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A new method for determining affinity constants on isolated organs when a threshold value and spare receptors are present

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Abstract

It was shown in previous studies that fixed ratio combinations of agonists with competitive antagonists emulate partial agonists and, therefore, mediate submaximal concentration–effect ($E/$[A]) curves. A linear plot was obtained by plotting the relative height (H) of these submaximal curves against ϕH, where $\phi =$ [antagonist]/[agonist]. In this study, it was shown that in an agonist–effector system comprising a possible threshold value, the use of fixed ratio agonist–competitive antagonist concentrations (artificial partial agonists) induces $E/$[A] curves of different heights which allows one to quantify the threshold value. A nonlinear parabolic-like plot may be obtained when a threshold value is present in an agonist–effector system. By quantifying the threshold value, the nonlinear plot was converted to a linear plot from which the apparent affinity (K_A) and an efficacy related parameter (e^{ES}) of an agonist could be estimated. The practical applicability of the method was shown by applying the method to $E/$[A] curves obtained for agonists (noradrenaline and acetylcholine) obtained in the presence and absence of increasing concentrations of their respective competitive antagonists on different effectors.

Keywords: Spare receptor; Threshold value; Agonist–competitive antagonist combination; Agonist affinity

1. Introduction

Although the simplest relationship between receptor stimulus and tissue response is a linear one, there is a wealth of evidence to suggest that in many isolated tissues the relationship between receptor occupancy and tissue response is nonlinear. Since the advent of pharmacological procedures to estimate the equilibrium dissociation constants of full agonists and the refinement of biochemical binding studies, quantitative evidence for nonlinear relationships between receptor occupancy and tissue response has accumulated. It is generally believed that the main reason for this nonlinearity may be connected to the presence of spare receptors in the system. If spare receptors are present in the system, then the relationship between stimulus and effect will be nonlinear and $EC_{50} \neq K_A$. Much effort has, therefore, been expended in the measurement and quantification of spare receptors in isolated organs (for an overview, see the work of Kenakin (1984)). Besides the occurrence of spare receptors a nonlinear relationship may also result when a threshold value is present in an effector. A threshold value may result when the contractile elements in the isolated organ have to contract to a certain degree before the organ as a whole is shortened, i.e., the effect does not start right away with the induction of the stimulus (Ariëns et al., 1964b). The estimation of K_A will be in error if the presence of a threshold value is not taken into account.

Although a method for the quantitative and qualitative assessment of a threshold value was reported (Kirschner and Stone, 1956; Ariëns et al., 1964b), it is not general practice in experimental pharmacology to take note of the possible presence of a threshold value. This may be due the fact that threshold values reported in the literature are generally relatively small and therefore it's influence on K_A may be considered to be unimportant. The primary objective of this study was to forward a new method for the quantitative and qualitative assessment of threshold values and the estimation of dissociation constants (K_A) of agonist–receptor complexes in isolated organs. The method may be applied when a threshold value, or a threshold value and receptor reserve, are present. The method is based on the properties of submaximal $E/$[A] curves

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mediated by fixed ratio agonist–competitive antagonist combinations which behaves as partial agonists.

2. Materials and methods

2.1. Theory

It is known that a mixture of a full agonist A and a competitive antagonist B mimics the effect of a partial agonist and results in a submaximal effect (Feuerstein et al., 1994; Venter, 1996, 1997a,b). If A and B are combined in a fixed concentration ratio (ϕ), then the relationship between [A] and [B] is given by:

$$[B]/[A] = \phi \quad (1)$$

It was further reported by Venter (1997b) that a linear relationship may exist between the relative height (H) of the submaximal $E/[A]$ curves mediated by an agonist–antagonist mixture and ϕH, i.e.,:

$$H = -\frac{K_A}{K_B}\phi H + e^{ES} \quad (2)$$

where $H = H_{AB}/H_m$. The quantity H_{AB} represents the height of the experimentally determined submaximal $E/[A]$ curve(s) mediated by the fixed ratio agonist–antagonist combination(s) and, H_m represents the maximal height of the $E/[A]$ curve of the full agonist A in the absence of B, thus, H_m represents the maximal value of H_{AB} at 100% effect (i.e., when $E_{AB}/E_m = 1$). The parameter e^{ES} represents a dimensionless quantity which is related to efficacy e and was defined as $e^{ES} = h_m/H_m$ (Venter, 1997a,b), where h_m signifies the height of the concentration–stimulus ($S/[A]$) curve of agonist A. This efficacy related parameter may be estimated graphically by employing Eq. (2) and, since the value of e^{ES} depends on the value of H, it is advisable (indeed it should be considered as a prerequisite) that the maximal value of H should always be taken as unity when estimating e^{ES}. Eq. (2) describes a straight line (i.e., it is equivalent to $y = mx + c$, where m = gradient of the line and c = intersection of the straight line with the y-axis), thus if the relationship between H and ϕH is linear, then a straight line should be obtained when H is plotted against ϕH. The value of e^{ES} would then be given by the intercept of the straight line with the ordinate (H-axis) while the dissociation constant, K_A, of the agonist–receptor complex may be calculated from the slope of the straight line if K_B is known: slope $= -K_A/K_B$ (Venter, 1997a,b).

However, in this study it was generally found that plotting pharmacological data obtained with isolated organs according to Venter, did not always result in the expected straight lines, and instead, the nonlinear plots shown in the insets in Figs. 2, 5 and 6 were actually obtained. It was shown by Venter (1996) that Eq. (2) describes a straight line only when the relative height of the $S/[A]$ curves equals that of the experimentally obtained $E/[A]$ curves, i.e., when the stimulus–effect relationship is linear. Eq. (2) will describe a nonlinear plot when the relationship for the stimulus–effect relationship is not a linear one, and, therefore, the relationship between H and ϕH should also be nonlinear when such nonlinear conditions are present in the transduction system. It will be shown that such a nonlinearity may occur when $H_{AB} \neq h_{AB}$, i.e., when the height of the experimentally determined submaximal curve heights H_{AB} differ from that of their corresponding $S/[A]$ curve heights h_{AB}. Such a nonlinear phenomena may occur in agonist–effector systems in which a threshold value exists.

2.2. Significance and estimation of threshold value

The influence of a threshold value upon $E/[A]$ curves is illustrated by comparing Figs. 1 and 2. Fig. 1 shows theoretical $E/[A]$ curves calculated for an agonist–effector system void of a threshold value, while Fig. 2 shows $E/[A]$ curves obtained for an agonist–effector system in which a threshold value is present. In the presence of a

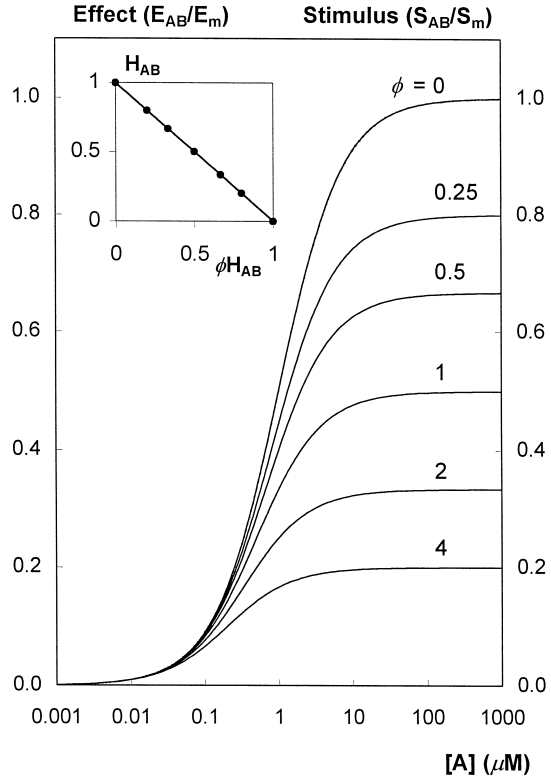

Fig. 1. Linear stimulus-effect relationship. Theoretical $S/[A]$ curves and simulated $E/[A]$ curves of a full agonist A combined with a competitive antagonist B in fixed ratios. The $S/[A]$ curves were calculated according to Eq. (2): $K_A = 1$ μM, $K_B = 1$ μM, $e = 1$, $\phi = [B]/[A] = 0$–4.0. The $E/[A]$ curves and $S/[A]$ curves coincide completely. The maximal curve height (obtained when $\phi = 0$, i.e., $[B] = 0$) was taken as unity and the $E/[A]$ curve heights H_{AB} are given in Table 1. Inset: Plot of H_{AB} (or H) against ϕH_{AB} (or ϕH (Table 1). Ordinate intercept: $e^{ES} = 1.0$; slope: $K_A/K_B = -1.0$.

