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Tissue Response as a Functional Discriminator of Receptor Heterogeneity: Effects of Mixed Receptor Populations on Schild Regressions

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SUMMARY

A model is described that predicts the behavior of competitive antagonists in tissues with more than one receptor mediating response. The receptor stimuli for two receptor types are summed and processed, via a cellular stimulus-response mechanism, into tissue response. The primary receptor is described by a standard Langmurian isotherm, and a secondary receptor input with a variable maximal strength, for which the agonist has variable sensitivity, is added. The prediction of drug effects in this system does not depend on the way in which the two stimuli are combined or on the absolute magnitudes of the parameters used to make the calculations. The model is maximally flexible, in that no pharmacological significance is put on the magnitudes of the inputs from the secondary receptor system (i.e., they can vary with either agonist intrinsic efficacy, receptor number, or efficiency of stimulus-response coupling). The theoretical Schild regressions for selective antagonists in two-receptor systems are calculated for various secondary receptor inputs. These regressions generally are curvilinear whenever the secondary receptor significantly contributes to agonist response. These calculated data also indicate that minor variations in biological input from secondary receptor systems would obscure curvature in the Schild regression and result in a seemingly linear regression with a slope of less than unity. However, further calculations indicate three possible ways to use Schild analysis to detect receptor heterogeneity in tissues.

One indicator of receptor heterogeneity is a change in the slope of the dose-response curve for the agonist in the presence of a selective antagonist. A second indicator would be a marked heteroscedasticity of errors in the Schild regression, i.e., the magnitude of the standard errors in the ordinate values would depend upon the concentration of the antagonist. A third, and most experimentally accessible, aspect of heterogeneous receptor systems predicts that changes in the overall sensitivity of organ response mechanisms will differentially alter the relative strength of two receptor inputs. This would be observed as a change in the potency of an antagonist. Under these circumstances, differences in the stimulus-response characteristics of a tissue would result in a change in the Schild regression for a selective antagonist. These concepts are discussed in terms of the use of Schild analysis in functional systems for the detection of physiologically relevant mixtures of receptors and the possible advantages over biochemical binding data.

There is evidence to suggest that heterogeneous receptor populations in various tissues mediate similar, and in some cases identical, cellular responses in tissues. With the use of selective ligands, agonists, and antagonists, receptor population heterogeneity can be identified with biochemical binding techniques, as well as in functional tissue studies. The important factors in biochemical binding studies for the detection of receptor heterogeneity are the binding selectivity of the radioactive ligand, the binding selectivity of the displacing drug, and the relative receptor densities of the various receptor populations.

There are theoretical reasons why functional tissue studies may be more sensitive indicators of mixed receptor populations. Whereas there are three factors involved in the selectivity of drugs in binding studies, there are three additional factors involved in functional studies. These are the relative intrinsic efficacy of the agonist for the receptors, the relative strength of the stimuli from each of the receptor systems, and the relative efficiency of the transduction mechanisms in the tissue that transform the receptor signal into tissue response (see Table 1). This paper describes a model in which the effects of these additional factors on the competitive antagonism of antagonists, as observed by Schild analysis, are calculated.

Methods

Dose-response curves for agonists. This model describes the effects of two receptor-mediated stimuli that contribute to the overall organ response. The relative strengths of the two stimuli are variable, as are their sensitivities to agonists and antagonists. The primary stimulus is arbitrarily set to a standard Langmurian isotherm:

$$S_1 = \frac{[A]/K_A}{[A]/K_A + 1} \quad (1)$$

It is assumed that a secondary stimulus (S_2) can be generated by the tissue via a secondary receptor system. The maximal strength of this stimulus, relative to the maximal strength of S_1, is defined by a proportionality factor μ. In addition, the sensitivity of the organ to the

TABLE 1

Discriminating factors for receptor subtypes in binding versus functional studies

Binding studies	Functional studies
Radioactive ligand	
Selective affinity	Selective affinity
	Selective intrinsic efficacy[a]
Antagonist	Antagonist
Selective affinity	Selective affinity
Receptors	Receptors
Relative density	Relative density
	Coupling to transducers[a]
	Effector transduction
	Efficiency of transduction[a]

[a] Additional discriminating factors for functional studies, compared with binding studies.

