} and ϕH_{AB} and, (2), the stimulus (S) generated by agonist A should be linearly proportional to the number of receptors occupied (ρ), i.e., $S = e.\rho$ (Stephenson, 1956), and (3), ρ should be described by the following equation:

$$\rho = \frac{1}{1 + \frac{K_A}{[A]}}$$

It is, however, generally accepted that the latter equation describes the receptor occupation (Ariëns et al., 1964; Van den Brink, 1977; Kenakin, 1993). As regards the first prerequisite, one would expect it to hold as long as the submaximal concentration–effect curves have the same heights (H_{AB}) as do their corresponding concentration-stimulus curves (h_{AB}), i.e., $H_{AB} = h_{AB}$ (Venter, 1997). Note, it is not required that the concentration–effect curves and concentration-stimulus curves should actually coincide. The theoretical curves shown in Fig. 2 and Fig. 3 were drawn in this manner simply for the sake of convenience. However, the exact value of K_A can only be determined if the concentration–effect curves coincide with their concentration-stimulus curves, because, if these curves do not coincide one would only be able to estimate apparent K_A values (Venter, 1996). It was found, however, that $H_{AB} \neq h_A$ for a large number of experimental cases studied, and for these cases plots of H_{AB} against ϕH_{AB} were nonlinear. Since the present study was limited only to those cases where a linear relationship exists between of H_{AB} and ϕH_{AB}, the treatment of such nonlinear relationships where considered to be beyond the scope of the present study and it will therefore be discussed in the following paper.

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