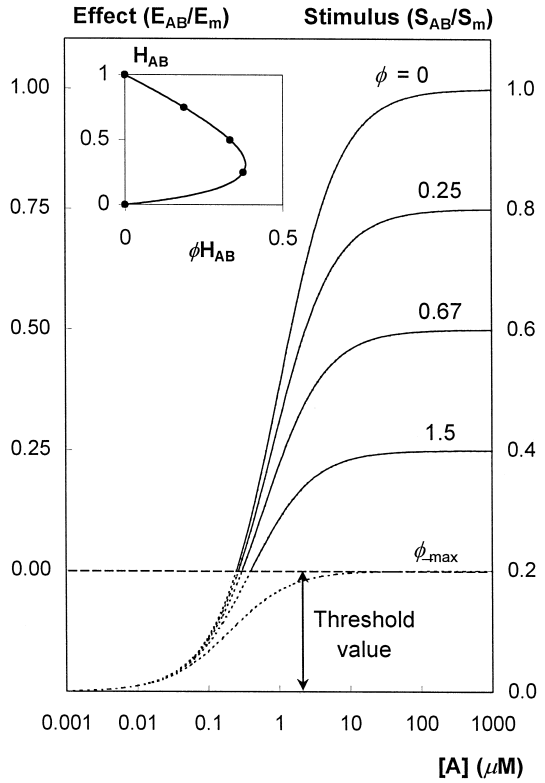

Fig. 2. Threshold value. Theoretical $S/$[A] curves (dotted lines) and simulated $E/$[A] curves (continues lines) of a full agonist A combined with a competitive antagonist B in fixed ratios. The $S/$[A] curves were calculated according to Eq. (2): $K_A = 1$ μM, $K_B = 1$ μM, $e = 1$, $\phi =$ [B]/[A] = 0–4.0. The $E/$[A] curves partly coincide with the $S/$[A] curves. The maximal curve height (obtained when $\phi = 0$, i.e., [B] = 0) was taken as unity and the $E/$[A] curve heights H_{AB}) are given in Table 2. Inset: Nonlinear parabolic-like plot of H_{AB} against ϕH_{AB} (Table 2).

threshold value, the $S/$[A] curve and the $E/$[A] curve of an agonist does not originate at the same point (compare Figs. 1 and 2), and an effect would only commence after the stimulus has reached a certain threshold value (Ariëns et al., 1964b). This particular stimulus value will be reached at the so-called threshold concentration (Ariëns et al., 1964b), i.e., the highest agonist concentration at which zero effect will still be observed. In the absence of a threshold value, a plot of H against ϕH (Table 1) yielded the expected straight line (inset: Fig. 1), indicating a linear relationship. On the other hand, when a threshold value is present in a agonist–effector system, a plot of H against ϕH (Table 2) did not yield a straight line, but instead it yielded the nonlinear parabolic-like curve shown in the inset of Fig. 2. Since the onset of a pharmacological effect in the presence of a threshold value commence at a stimulus value greater than zero (see Fig. 2), it follows that the heights of the $E/$[A] curves differ from the heights of their corresponding $S/$[A] curves, i.e., $H_{AB} \neq h_{AB}$. It is obvious that Eq. (2) cannot be applied in this particular case because the height of the submaximal $S/$[A] curves are unknown. It follows that the threshold value should be known before an equation of the form of Eq. (2) would be applicable. The threshold value is a stimulus value (Ariëns

Table 1
$E/$[A] curve heights of fixed agonist–competitive antagonist combinations for different values of ϕ in the absence of threshold value in the agonist–effector system (Fig. 1)

ϕ[a]	H_{AB}[b]	ϕH_{AB}	H[c]	ϕH
0	1.0	0	1.0	0
0.25	0.8	0.2	0.8	0.2
0.5	0.667	0.333	0.667	0.333
1.0	0.5	0.5	0.5	0.5
2.0	0.333	0.667	0.333	0.667

[a] ϕ = [B]/[A]; $\phi = 0$ if [B] = 0.
[b] Measured $E/$[A] curve heights; heights were measured relative to maximal curve height which was taken as unity.
[c] Values for H_{AB} and H are equivalent in the absence of a threshold value.

Table 2
$E/$[A] curve and $S/$[A] curve heights of fixed agonist–competitive antagonist combinations for different values of ϕ when a threshold is present

ϕ[a]	H_{AB}[b]	ϕH_{AB}	H'_{AB}[c]	$\phi H'_{AB}$	H[d]	ϕH
0	1.0	0	1.25	0	1.0	0
0.25	0.75	0.188	1.0	0.25	0.8	0.2
0.667	0.5	0.333	0.75	0.5	0.6	0.4
1.5	0.25	0.375	0.5	0.75	0.4	0.6

[a] ϕ = [B]/[A]; $\phi = 0$ if [B] = 0.
[b] Measured $E/$[A] curve heights; maximal curve height where taken as unity.
[c] $H'_{AB} = H_{AB} + H_T$, where $H_T = 0.25$. H_T may be calculated according to Eq. (viii).
[d] Relative curve height $H = H'_{AB}/1.25$.
These curve heights where obtained from fixed agonist–competitive antagonist combinations (Fig. 2) and a set of agonistic $E/$[A] curves determined in the presence and absence of increasing concentrations of a competitive antagonist (Fig. 5).

Table 3
$E/$[A] curve and $S/$[A] curve heights of fixed agonist–competitive antagonist combinations for different values of ϕ (from Fig. 6) in the presence of a threshold and spare receptors

ϕ[a]	H_{AB}[b]	ϕH_{AB}	H'_{AB}[c]	$\phi H'_{AB}$	H[d]	ϕH
0	1.0	0	1.4	0	1.0	0
0.667	0.8	0.533	1.2	0.8	0.857	0.571
1.0	0.6	0.6	1.0	1.0	0.714	0.714
1.5	0.4	0.6	0.8	1.2	0.571	0.857
2.333	0.2	0.467	0.6	1.4	0.429	1.0

[a] ϕ = [B]/[A]; $\phi = 0$ if [B] = 0.
[b] Measured $E/$[A] curve heights; maximal curve height where taken as unity.
[c] $H'_{AB} = H_{AB} + H_T$, where $H_T = 0.4$. H_T may be calculated according to Eq. (viii).
[d] Relative curve height; $H = H'_{AB}/1.4$.
These curve heights where obtained from a set of agonistic $E/$[A] curves determined in the presence and absence of increasing concentrations of a competitive antagonist.

Table 4
$E/$[A] curve and $S/$[A] curve heights of fixed noradrenaline–prazosin combinations for different values of ϕ

$\phi^a \times 10^{-4}$	H_{AB}^b	$\phi H_{AB} \times 10^{-5}$	H'^c_{AB}	$\phi H'_{AB} \times 10^{-4}$	H^d	$\phi H \times 10^{-4}$
0	1.00	0	1.13	0	1.0	0
0.457	0.80	3.66	0.93	0.376	0.82	0.376
0.575	0.75	4.32	0.88	0.448	0.78	0.448
0.708	0.70	4.96	0.83	0.520	0.73	0.520
0.871	0.65	5.66	0.78	0.601	0.69	0.601
1.05	0.60	6.28	0.73	0.676	0.65	0.676
1.24	0.55	6.84	0.68	0.749	0.60	0.749
1.45	0.50	7.23	0.63	0.806	0.56	0.806
1.72	0.45	7.73	0.58	0.882	0.51	0.882
2.02	0.40	8.07	0.53	0.947	0.47	0.947
2.41	0.35	8.43	0.48	1.02	0.42	1.02
2.88	0.30	8.65	0.43	1.10	0.38	1.10
3.47	0.25	8.67	0.38	1.17	0.34	1.17
4.27	0.20	8.53	0.33	1.25	0.29	1.25

[a] ϕ = [prazosin]/[noradrenaline].
[b] Experimentally determined $E/$[A] curve heights determined relative to the maximal curve height. The height of the noradrenaline curve (in the absence of prazosin) where taken as maximal and was set equal to unity.
[c] $H'_{AB} = H_{AB} + H_T$, where $H_T = 0.13$. H_T was determined by the incremental increasement (δH_{AB}) of H_{AB}.
[d] Relative curve height $H = H'_{AB}/1.13$.
The noradrenaline–prazosin curves were deduced from noradrenaline $E/$[A] curves determined in the absence and presence of different concentrations of the competitive antagonist, prazosin, on a receptors of rat vas deferens (Ko et al., 1994)