secondary stimulus S_2 is variable. Thus, the concentrations of agonist that activate the receptor mechanisms to produce the secondary stimulus are related to those that produce the primary stimulus by a factor L. Therefore, the secondary stimulus is given by:

$$S_2 = \frac{\mu \cdot [A]/(L \cdot K_A)}{[A]/(L \cdot K_A) + 1} \quad (2)$$

It is further assumed that the total stimulus presented to the tissue stimulus-response machinery is the additive sum of S_1 and S_2 ($S_t = S_1 + S_2$). It should be stressed at this point that the manner in which the two stimuli are combined does not affect the subsequent predictions of this model and that a more complex relationship could exist between S_1 and S_2, with possible interdependence between tissues. However, the simplest case of additivity was used in these calculations to explore the effect of the secondary input on competitive antagonism. Differences in the way in which S_1 and S_2 combine would affect where the deviations from simple competitiveness would occur along the concentration axis of the Schild regression and also the absolute magnitude of the deviations, but not the general observations (see below), which may be used to detect heterogeneity in receptor populations. Under these circumstances, the method of combining S_1 and S_2 has no relevance to the predictions of this model regarding the behavior of heterogeneous receptor systems.

The relationship between the total receptor stimulus S_t and tissue response is a rectangular hyperbolic function. Thus, the tissue response is given by a general logistic function (1)

$$R = \frac{S_t}{S_t + \beta} \quad (3)$$

where β is a fitting parameter with which the relative "efficiency" of tissue processing of the receptor stimulus into response can be manipulated. Thus, when the magnitude of β is small, relative to S_t, a large response will be produced by a relatively small stimulus. This would characterize an "efficiently coupled" tissue response system with a large effective receptor reserve. Larger values of β would create tissue response systems of lower efficiency.

It should be made clear that, although the term stimulus is used for S_1 and S_2, this should not be equated with the formal pharmacological term defined by Stephenson (2). Eq. 1 defines a quantity of response-generating input that results from the activation of a population of receptors by an agonist with a given intrinsic efficacy for that receptor. The binding of the agonist to the receptor is normalized ($[A]/K_A$); thus, the affinity of the agonist for the receptor is not a variable. This model of receptor activation is compatible with both the classical receptor theory and the operational model of Black and Leff (3).

Competitive antagonism. Responses were calculated for the two-receptor system described above in the presence of a competitive antagonist. Under these circumstances, the total stimulus to a tissue is given by:

$$S = \frac{[A]/K_A}{[A]/K_A + [B]/K_B + 1} + \frac{\mu \cdot [A]/(L \cdot K_A)}{[A]/(L \cdot K_A) + [B]/(\theta \cdot K_B) + 1} \quad (4)$$

where $[B]$ denotes the molar concentration of the antagonist. All equilibrium dissociation constants for both the agonist and the antagonist are for the primary receptor system (S_1). Under these conditions, the relative equilibrium dissociation constant for the antagonist-receptor complex for the primary receptor generating S_1 is denoted by K_B and for the secondary receptor (S_2) by $\theta \cdot K_B$. Therefore, the relative affinity of the antagonist for receptor 1 versus receptor 2 is θ^{-1}.

Eq. 4 was used to calculate dose-response curves in the absence and presence of various concentrations of antagonist. A Research Programming Language program was written to calculate the concentrations of agonist required for half-maximal response, and these were used to calculate dose ratios for regressions, according to the Schild equation (4)

$$\log (DR - 1) = \log [B] - \log K_B \quad (5)$$

where DR refers to the ratio of equiactive concentrations of agonist in the presence and absence of antagonist $[B]$ and K_B is the equilibrium dissociation constant of the antagonist-receptor complex.

Results

Dose-Response Curves for Agonists

Eq. 2 describes a secondary input from another receptor system in the same tissue and scales that input by a factor μ, for the maximal strength of the secondary stimulus. Fig. 1A shows theoretical dose-response and stimulus-response curves for a two-receptor system with variable μ values for S_2. A location factor L is used to define the relative potency of the agonist for the second system. The effects of varying L are shown in Fig. 1B. The biochemical outputs of these two receptor systems are assumed to be summed in the cytosol (i.e., two receptors that increase cAMP or intracellular calcium) and fed into a further biochemical cascade to produce the tissue response. The mathematical interpretation of this second process is given by the general logistic function (eq. 3). This allows for the maximal flexibility of the system, because tissue sensitivity can be manipulated without assuming specific changes in either receptor system. Fig. 1 shows the translation of the summed stimulus into tissue response for a tissue with an efficiency factor of $\beta = 0.03$.