Table 5
Heights of $E/$[A] curves of carbachol–liriodenine combinations for different values of ϕ

ϕ^a	H_{AB}^b	$\phi H_{AB} \times 10^{-1}$	H'^c_{AB}	$\phi H'_{AB} \times 10^{-1}$	H^d	$\phi H \times 10^{-1}$
0	1.00	0	1.11	0	1	0
0.155	0.80	1.24	0.91	1.41	0.82	1.27
0.201	0.75	1.51	0.86	1.73	0.77	1.56
0.248	0.70	1.73	0.81	2.01	0.73	1.81
0.294	0.65	1.91	0.76	2.23	0.68	2.01
0.342	0.60	2.05	0.71	2.43	0.64	2.19
0.401	0.55	2.21	0.66	2.65	0.59	2.39
0.466	0.50	2.33	0.61	2.84	0.55	2.56
0.542	0.45	2.44	0.56	3.04	0.50	2.73
0.636	0.40	2.54	0.51	3.24	0.46	2.92
0.747	0.35	2.62	0.46	3.44	0.41	3.10
0.899	0.30	2.70	0.41	3.69	0.37	3.32
1.10	0.25	2.75	0.36	3.96	0.32	3.57
1.36	0.20	2.72	0.31	4.22	0.28	3.80

[a] ϕ = [liriodenine]/[carbachol].
[b] Experimentally determined $E/$[A] curve heights determined relative to the maximal curve height. The height of the carbachol curve (in the absence of liriodenine) where taken as maximal and was set equal to unity.
[c] $H'_{AB} = H_{AB} + H_T$, where $H_T = 0.11$. H_T was determined by the incremental increasement (δH_{AB}) of H_{AB}.
[d] Relative curve height $H = H'_{AB}/1.11$.
The carbachol–liriodenine curves are deduced from carbachol curves determined in the absence and presence of different fixed liriodenine concentrations on the guinea-pig ileum (Lin et al., 1994).

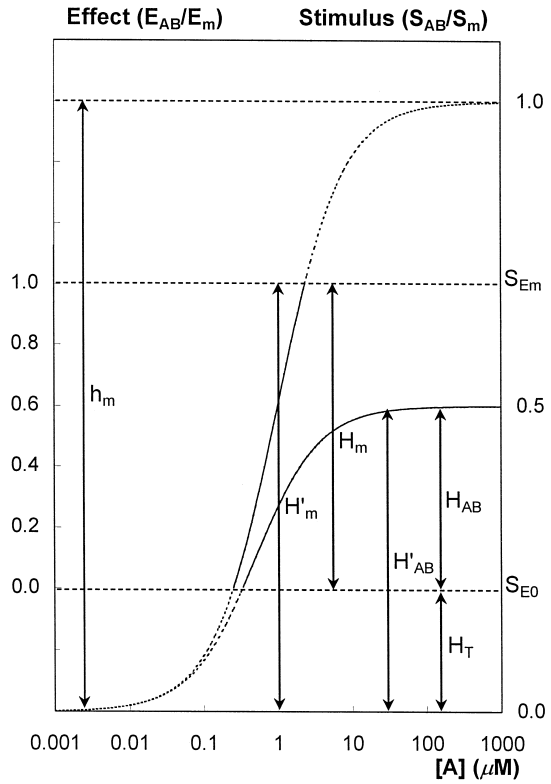

Fig. 3. This figure illustrates the meaning of the various symbols used. Note: in the absence of a threshold $H_T = 0$ and thus $H = H_{AB}/H_m$, because $H_{AB} = H'_{AB}$ and $H_m = H'_m$. In the presence of a threshold, $H' = H'_{AB}/H'_m$. Heights indicated by H are experimentally determinable, while heights of $S/$[A] curves (usually signified by 'h') are not directly determinable.

et al., 1964b), and it may be quantified if the height of the $S/$[A] curve mediated by $\phi = \phi_{max}$ (see Section 2.2.2 and Fig. 2) is known. The 'height' of this specific $S/$[A] curve is signified by H_T which may be regarded as a 'height compensating factor'; H_T, therefore, represents a quantity that should be added to the measured $E/$[A] curve heights H_{AB}. The quantity H_T should now be added to the experimentally determined $E/$[A] curve heights H_{AB} to compensate for the threshold value (see Tables 2–5 and Fig. 3). Therefore,

$$H'_{AB} = H_{AB} + H_T \quad (3)$$

where H'_{AB} represents the 'corrected' curve heights which incorporate threshold value.

The relative curve heights H' (Tables 2–5) may be calculated according to:

$$H' = H'_{AB}/H'_m = (H_{AB} + H_T)/(H_m + H_T) \quad (4)$$

where $H'_m = H_m + H_T$ represents the maximal height of the corrected submaximal curve heights. If the value H_m is arbitrarily taken as unity (as is generally the case), then the previous equation may be written as:

$$H' = (H_{AB} + H_T)/(1 + H_T) = H'_{AB}/(1 + H_T) \quad (5)$$

which may be employed directly for the calculation of H'.

Note that the meaning of the various symbols reflecting different curve heights, are illustrated in Fig. 3. Analogous to Eq. (2), it follows that:

$$H' = -\frac{K_A}{K_B}\phi H' + e^{ES} \quad (6)$$

A plot of H' against $\phi H'$ should result in a straight line if the threshold phenomenon is the main cause of the nonlinear plots shown in Figs. 2, 5 and 6. Straight lines obtained from the plots of H' against $\phi H'$, when a threshold is present, are shown in Figs. 7b and 8b which were plotted by employing data from Tables 4 and 5. Straight lines were also obtained by plotting the data from Tables 2 and 3 (the latter linear plots are not shown). The value of e^{ES} was obtained from the intercepts of the straight lines with their respective ordinates (H'-axis) while the (apparent) K_A may be obtained from the slope of the straight line (slope $= -K_A/K_B$).

2.2.1. Determination of H_T by incrementally increasing the value of H_{AB}

The value of H_T may be determined by gradually increasing the value of the experimentally measured $E/[A]$ curve heights (H_{AB}) with small increments (δH_{AB}). This action is readily carried out by employing an appropriate computer program. As the curve height increases, the nonlinear plot of $H_{AB} + \delta H_{AB}$ against $\phi(H_{AB} + \delta H_{AB})$ gradually changes to a linear plot as δH_{AB} approach H_T. A linear plot will eventually be obtained when $\delta H_{AB} = H_T$. In practice, H is taken equal to δH_{AB} when a plot of $\phi(H_{AB} + \delta H_{AB})$ on $H_{AB} + \delta H_{AB}$ results in a linear regression which gives the best overall fit (the value of the correlation coefficient, R^2, should then be as close as possible to unity).

In addition to above mentioned procedure H_T, and thus threshold value, may also be calculated via a mathematical equation (see Eq. (viii) in Section 2.4).

2.2.2. Drug parameters denoting threshold value: S_{EO}/S_M and ϕ_{max}

The threshold value, which is a stimulus value, may be represented as the stimulus fraction S_{EO}/S_m, where S_{EO} represents the stimulus value prevailing at the threshold concentration of the agonist. Thus, S_{EO}/S_m signifies the fraction of the maximal stimulus produced by the highest agonist concentration (i.e., the threshold concentration) which still produces a zero effect ($E_{AB}/E_m = 0$) and it may by calculated by employing the following equation:

$$\frac{S_{EO}}{S_m} = \frac{H_T}{(H_m + H_T)e^{ES}} = \frac{H_T}{H'_{m}e^{ES}} \quad (7)$$

The value of ϕ which produces a submaximal stimulus equal to S_{EO}/S_m is signified as ϕ_{max} (see Fig. 2). The quantity ϕ_{max} may be calculated according to Eq. (8) which was derived by replacing H' with H'_T ($= H_T/H'_m$) in Eq. (6) and rearranging the equation to give:

$$\phi_{max} = \frac{e^{ES} - H'_T}{H'_T(K_A/K_B)} \quad (8)$$

where $-K_A/K_B$ represents the slope of the straight line described by Eq. (6). It is important to note that H'_T represent a relative height, thus; $H'_T = H_T/H'_m$, $H'_m = H_m + H_T$ and since H_m is usually taken as unity, it follows that $H'_m = 1 + H_T$.

Fig. 4 illustrates the influence of different threshold values S_{EO}/S_m, and H_T values, on the character of a curve obtained by plotting H' against $\phi H'$. The value of S_{EO}/S_m varied from zero to 0.2. $S_{EO}/S_m = 0$ represents an agonist–effector system void of threshold value, while $S_{EO}/S_m = 0.2$ represents the greatest threshold value. The $S/[A]$ curves in Fig. 1 and the values shown in Table 1 were used as a bases for the calculation of these curves. The curve heights in the presence of the supposed threshold values ($S_{EO}/S_m = 0.1$ and $S_{EO}/S_m = 0.2$) were determined by subtracting these values of S_{EO}/S_m from the original curve heights H_{AB}.

2.3. Practical estimation of threshold value and drug parameters

It was previously pointed out by Venter (1996) that the utilization of fixed ratio agonist–antagonist combinations

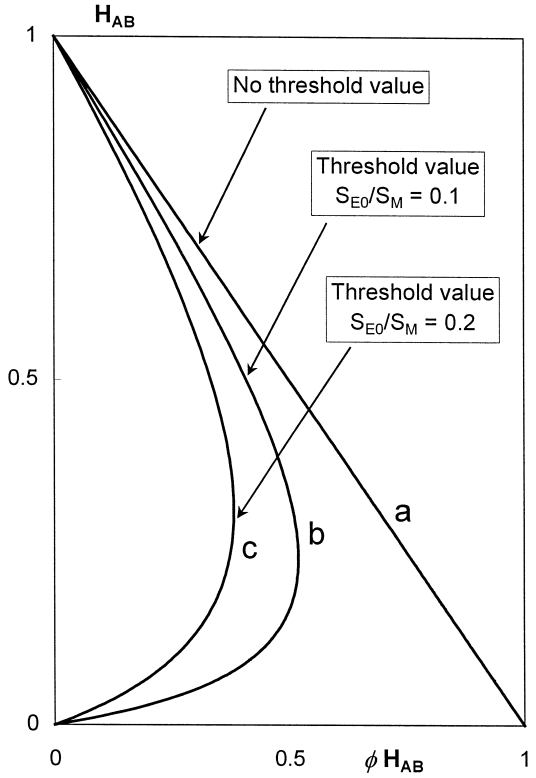

Fig. 4. A comparison of curves obtained from agonist–effector systems; (a) no threshold present, (b) relatively small threshold ($S_{EO}/S_m = 0.1$) is present, and (c) relative large threshold ($S_{EO}/S_m = 0.2$) is present.

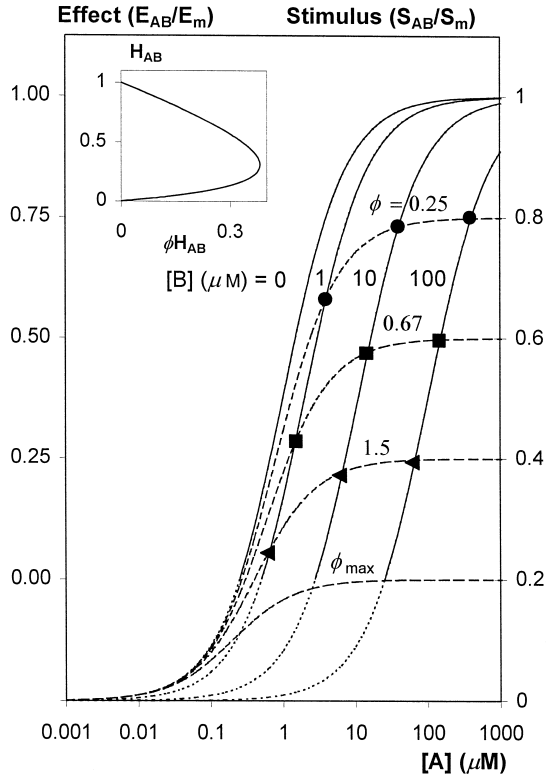

Fig. 5. Threshold value. Theoretical $S/$[A] curves (dotted lines) and simulated $E/$[A] curves (continues lines) of a full agonist A in the absence and presence of increasing concentrations of a competitive antagonist B. The $S/$[A] curves were calculated according to Eq. (2): $K_A = 1$ μM, $K_B = 1$ μM, $e = 1$, [B] = 0, 1, 10 and 100 μM. The $E/$[A] curves partly coincide with the calculated $S/$[A] curves. The broken lines represent deduced $E/$[A] curves of fixed [antagonist]/[agonist] ratios (ϕ). The various markers represent positions of constant ϕ and were calculated according to ϕ = [B]/[A] (●, $\phi = 0.25$; ■, $\phi = 0.66667$; ◄, $\phi = 1.5$). Submaximal $E/$[A] curve heights H_{AB} (broken lines) are given as fractions of maximal effect ($E_{AB}/E_m = 1.0$) (Table 2). Inset: Non-linear parabolic-like plot of H_{AB} against ϕH_{AB} (Table 2).

present certain difficulties such as differences in the equilibrium rate of the agonist and competitive antagonist in question. For experiments performed on isolated effectors with fixed agonist–antagonist concentration ratios it is therefore necessary that the equilibrium rate of the antagonist should be equal or faster than that of the agonist (Venter, 1996). Unfortunately, it was generally found that the equilibrium rate between an agonist and its receptors is much higher than the equilibrium rate between the competitive antagonist and the receptors. Obviously, these differences in equilibrium rates will lead to adverse pharmacological results when fixed ratio agonist-antagonist combinations are use indiscriminately. Therefore, this study concentrates mainly on agonistic $E/$[A] curves determined in the presence of different competitive antagonist concentrations. These sets of $E/$[A] curve were also used because of practical reasons; since $E/$[A] curves of agonists determined in the presence of increasing concentrations of competitive antagonists appears abundantly in the pharmacological literature, the versatility and usefulness of the model can be readily illustrated by utilizing this readily available data. The procedure for analyzing these sets of agonistic $E/$[A] curves was reported by Venter (1996, 1997a,b).

By analyzing these sets of $E/$[A] curves (see Figs. 5 and 6), one could readily estimate the various drug parameters, such as e^{ES}, apparent agonist affinity K_A, S_{EO}/S_m, ϕ_{max}, ϕ_{min} and S_{Em}/S_m. The values of S_{Em}/S_m and ϕ_{min} were calculated according to the equations reported by Venter (1997a,b). If the maximal stimulus is taken as unity, i.e., $S_{AB}/S_m = 1$, then the value of S_{Em}/S_m may be calculated by employing the following equation (Venter, 1997a,b):

$$S_{Em}/S_m = 1/e^{ES} \quad (9)$$

while the value of ϕ_{min} may be calculated by:

$$\phi_{min} = (e^{ES} - 1)/(K_A/K_B) \quad (10)$$

where $-K_A/K_B$ represents the slope of the straight line described by Eq. (6).

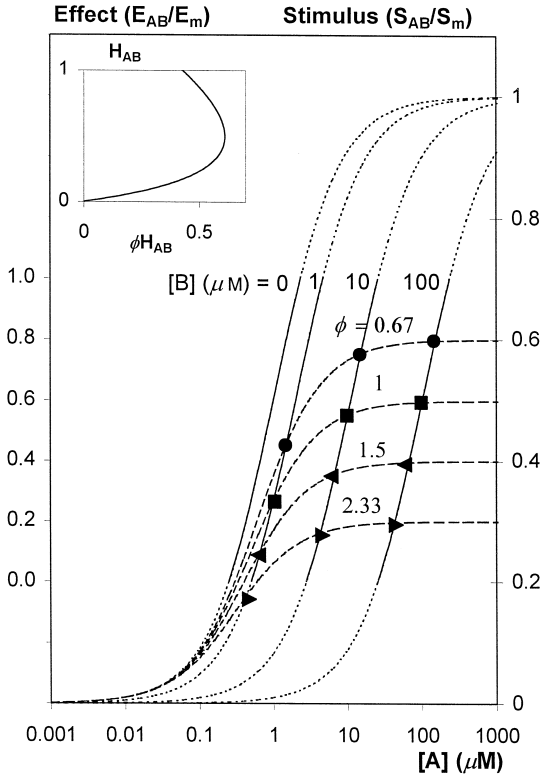

Fig. 6. Threshold value and spare receptors for maximal effect. Theoretical $S/$[A] curves (dotted lines) and simulated $E/$[A] curves (continues lines) of a full agonist A, in the absence and presence of increasing concentrations of a competitive antagonist B. The $S/$[A] curves were calculated according to Eq. (2): $K_A = 1$ μM, $K_B = 1$ μM, $e = 1$, [B] = 0, 1, 10 and 100 μM. The $E/$[A] curves partially coincide with the calculated $S/$[A] curves. The broken lines represent deduced $E/$[A] curves of fixed [antagonist]/[agonist] ratios (ϕ). The various markers represent positions of constant ϕ and were calculated according to ϕ = [B]/[A] (●, $\phi = 0.66667$; ■, $\phi = 1.0$; ◄, $\phi = 1.5$; ►, $\phi = 2.3333$). Submaximal $E/$[A] curve heights H_{AB} (broken lines) are given as fractions of maximal effect ($E_{AB}/E_m = 1.0$) (Table 3). Inset: Non-linear parabolic-like plot of H_{AB} against ϕH_{AB} (Table 3).