Fig. 2A shows the calculated response in a tissue possessing two receptor systems of different input strengths. A notable feature of this figure is the fact that the response curve resulting from two stimulus inputs with different locations (L) and maximal strengths (μ) is monophasic. Therefore, it is not apparent from the shape of this dose-response curve that it is a composite of two separate receptor inputs. The values of μ, L, and β have all been chosen so that, at the concentrations required to produce the dose-response curve, the curve corresponds completely to the response generated by the primary stimulus S_1 and is not modified by the input of S_2. This is in contrast to the dose-response curve shown in Fig. 2B. In this figure, the values of μ, L, and β have been chosen to cause the total response function to be shifted to the left of the response generated by S_1. This is because the secondary stimulus S_2 does contribute to the total response. However, as in Fig. 2A, the total response curve is still monophasic. In contrast, Fig. 2C shows that, when the transducer function (eq. 3) is of low

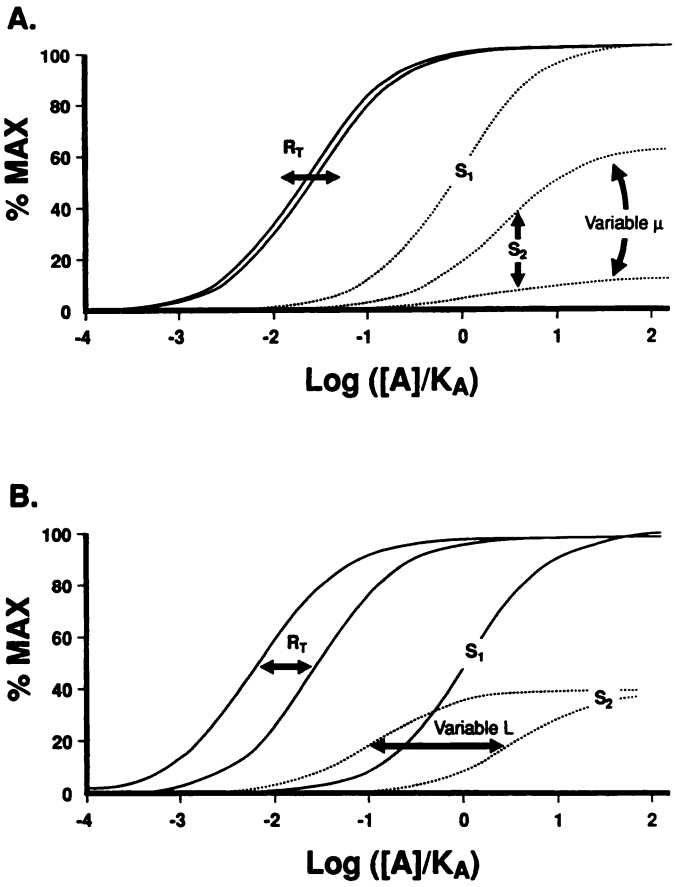

Fig. 1. Dose-response curves for an agonist activating two receptors in a single tissue. *Ordinates*, response as a percentage of the maximal response. *Abscissae*, logarithm of molar concentrations of agonist expressed as a fraction of the agonist-receptor equilibrium dissociation constant for receptor 1. Total response is designated as R_t, emanating from the summation of two stimuli, S_1 and S_2. A, Variation in μ. The maximal strength of S_2 is variable by a factor μ. The variance in R_t results from differences in S_2. $\beta = 0.03$, $L = 3$, μ varies from 0.1 to 0.6. B, Variation in L. The location parameter of the dose-stimulus curve for S_2 is variable by a factor L. The variance in R_t results from the differences in the location of the curve for S_2 along the concentration axis. $\beta = 0.03$, $\mu = 0.4$, L varies from 0.1 to 3.

efficiency, such that each of the stimuli does not produce the maximal tissue response, the relative contributions of S_1 and S_2 may become apparent in an obviously biphasic dose-response curve. The Hill plots for the three dose-response curves shown in Fig. 2, A–C, are shown in Fig. 2D. Here it can be seen that, when the primary stimulus S_1 is dominant (Fig. 2A) or when the efficiency of the transducer function is high (Fig. 2B), the resulting dose-response curve for the agonist appears to be a single Langmurian binding isotherm, with a uniform Hill plot slope. However, as seen in Fig. 2D, when the efficiency of the transducing function is low and the two stimuli are sufficiently separated with respect to either their relative maximal strengths or the location along the concentration axis at which they are activated, a change in the slope of the dose-response curve can be observed. This was evident in the curvilinear Hill plot (Fig. 2D) for the dose-response curve shown in Fig. 2C.