Estimation of the threshold value and various drug parameters in the absence and presence of spare receptors will be discussed on hand of theoretical (Section 2.3.1) and experimental data (Section 2.3.2).

2.3.1. Theoretical E / [A] curves

In this section, theoretical cases for agonist–effector systems will be discussed: (1) when only a threshold value is present; and (2) when a threshold value plus spare receptors for the maximal effect are present. Sets of theoretical agonist $S/$[A] curves determined in the absence and presence of increasing antagonist concentrations, shown in Figs. 5 and 6, were calculated according to Eq. (11). Theoretical $S/$[A] curves (dotted lines) for agonist A were calculated for constant values of K_A, K_B and e ($K_A = K_B = 1$ μM, $e = 1$) while the value of [B] was increased from zero to 100 μM.

When spare receptors for maximal effect where supposed to be absent in a particular agonist–effector system, then the theoretical $E/$[A] curves (continues lines; Fig. 5) simulating experimental $E/$[A] curves were constructed according to the assumption that the maximal effect ($E_{AB}/E_m = 1$) on the effector is obtained at $S_{AB}/S_m = 1.0$ (also see the work of Venter, 1996, 1997a). On the same effector, a threshold value was visualized by assuming that $S_{AB}/S_m = 0.2$ ($= S_{EO}/S_m$), generated by the threshold concentration, produced zero effect ($E_{AB}/E_m = 0$). The submaximal $E/$[A] curve heights H_{AB} (Table 2) were plotted against ϕH_{AB}, yielding the expected nonlinear parabolic-like curve (inset: Fig. 5).

When a threshold value and spare receptors for maximal effect were assumed to be present in an agonist–effector system, then theoretical $E/$[A] curves (continues lines; Fig. 6) simulating experimental $E/$[A] curves were constructed according to the assumption that the maximal effect ($E_{AB}/E_m = 1$) on the effector is generated by $S_{AB}/S_m = 0.7$ ($= S_{Em}/S_m$), while zero effect ($E_{AB}/E_m = 0$) and $S_{AB}/S_m = 0.2$ ($= S_{EO}/S_m$) was produced by the threshold concentration (Fig. 6). The notation S_{Em}/S_m signifies the fraction of the maximal stimulus generating maximal effect in the presence of spare receptors for maximal effect (Venter, 1997a,b). The presence of a threshold value was indicated by a plot of the $E/$[A] curve heights ϕH_{AB} against ϕH_{AB} (Table 3) which yielded the nonlinear curve shown in the inset of Fig. 6.

2.3.1.1. Estimation of drug parameters in the presence of a threshold value. From sets of $E/$[A] curves shown in Figs. 5 and 6 as continues lines one could easily determine the curve height H'_{AB} from the experimentally determined submaximal $E/$[A] curve height H_{AB} mediated by fixed agonist–antagonist combinations (for constant ϕ). The positions of constant values for ϕ, calculated by means of Eq. (12), were marked with identical markers on the $E/$[A] curves of A (determined in presence of B). By connecting these markers an agonist–antagonist $E/$[A] curve is obtained for which $\phi = [B]/[A]$ is constant. $E/$[A] curves which have been deduced by this approach are shown in Figs. 5 and 6 as broken lines.

The measured experimental curve heights H_{AB} shown in Figs. 5 and 6 were corrected to H'_{AB} by adding the quantity H_T which was calculated according to Eq. (viii) (Tables 2 and 3). The relative curve heights H' were calculated according to Eq. (5) (Tables 2 and 3) and a plot of H' against $\phi H'$ resulted in the expected straight lines as was predicted by Eq. (6) (plots are identical to the inset in Fig. 1 and are therefore not shown). The e^{ES} values were obtained from the intercept of the respective straight lines with the ordinate (H'-axis) while apparent K_A were obtained from the slope of the respective straight lines, slope $= -K_A/K_B$.

2.3.2. Estimation of threshold value and receptor reserve from experimental data

By analyzing literature data it was found that a threshold value was present in a number of cases studied. Only two cases encompassing different effectors and the agonists, noradrenaline and acetylcholine, were selected for discussion in the present paper. The sets of noradrenaline and acetylcholine $E/$[A] curves used in this study were determined in the absence and presence of increasing concentrations of the competitive antagonists prazosin and liriodenine, respectively. Noradrenaline $E/$[A] curves were determined on a receptors of rat vas deferens (Ko et al., 1994), while carbachol $E/$[A] curves were determined on cholinergic receptors of guinea-pig ileum (Lin et al., 1994).

From these sets of $E/$[A] curves were determined the experimental heights (H_{AB}) of the various submaximal $E/$[A] curves that would have been reached by submaximal $E/$[A] curves if fixed noradrenaline–prazosin and carbachol–liriodenine ratios were directly employed. The submaximal $E/$[A] curves were calculated for constant values of ϕ according to the procedure described by Venter (1996, 1997b). It was found, however, that only

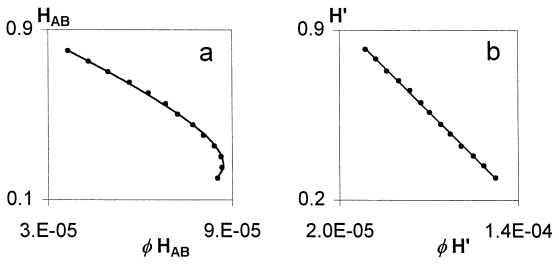

Fig. 7. (a) Experimental. A plot of experimentally determined curve heights H_{AB} against ϕH_{AB} for fixed noradrenaline–prazosin combinations on a adrenoceptors of rat vas deferens (Table 4) (Ko et al., 1994). (b) Experimental. A plot of relative curve heights H' against $\phi H'$ for fixed noradrenaline–prazosin combinations on a adrenoceptors of rat vas deferens (Table 4) (Ko et al., 1994). The data points were fitted to a straight line by the method of least squares. Ordinate intercept: $e^{ES} = 1.054 \pm 0.005$; slope: $-K_A/K_B = -6131.41 \pm 54.52$; correlation coefficient, R^2: 0.999.

maximal effects were obtained for all possible ϕ values used in deducing series of noradrenaline–prazosin and carbachol–liriodenine $E/[A]$ curves. Therefore, the ϕ values for several noradrenaline–prazosin and carbachol–liriodenine submaximal $E/[A]$ curves could readily be estimated at different curve heights (effects) of choice (Tables 4 and 5). For measurement of curve heights, care should be taken to ascertain that curve heights are only obtained from the 'linear' part of the agonistic curves (see Fig. 6), therefore, the different values of ϕ were only determined from this 'linear' section of the sigmoidal shaped $E/[A]$ curves. By measuring curve heights in the linear section, one hopefully avoids possible unintentional introduction or exclusion of a threshold or receptor reserve.

Plots of the experimentally determined submaximal $E/[A]$ curve heights H_{AB} against the product ϕH_{AB} resulted in a parabolic-like curve indicative of threshold values (Figs. 7a and 8a). The value of H_T was calculated according to Eq. (viii). However, this method seems to be of little use since it afforded inconsistent results (different values) when experimentally determined data were used (Tables 4 and 5). Unfortunately, small experimental errors severely influences the calculated H_T value, and therefore the use of Eq. (viii) is limited when experimental curve heights are being employed. Because of this limitation, the curve heights H'_{AB} (Tables 4 and 5) were obtained by estimating H_T via the method of incremental increasement of H_{AB} (see Section 2.2.1). Plots of the relative curve heights $\boldsymbol{H}'$ against the product $\phi\boldsymbol{H}'$ resulted in straight lines (Figs. 7b and 8b) from which the various drug parameters were determined. The data points shown in Figs. 7b and 8b were fitted to the straight lines by the method of least squares.