Schild Regressions in Two-Receptor Systems

For a one-receptor system at equilibrium, the Schild regression according to eq. 5 is linear and has a slope of unity and an intercept of K_B for a simple competitive antagonist. However, in a two-receptor system, unless the affinity of the antagonist for the two receptors is identical, at some point along the Schild regression a deviation from the single-receptor regression will occur. This is because the agonist will encounter the secondary receptor, which will not be blocked to the same extent as the primary receptor. Under these circumstances, more response will be generated than that predicted by a competitively antagonized single receptor population. This is predicated on the premise that the secondary receptor is less sensitive to the antagonist than is the primary receptor. If the antagonist is a more potent blocker of the secondary receptor, then the second receptor stimulus will not contribute to the overall response throughout the Schild regression and the receptor heterogeneity will not be detected by the antagonist (i.e., the Schild regression will be a straight line with a slope of unity).

Varying Maximal Secondary Signal Strength

Fig. 3A shows theoretical Schild regressions for a single-receptor system (Fig. 3A, *curve 1*) and for tissues with various increasing strengths of a secondary stimulus for an antagonist with a 100-fold greater affinity for the primary receptor, compared with the secondary receptor. It should be noted that the differences in the magnitude of the secondary stimulus (μ) can be brought about by different agonists (having different relative efficacies for the two receptors), differences in the receptor density of the secondary receptor population, and/or differences in the transducer functions relating receptor activation to production of S_2. As can be seen from Fig. 3A, there are nonlinear portions in these regressions. Fig. 3B shows the first differentials of the Schild regressions and indicates that in no case do the slopes increase above unity. Fig. 3B also shows that the portion of the Schild regression demonstrating a slope of less than unity and the point of minimal slope both vary with μ, i.e., there is more nonlinearity with increasing input from the secondary receptor system. It can been seen that, when the secondary receptor system becomes dominant (i.e., $\mu > 1$), the regression again tends towards linearity, with a pK_B equal to the equilibrium dissociation constant of the antagonist for the secondary receptor. Under these circumstances, nonlinear portions of the Schild regression occur at dose ratios at or below the pA_2.

Varying Relative Location of Secondary Signal

Qualitatively, the same effects are observed when the relative location (along the agonist concentration axis), rather than the relative strength of the secondary receptor effects, is varied. Fig. 4A shows the effects of a standard maximal strength contribution from a secondary receptor system ($\mu = 0.5$), when those effects are activated at concentrations 0.03–10 times the concentrations required to activate the primary receptor system ($L = 0.03$–10). As with changes in μ, the regressions are linear, with nonlinear portions occurring when the secondary receptor system is activated by the agonist. The slopes of these regressions are shown in Fig. 4B, where it can be seen that the span of the nonlinear portions and the decrease in slope are dependent upon L, the relative location of the secondary receptor activation.

Varying Efficiency of Transducer Function

Another potential point of variability is the transducer function into which S_1 and S_2 feed (eq. 3). The physiological

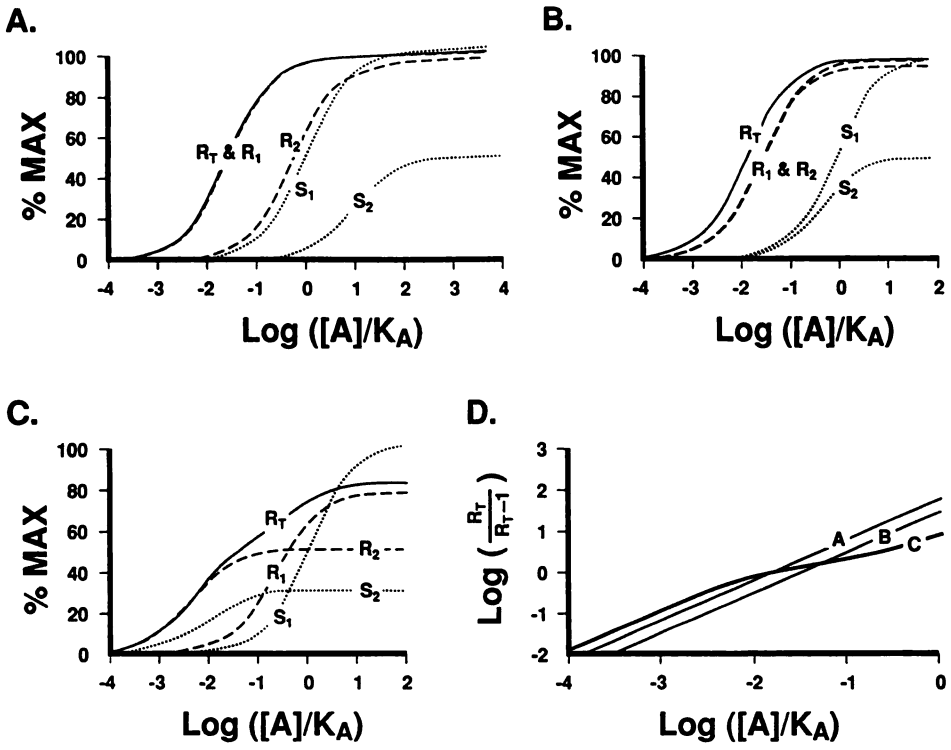

Fig. 2. Effects of varying combinations of μ and L on total response. *Ordinates* and *abscissae*, as for Fig. 1. A, The magnitude of S_2 is such that total response, R_t, completely depends upon S_1, and the curve for R_t is monophasic. $\beta = 0.03$, $\mu = 0.5$, $L = 10$. B, S_2 contributes to total response, R_t, but the curve for R_t is still monophasic. $\beta = 0.03$, $\mu = 0.5$, $L = 0.5$. C, Both S_1 and S_2 contribute to total response, R_t, and the dose-response curve for R_t deviates from a standard Langmurian isotherm. $\beta = 0.3$, $\mu = 0.3$, $L = 0.01$. D, Hill plots for dose-response curves shown in A–C. *Ordinates*, logarithms of $(R_t/(R_t - 1))$, where R_t is expressed as a fraction of the maximal response. *Abscissae*, as for A–C. Hill plot shown for the curve shown in A is designated A, with B and C labeled similarly.

rationale for such variability would be a difference in the sensitivities of cellular mechanisms to second messengers. Unlike differences in μ and L, which can occur because of both tissue and agonist factors, differences in the efficiencies of transducer functions would be solely tissue related. As can be seen from the preceding figures in which μ and L are varied, relatively small secondary stimuli can significantly modify Schild regressions if the amplification in the transducer function is large. One example of such a tissue system is one in which the secondary stimulus is small (i.e., 5% of the primary stimulus) and activated by an agonist at concentrations one third of those required to activate the primary stimulus ($L = 0.3$). If the 5% secondary stimulus were not amplified by a cellular mechanism, it would produce very little effect on the antagonism of the primary receptor system (i.e., very little perturbation of a binding curve in a radioligand binding experiment). However, if there is cellular amplification of both the primary and secondary stimuli, then a 5% secondary stimulus would, in fact, result in a very substantial total contribution to tissue response. Fig. 5A shows the effects of varying coupling efficiencies of the total stimulus to the tissue response (varying β in eq. 3). As can be seen from Fig. 5A, a 5% secondary stimulus could produce significant nonlinearity in the Schild regression. Fig. 5B shows the slopes of the Schild regressions.

Detection of Two-Receptor Systems

It was clear from the calculations that two-receptor systems often could yield nonlinear Schild regressions. Therefore, it could be assumed that curved Schild regressions with slopes of less than unity indicate the presence of two or more receptor populations in a tissue. However, it should be noted that the degree of curvature depends upon the relative strength of the input from the secondary receptor and the efficiency of the stimulus transducer and that these are unique features of a given tissue. Therefore, if more than one tissue is used to construct a Schild regression, a variation of values for β, μ, and L would be operative throughout the analysis. Under these circumstances, the resulting mean Schild regression may not uniformly reflect a fixed receptor mixture. Fig. 6A shows calculated data points for five experiments in five tissues with a fixed transducer efficiency ($\beta = 0.03$) and fixed relative location for secondary receptor input ($L = 0.3$). However, it was assumed that the maximal secondary receptor inputs varied from 1% to 5% ($\mu = 0.01$, 0.02, 0.03, 0.04, and 0.05) from tissue to tissue. The curved line in Fig. 6B indicates the true mixed receptor regression line for a 3% maximal secondary receptor input, and the straight line indicates the best fit straight line through the data points. It can be seen from Fig. 6B that the curved nature of the Schild regression is difficult to detect over a 100-fold range of antagonist concentrations and that the data points simply indicate a straight line with a slope less than unity. Fig. 6C shows the same analysis for a two-receptor system with a fixed maximal input ($\mu = 0.2$) but variable L, where L ranges from 1 to 3. As with variations in μ, this leads to data points that do not clearly indicate nonlinearity.