2.4. Appendix

Theoretical $S/[A]$ curves of agonists in the presence of increasing concentrations of a competitive antagonist may be calculated by employing the following equation (Arunlakshana and Schild, 1959; Ariëns et al., 1964a; Venter, 1996):

$$\frac{S_{AB}}{S_m} = \frac{e}{1 + \left(1 + \frac{[B]}{K_B}\right)\frac{K_A}{[A]}} \tag{11}$$

in which e represents the efficacy of the full agonist A. The relationship S_{AB}/S_m signifies the fraction of the maximal stimulus of agonist A in the presence of competitive antagonist B, S_{AB} represents the stimulus of A in the presence of B and S_m represents the maximal stimulus of A. [A] and [B] are the concentrations of agonist A and competitive antagonist B, respectively, while K_A and K_B are the dissociation constants of the agonist–receptor complex and competitive antagonist–receptor complex, respectively.

The stimulus mediated by a fixed agonist–competitive antagonist combination (ϕ) may be calculated by employing Eq. (12), which was obtained by combining Eqs. (1) and (11) (Venter, 1996, 1997a).

$$\frac{S_{AB}}{S_m} = \frac{\left(\frac{e}{1 + \phi K_A/K_B}\right)}{1 + \left(\frac{1}{1 + \phi K_A/K_B}\right)\frac{K_A}{[A]}} \tag{12}$$

Eq. (12) can be employed to calculate $S/[A]$ curves of for various values of ϕ.

For practical reasons, it is generally preferable to illustrate the presence or absence of a threshold value by simply plotting curve heights H_{AB} on ϕH_{AB}. Since $H = H_{AB}/H_m$, it follows that Eq. (2) may be converted to the following equation (Venter, 1997a):

$$H_{AB} = -\frac{K_A}{K_B}\phi H_{AB} + E \tag{13}$$

where $E = e^{ES}H_m$. The intercept with the ordinate, namely E represents the actual height of the $S/[A]$ curve of agonist A in the absence of the competitive antagonist B. Note that (1) the value of E depends on that of H_{AB} and the therefore E and H_{AB} would have the same units (i.e., cm, mm etc.) and (2) the maximal height H_m represent the curve height in the measured units, which means that the value of H_m need not be equal to one. The value of E is given by the intercept of the straight line with the ordinate (H_{AB}-axis) while the (apparent) dissociation constant, K_A, of the agonist–receptor complex may be calculated from the slope of the straight line if K_B is known: slope $= -K_A/K_B$ (Venter, 1997a,b). It is important to realize that Eq. (13) describes a linear relationship only when the submaximal $E/[A]$ curve heights H_{AB} are equal to their corresponding $S/[A]$ curve heights h_{AB}, i.e., $H_{AB} = h_{AB}$.

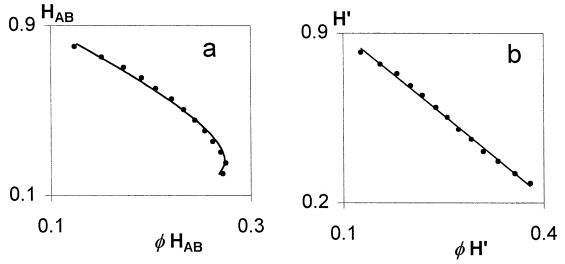

Fig. 8. (a) Experimental. A plot of experimentally determined curve heights H_{AB} against ϕH_{AB} for fixed carbachol–liriodenine combinations on cholinergic receptors of guinea-pig ileum (Table 5) (Lin et al., 1994; Ko et al., 1994). (b) Experimental $E/[A]$ curves. A plot of relative curve heights H' against $\phi H'$ for fixed carbachol–liriodenine combinations on cholinergic receptors of guinea-pig ileum (Table 5) (Lin et al., 1994; Ko et al., 1994). The data points were fitted to a straight line by the method of least squares. Ordinate intercept: $e^{ES} = 1.122 \pm 0.011$; slope: $-K_A/K_B = -2.246 \pm 0.039$; correlation coefficient, R^2: 0.997.

Analogous to Eq. (13), the following equation was derived from Eq. (6).

$$H'_{AB} = -\frac{K_A}{K_B}\phi H'_{AB} + E' \quad (14)$$

where $E' = e^{ES}H'_m$. Eq. (14) may be employed for plotting the corrected curve heights H'_{AB} on $\phi H'_{AB}$. The intercept with the ordinate is given by E'.

2.4.1. Mathematical equation for calculating H_T

It follows from Section 2.2 that the calculated curve height H'_{AB} should be equal to the experimental curve height H_{AB} plus the additional value H_T (see Fig. 2). The addition of H_T to the various experimental curve heights H_{1AB}, H_{2AB}, H_{3AB},..., H_{NAB}, should hence yield the various calculated curve heights:

$$H'_{1AB} = H_{1AB} + H_T,\ H'_{2AB} = H_{2AB} + H_T,\ \ldots\ H'_{NAB} = H_{NAB} + H_T.$$

If the actual value of $\phi H'_{AB}$ is represented by a, i.e., $\phi H'_{AB} = a$, then it follows that:

$$\phi_1(H_{1AB} + H_T) = \phi_1 H'_{1AB} = a_1 \quad (i)$$

$$\phi_2(H_{2AB} + H_T) = \phi_2 H'_{2AB} = a_2 \quad (ii)$$

$$\phi_3(H_{3AB} + H_T) = \phi_3 H'_{3AB} = a_3 \quad (iii)$$

$$\vdots$$

$$\phi_N(H_{NAB} + H_T) = \phi_N H'_{NAB} = a_N \quad (N)$$

where a_1, a_2, a_3,..., a_{N-1}, a_N represent the various actual values of the product $\phi H'_{AB}$ for different values of H'_{AB}. These actual values of $\phi H'_{AB}$ are of course unknown. If the submaximal $S/[A]$ curve heights increase (or decrease) with a constant factor (see Figs. 5 and 6 and Tables 2 and 3), namely H_C, then:

$$H_{1AB} - H_{2AB} = H_{2AB} - H_{3AB} = \ldots = H_{N-1AB} - H_{NAB} = H_C \quad (iv)$$

It follows now that:

$$H'_{1AB} - H'_{2AB} = H'_{2AB} - H'_{3AB} = \ldots = H'_{N-1AB} - H'_{NAB} = H_C$$

Eq. (ii)–Eq. (i) gives:

$$a_2 - a_1 = \phi_2 H'_{2AB} - \phi_1 H'_{1AB} \quad (v)$$

According to Eq. (13); $\phi H'_{AB} = (H'_m - H'_{AB})/m$, where $m = K_A/K_B$. Thus, it follows from Eq. (v) and the previous equation that:

$$\phi_2 H'_{2AB} - \phi_1 H'_{1AB} = (H'_m - H'_{2AB})/m - (H'_m - H'_{1AB})/m = (H'_{1AB} - H'_{2AB})/m = H_C/m$$

If the constant H_C/m is set equal to a, then it follows from the latter equation and Eq. (v) that $H_C/m = a_2 - a_1 = a$. If the same manner of reasoning is followed for all equations, i.e., from Eqs. (i), (ii), (iii) and (N), then it clearly follows that:

$$a = a_2 - a_1 = a_3 - a_2 = \ldots = a_N - a_{N-1}$$

Thus, Eq. (ii) minus Eq. (i) would result in:

$$\phi_2 H_{2AB} + \phi_2 H_T - \phi_1 H_{1AB} - \phi_1 H_T = a_2 - a_1 = a \quad (vi)$$

while Eq. (iii) minus Eq. (ii) results in:

$$\phi_3 H_{3AB} + \phi_3 H_T - \phi_2 H_{2AB} - \phi_2 H_T = a_3 - a_2 = a \quad (vii)$$

By subtracting Eq. (vi) from Eq. (vii) one can dispose of the unknown quantity a, thus Eq. (vii) − Eq. (vi) gives rise to:

$$\phi_1 H_{1AB} - 2\phi_2 H_{2AB} + \phi_3 H_{3AB} + \phi_1 H_T - 2\phi_2 H_T + \phi_3 H_T = 0$$

which rearranges to:

$$H_T = \frac{-\phi_1 H_{1AB} + 2\phi_2 H_{2AB} - \phi_3 H_{3AB}}{\phi_1 - 2\phi_2 + \phi_3} \quad (viii)$$

The quantities ϕ and H_{AB} are measurable quantities. The quantity H_T may be calculated according to Eq. (viii). It is important to note that Eq. (viii) is only valid when the experimental curve heights (H_{AB}) are chosen such that Eq. (iv) is valid.