Tests for Two-Receptor Systems

Calculations with this model predict three distinctive behaviors of two-receptor systems, which may be detected experimentally and used as tests for receptor heterogeneity. These are 1) changes in slopes of dose-response curves, with rightward displacement, 2) heteroscedacity of error in ordinate values of Schild regressions, and, perhaps most accessible experimentally, 3) sensitivity of antagonist potency to stimulus-response coupling.

Dependence of slope of dose-response curves on antagonist concentration. One possible indicator of receptor heterogeneity is a change in the slope of the dose-response curve in the presence versus the absence of selective receptor antagonists. As seen in Fig. 2, the character of the dose-response

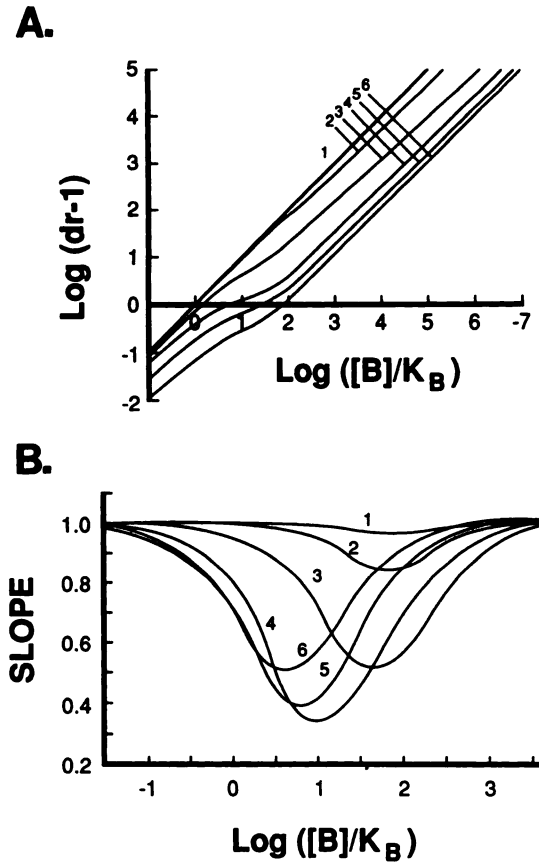

Fig. 3. Theoretical Schild regressions for two receptor systems with varying strength of secondary stimulus (S_2 varies with μ). *Ordinates*, logarithms of equiactive dose ratios of agonist minus one. A, Schild regressions for a system of $\beta = 0.01$ and $L = 10$, for an antagonist with 100 times greater affinity for receptor 1 than receptor 2. *Abscissae*, logarithms of molar concentrations of antagonist expressed as fractions of the equilibrium dissociation constant of the antagonist-receptor complex for receptor 1. Regressions are shown for $\mu = 0$ (*curve 1*), 0.01 (*curve 2*), 0.10 (*curve 3*), 10 (*curve 4*), 100 (*curve 5*), and 1000 (*curve 6*). B, Slopes of the Schild regressions shown in A. *Ordinates*, slope of regression line. *Abscissae*, as for A.

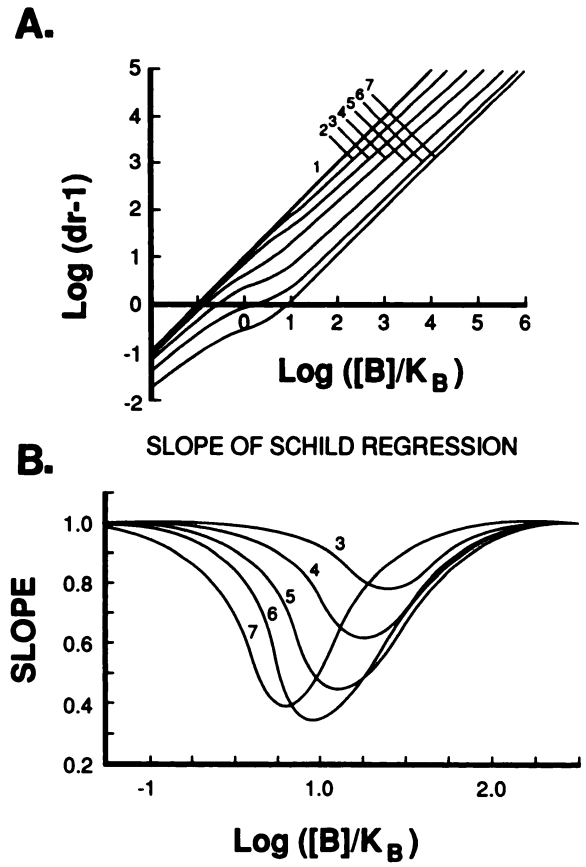

Fig. 4. Variable L for secondary stimulus, S_2, of constant maximal strength ($\mu = 0.5$). A, *Ordinates* and *abscissae*, as for Fig. 3A. $\beta = 0.01$, $\theta = 100$. *Curve 1*, single-receptor system ($\mu = 0$); *curves 2–6*, tissue with $\mu = 0.5$ and L variable; $L = 10$ (*curve 2*), 3 (*curve 3*), 1 (*curve 4*), 0.3 (*curve 5*), 0.1 (*curve 6*), or 0.03 (*curve 7*). B, *Ordinates* and *abscissae*, as for Fig. 3B. Slopes for the Schild regressions shown in A.

curve for an agonist activating two receptors in a tissue depends upon the relative strength of input from the two receptors. Depending on the relative values of β, μ, and L, there may be no indication of receptor heterogeneity from the shape of this curve. Also, the total response may not be modified by the secondary receptor in the absence of the antagonist. However, if the relative receptor occupancy of the agonist was modified by a selective receptor antagonist, then there would be a corresponding change in the relative inputs of S_1 and S_2 to the dose-response curve. This could be manifested in a change of shape of the dose-response curve in the presence of the antagonist. Fig. 7 shows the dose-response curve for a two-receptor system (Fig. 7, *curve 1*); the dotted line indicates the expected change in location of this curve in the presence of a simple competitive antagonist at 100 times the K_B for the primary receptor. Fig. 7, *curve 2*, indicates the curve observed in the presence of an antagonist with 100 times greater affinity for receptor 1, compared with receptor 2. It can be seen from Fig. 7 that, because curve 2 reflects more of the secondary receptor input (less antagonism of this site), its shape changes accordingly.

Heteroscedacity of Schild regression error. A second possible indicator of receptor heterogeneity is the dependence of the magnitude of the standard error of the ordinate values for Schild regressions upon the independent variable of antagonist concentration. Fig. 8 shows the effect of a 20% random error in the independent variable (antagonist concentration) of a Schild regression in a one-receptor system. As can be seen from Fig. 8, the magnitude of this error does not change across a wide range of values for the independent variable (antagonist concentration). The smoothed curve shown in Fig. 8 illustrates that the error is homeoscedastic. However, in a two-receptor system, minor differences in receptor coupling, receptor number, and efficiency of stimulus-receptor transduction produce errors in Schild regressions that do depend upon antagonist concentration. This is because the deviation from linearity of Schild regressions in two-receptor systems depends upon the concentration of antagonists, because these change the relative activation of the receptor subpopulations by the agonist.

Fig. 9A shows the dependence of the error of Schild regression ordinate values on antagonist concentration for tissues of differing maximal secondary receptor input ($\mu = 0.1$–100). Although there is no uniform relationship between the magnitude of the error and antagonist concentration, it can be seen that the magnitude of the error is not constant across a range of antagonist concentrations. This also is evident in two-receptor systems where the maximal secondary receptor input is constant but the sensitivity of this secondary input to the agonist