3. Results

The simulated $E/[A]$ curves in Figs. 1 and 2 show the influence of a competitive antagonist B on a full agonist A for different values of $\phi = [B]/[A]$. The combination of agonist A and competitive antagonist B in fixed concentration ratios resulted in submaximal $E/[A]$ curves. The heights (H_{AB}) of the simulated $E/[A]$ curves in Fig. 1 are shown in Table 1. A plot of H_{AB} against ϕH_{AB} resulted in a straight line, pointing to the absence of a threshold value (inset: Fig. 1). Since the maximal curve height was taken equal to unity, the values of H_{AB} would in this case also be equal to the relative curve heights H. Regression analysis of the straight line afforded the following equation:

$$H = -\phi H + 1.0 \quad (15)$$

It followed from the slope and ordinate intercept of the straight line that $K_A = 1\ \mu M$ (if $K_B = 1\ \mu M$) and $e^{ES} = 1.0$, respectively.

The $E/[A]$ curve heights (H_{AB}) obtained from Fig. 2, the corrected curve heights H'_{AB} and the relative curve heights H' are shown in Table 2. In contrast to the previous case, a plot of H_{AB} against ϕH_{AB} resulted in a parabolic-like curve which is indicative of a threshold

value (inset: Fig. 2). A plot of the relative curve heights H' against $\phi H'$ (not shown) resulted in a straight line identical to the inset in Fig. 1, and of course, as can be expected, this straight line is described by the following equation which is analogous to the previous equation:

$$H' = -\phi H' + 1.0 \quad (16)$$

It followed from the intercept of the straight line with the ordinate that $e^{ES} = 1.0$. The maximal effect ($E_{AB}/E_m = 1$) was obtained at $S_{Em}/S_m = 1.0$, while, according to Eq. (7) the zero effect ($E_{AB}/E_m = 0$) was obtained at $S_{EO}/S_m = 0.2$ and according to Eq. (8) $\phi_{max} = 4$. From the slope of the straight line it followed that $K_A = 1$ μM if $K_B = 1$ μM.

Figs. 5 and 6 show $S/$[A] curves (dotted lines) calculated according to Eq. (11) and simulated $E/$[A] curves (solid lines) of a full agonist A in the absence and presence of increasing concentrations of a competitive antagonist B. The broken lines represent deduced submaximal $E/$[A] curves of fixed ratio agonist–competitive antagonist combinations. These submaximal $E/$[A] curves were deduced from data obtained from the simulated $E/$[A] curves of A determined in the presence of increasing [B]. The submaximal $E/$[A] curve heights (H_{AB}) are shown in Tables 2 and 4 as fractions of the maximal effect of the deduced agonist–antagonist submaximal $E/$[A] curves (broken lines). A plot of H_{AB} against ϕH_{AB} resulted in a parabolic-like curve which is typical of a threshold value (Table 2; inset: Fig. 5). The quantity $H_T = 0.25$ was calculated by employing Eq. (viii) and the relative curve heights H' were calculated from the corrected curve heights H'_{AB} by employing Eq. (5) (Table 2). By plotting H' against H' a straight line (not shown) was obtained which is identical to that shown in the inset of Fig. 1. As expected, data from Fig. 5 produced the same linear equation as was obtained from Figs. 1 and 2. The maximal effect ($E_{AB}/E_m = 1.0$) of the full agonist A was obtained at $S_{AB}/S_m = 1.0$, while, according to Eq. (7) zero effect was predicted to occur at $S_{EO}/S_m = 0.2$.

The curve heights H_{AB} obtained from Fig. 6 and the relative curve heights H' calculated according to Eq. (5) are shown in Table 3. The presence of a threshold value in the agonist–receptor system was illustrated by plotting H_{AB} against ϕH_{AB} which resulted in the parabolic-like curve shown in the inset of Fig. 6. By comparing this curve to the curve shown in the inset of Fig. 5, one would notice that the upper part of curve in the inset of Fig. 6 does not intersect the ordinate. This phenomenon is indicative of the presence of spare receptors at maximal effect. $H_T = 0.4$ was calculated by employing Eq. (viii), while the relative curve heights H' were calculated by employing the corrected curve heights H'_{AB}. A plot of H' against $\phi H'$ resulted in a straight line (not shown) of which a linear regression analyses afforded the following equation:

$$H' = -\phi H' + 1.429 \quad (17)$$

It followed from the intercept of the straight line with the ordinate that $e^{ES} = 1.429$. The maximal effect ($E_{AB}/E_m = 1$) was obtained at $S_{Em}/S_m = 1/e^{ES} = 0.7$ (Eq. (10)), and according to Eq. (7) zero effect ($E_{AB}/E_m = 0$) was obtained at $S_{EO}/S_m = 0.2$. According to Eq. (8) $\phi_{max} = 4.0$ and from the slope it followed that $K_A = 1$ μM if $K_B = 1$ μM.

The same information obtained by plotting H' against $\phi H'$, may, however, be obtained by plotting corrected curve heights H'_{AB} against $\phi H'_{AB}$ (Table 3). The plot of the curve heights H'_{AB} against $\phi H'_{AB}$, according to Eq. (14), resulted in a straight line which was described by the following equation:

$$H'_{AB} = -\phi H'_{AB} + 2 \quad (18)$$

Note, the heights of submaximal $E/$[A] curve heights H_{AB} (from which H'_{AB} were calculated) may of course be measured in any unit that denotes length. In this instance H_{AB} were not measured in any particular unit and it was actually expressed as relative to the maximal height H_m (see Table 3). It follows from the intercept of the straight line with the H'-axis that $E' = e^{ES}H'_m = 2$. The e^{ES} may be calculated from E': $e^{ES} = E'/H'_m = 2/1.4 = 1.429$. The value of H'_m were calculated according to: $H'_m = H_m + H_T$, where $H_m = 1$ and $H_T = 0.4$ (see Table 3). The maximal effect ($E_{AB}/E_m = 1$) of the $H'_{AB}/\phi H'_{AB}$ plot would be obtained at of $S_{Em}/S_m = 1/e^{ES} = 0.7$.

Fig. 7a shows a plot of submaximal $E/$[A] curve heights H_{AB} against ϕH_{AB} for a fixed concentration ratio of the agonist noradrenaline and the competitive antagonist prazosin (Table 4). The parabolic-like curve points to the possibility of a threshold value in noradrenaline-α receptor system. $H_T = 0.13$ was determined according to the incremental height increasing method (see Section 2.2.1) and the relative curve heights H' were calculated according to Eq. (5) (Table 4). As predicted by receptor theory, i.e., Eq. (6), a plot of the relative curve heights H' against $\phi H'$ resulted in a straight line (Fig. 7b). Linear regression analyses of the noradrenaline–prazosin results yielded the following linear equation:

$$H' = -2.2464\phi H' + 1.12 \quad (19)$$

From the slope and intercept of this linear plot were estimated the apparent K_A of the noradrenaline–α receptor complex and the e^{ES} value of noradrenaline on rat vas deferens. For calculation of apparent K_A (= 3.29 mM) a K_B value (for prazosin–receptor complex) of 0.537 nM was used (Ko et al., 1994), while the EC_{50} for noradrenaline was found to be 2.344 mM. The intercept of the straight line with the ordinate gave $e^{ES} = 1.05$ which is indicative of relatively few spare receptors for maximal effect. According to Eq. (7) zero effect ($E_{AB}/E_m = 0$) was obtained at $S_{EO}/S_m = 0.11$, and 100% effect ($E_{AB}/E_m = 1$) was obtained at $S_{Em}/S_m = 1/e^{ES} = 0.949$. It was of course assumed that the maximal stimulus would be ob-

tained at a value of $S_{AB}/S_m = 1.0$. The parameters $\phi_{max} = 1.332 \times 10^{-3}$ and $\phi_{min} = 8.85 \times 10^{-6}$ were calculated by employing Eq. (8) and Eq. (10), respectively.

Fig. 8a shows a partial parabolic-like plot of submaximal $E/[A]$ curve heights H_{AB} against ϕH_{AB} for fixed combinations of the agonist (carbachol) and the competitive antagonist (liriodenine) (Table 5). $H_T = 0.11$ was determined according to the incremental height increasing method (see Section 2.2.1) and the relative curve heights H' were calculated according to Eq. (5) by employing H'_{AB} (Table 5). The plot of the relative curve heights H' against $\phi H'$ resulted in a straight line (Fig. 8b) and the linear regression analyses of the line resulted in:

$$H' = -2.246\phi H' + 1.122 \quad (20)$$

From the slope and intercept of this linear plot were estimated the apparent K_A of the carbachol–cholinergic receptor complex and the e^{ES} value of carbachol on guinea-pig ileum. For calculation of apparent K_A (= 1.35 νM) a K_B value (for liriodenine–receptor complex) of 0.603 νM was used (Lin et al., 1994), while the EC_{50} for carbachol was found to be 5.128 νM. The intercept of the straight line with the ordinate gave $e^{ES} = 1.12$ which indicate the presence of spare receptors for maximal effect. According to Eq. (7) zero effect ($E_{AB}/E_m = 0$) was obtained at $S_{EO}/S_m = 0.088$, and 100% effect ($E_{AB}/E_m = 1$) was obtained at $S_{Em}/S_m = 1/e^{ES} = 0.89$. It was of course assumed that the maximal stimulus would be obtained at a value of $S_{AB}/S_m = 1.0$. The parameters $\phi_{max} = 4.594$ and $\phi_{min} = 5.427^{-2}$ were calculated by employing Eq. (8) and Eq. (10), respectively.

4. Discussion

Plotting the data in the way described by Venter (1996) implies that the relative height of $S/[A]$ curves (h_{AB}/h_A) is plotted against $\phi h_{AB}/h_A$. Linear plots are expected if the $S/[A]$ relationship exactly obeys the equations based on the mass action law. Since the experimenter has only $E/[A]$ curves at his disposal, it is necessary to make certain suppositions regarding the stimulus–effect relationship, namely, it is assumed that the submaximal $E/[A]$ curves and their corresponding submaximal $S/[A]$ curves have the same heights, although it is not necessary that the $S/[A]$ and $E/[A]$ curves should coincide as illustrated in Figs. 1, 2, 5 and 6 (also see the works of Venter, 1997a,b). For the sake of convenience and simplicity the $S/[A]$ curves and $E/[A]$ curves shown in Figs. 1, 2, 5 and 6 are actually shown to coincide. In the absence of a threshold value (Fig. 1), the $E/[A]$ curves have a common point of origin, at least in theory, and in this instance the measured $E/[A]$ curve heights may be employed directly for estimating the various drug parameters. Experimental $E/[A]$ curves adhering to this prerequisite were described by Venter (1996, 1997a).

In a number of cases were experimental $E/[A]$ curves were analyzed, nonlinear plots (parabolic-like curves) instead of linear plots were obtained when H_{AB} was plotted against ϕH_{AB}. All of these data were obtained by making use of isolated organs. This deviation from linearity may be caused by a lack of linear proportionality between the concentration of the drug in the bath fluid and that in the biophase. However, in this regard it is generally believed that the drug concentration is in equilibrium between the bath fluid and the biophase (Van den Brink, 1977). On the other hand, it may be that the relation between drug and receptor does not follow the simple equations used, or that the relation between the stimulus evoked and the effect obtained is not linearly proportional. Such a nonlinearity may also result in the stimulus–effect relationship when spare receptors and/or threshold value is present for the drug–effector system. It was shown by Venter (1997a,b) that in the presence of spare receptors a linear plot would still be obtained when H_{AB} is plotted against ϕH_{AB}. Spare receptors would cause the value of e^{ES} to be greater than unity. However, if a threshold value should actually be present in a particular agonist–effector system, then the submaximal $E/[A]$ curves and their corresponding $S/[A]$ curves would possess different heights. This is to be expected because in the presence of a threshold value the actual location of the zero point of the $S/[A]$ curves would generally be unknown. Inspection of the $E/[A]$ curves shown in Fig. 2 demonstrate clearly that the relative $S/[A]$ curve heights h_{AB}/h_m differs from the experimentally determined relative $E/[A]$ curve heights H_{AB}/H_m. In the presence of a threshold value $H_{AB}/H_m < h_{AB}/h_m$ and the relationship between H_{AB} and ϕH_{AB} will be nonlinear giving rise to the typical parabolic-like nonlinear plots shown in the insets of Figs. 2, 5 and 6. If the hypothesis in this study, on which the theory of threshold value estimation is based, holds, then a plot of H' against $\phi H'$ should result in a linear plot. Tables 4 and 5 (Figs. 7b and 8b) represent experimental data, and Tables 2 and 3 (plots not shown) represent theoretical data which favors the assumption made for the threshold and at the same time shows that the hypothesis used fits the experimental results. In these cases the original parabolic-like curves were converted into straight lines by following the procedure described in this paper.

It follows from this study that the quantity H_T is indicative of a threshold value and that the estimation of a threshold value is thus dependant on H_T. The value of H_T may be estimated by employing Eq. (viii). The applicability of this equation is however restricted to cases where the heights of the $E/[A]$ curves increases (or decreases) with fixed increments and it should therefore be ideally suited for utilizing $E/[A]$ curves determined in the presence of increasing competitive antagonist concentrations. By applying this method one can actually choose the curve height and then calculate the value of ϕ. Unfortunately, for the determination of H_T the practical applicability of

Eq. (viii) is, however, limited because it is very sensitive for relative small experimental deviations. In this case, one should make use of the trail-and-error method whereby the value of H_T is determined by employing a suitable computer program.

By employing the procedure described in this paper, and analyzing a number of agonist curves determined in the absence and presence of a competitive antagonist, it was found that threshold values occurred generally in many of the cases studied. Ariëns et al. (1964b) also reported a number of cases where threshold values are present. It seems, therefore, that the occurrence of a threshold value is possibly a fairly general phenomenon which should most certainly be taken into account when estimating drug parameters. Although Ariëns et al. (1964b) described a technique whereby threshold values could be estimated via hyperbolic $E/[A]$ curves, threshold values are not readily determined in experimental pharmacology. The lack of interest for determination of threshold values may be ascribed to: (1) the inherent inaccuracy of these techniques which are based on extrapolation of the hyperbolic $E/[A]$ curve of an agonist, or (2) a general acceptance that threshold values are trivial or even absent. However, whatever the reason, the findings made by employing the new technique had shown that a threshold value may be an important agonist–effector phenomenon which should not be ignored.

If the proposed method is compared with that of Ariëns et al. (1964b), it can easily be seen that in principle the idea of threshold value estimation is the same. However, since the method proposed by Ariëns et al. comprise the extrapolation of a nonlinear curve (hyperbolic curve), it is by far less accurate than the method of incremental increasement of H_T proposed in this paper. Ariëns et al. also showed that for drugs with a threshold value, the Linweaver–Burk plot (i.e., a plot of $1/E$ on $1/[A]$, where E is the measured effect and $[A]$ is the agonist concentration) yields a nonlinear plot. If, however, the effect was corrected for threshold value, then the Linweaver–Burk plot yielded a linear plot.

The versatility of the new procedure was shown be analyzing data obtained from isolated organ experiments. For this purpose $E/[A]$ curves of the agonists noradrenaline (on rat vas deferens) and carbachol (on guinea-pig ileum) were employed. Both these cases encompassed a threshold and spare receptors. The latter may be concluded since $e^{ES} = 1.12$ for carbachol and $e^{ES} = 1.05$ for noradrenaline. Experimental data reported by Ariëns et al. (1960) deny, while more recent results suggest the existence of spare α-receptors for noradrenaline in the rat vas deferens (Diaz-Toledo and Marti, 1988). The results obtained in the present study point to the possibility that a rather small amount of spare α-receptors may be present in the noradrenaline–vas deferens system.

The K_A values that were estimated for noradrenaline and acetylcholine should be regarded as apparent K_A values, since it was previously reported by Venter (1997b) that the actual K_A values can only be estimated via this method if the submaximal $E/[A]$ curves and their corresponding $S/[A]$ curves coincide. Since the latter possibility cannot be readily established, it was suggested by Venter (1997b) that one should preferably determine relative K_A values by comparing apparent K_A values. Note, however, that for the estimation of e^{ES}, S_{Em}/S_m and S_{EO}/S_m the only prerequisite is that the submaximal $E/[A]$ curve heights and their corresponding submaximal $S/[A]$ curve heights should be equal.

It may be concluded that the new method reported in this paper encompasses a useful procedure for the quantitative and qualitative assessment of threshold values.

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