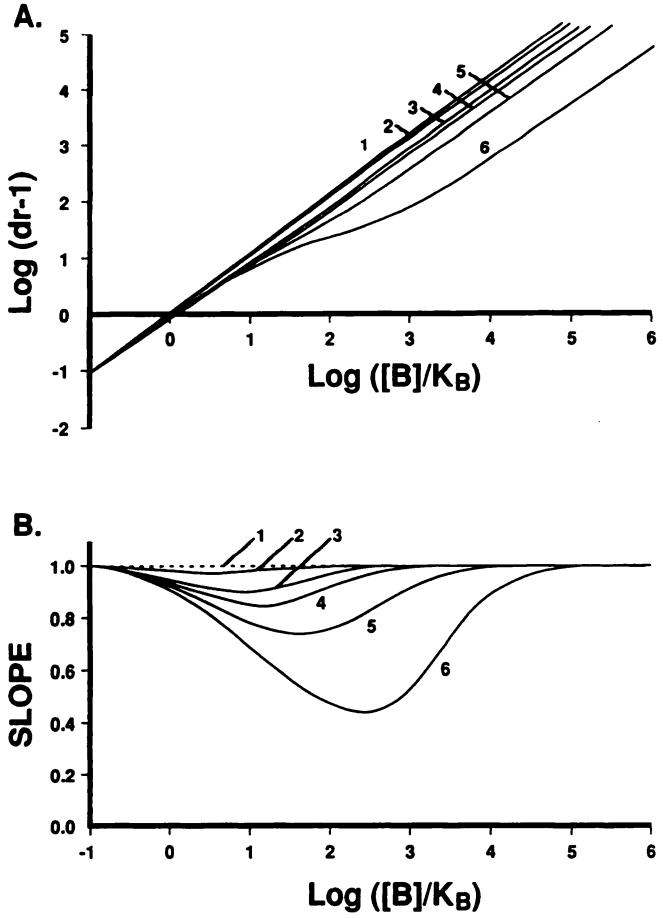

Fig. 5. Effect of varying efficiency of stimulus transduction in two receptor systems. A, Schild regressions for systems with fixed secondary stimulus input ($\mu = 0.05$, $L = 0.3$) and variable efficiency of stimulus transduction into tissue response, for an antagonist with a 1000 times greater affinity for receptor 1, compared with receptor 2 ($\theta = 1000$). *Ordinates* and *abscissae*, as for Fig. 3A. Curves are for a single-receptor system (*curve 1*) and a dual-receptor system with efficiency of transduction (β in eq. 3) of $\beta = 0.2$ (*curve 2*), 0.1 (*curve 3*), 0.08 (*curve 4*), 0.06 (*curve 5*), or 0.04 (*curve 6*). B, Slopes for regressions shown in A.

concentration is variable (variable L; see Fig. 9B). Fig. 9C shows the effect of antagonist concentration on the magnitude of the ordinate error in a two-receptor system. For this particular system, the magnitude of the error is bell-shaped over the concentration of antagonist shown. However, if a random concentration error is superimposed upon this biological error, it can be seen that the dependence upon antagonist concentration is reduced and the error becomes more homeoscedastic. Therefore, random concentration errors reduce the sensitivity of this method to detect receptor heterogeneity.

Sensitivity of antagonist potency to transducer amplification. As seen in Fig. 5, even a minimal secondary receptor input of 5% can significantly modify a Schild regression, if that secondary input is sufficiently amplified by the stimulus-response characteristics of the tissue. The dependence of the Schild regression on the amplifying properties of the tissue can be used to detect receptor heterogeneity. Fig. 10A shows two Schild regressions for a two-receptor system. Fig. 10A, *curve 2*, is for a tissue with efficient amplification of receptor stimulus ($\beta = 0.03$). As can be seen from Fig. 10A, the regression is curved, with portions having slopes less than unity. Fig. 10A, *curve 1*, is for the same tissue after a reduction in the amplification factor for stimulus-response ($\mu = 0.3$). Such a change could be produced experimentally by altering the experimental conditions needed for optimal tissue response (i.e., reducing the extracellular calcium concentration for tissues that require calcium entry for contraction, etc.). As seen in Fig. 10, *A* and *B*, a reduction in β results in a disproportionate modulation of the secondary receptor input. This occurs because the secondary input is intrinsically weaker than the primary input. The result is a regression nearly characteristic of a single-receptor system, with properties of the primary receptor.

Discussion

It is of practical importance to determine whether the response of a given organ to hormones, neurotransmitters, or foreign ligands is mediated by a homogeneous receptor population or a mixture of receptor subtypes. The latter circumstance leaves open the possibility of selective organ activation or inactivation via one of the receptors. The selective antagonism of receptors with simple competitive antagonists offers a method of determining whether receptor populations are heterogeneous, by comparison with a model that assumes receptor homogeneity. Thus, Schild analysis predicts what should be observed when a homogeneous receptor population of receptors is blocked competitively by a drug. Under these circumstances, the equilibrium dissociation constant of the antagonist-receptor complex can be estimated. Deviation from the predictions of this model can be due to either the presence of nonequilibrium steady states or a mixture of receptors (5, 6). This paper attempts to delineate some general guidelines for the use of Schild analysis to detect heterogeneous receptor populations in tissues.

Biochemical binding studies are widely used to determine receptor homogeneity. Thus, with the use of computer programs, such as LIGAND, the relative affinity constants of antagonists for each receptor population can be estimated according to a given model. As summarized in Table 1, the relative selectivity of the radioactive and competing ligands and the relative receptor density of the two sites determine the ability of this approach to detect receptor heterogeneity. It should be noted that, with this technique, the relative signals from the two receptor types are of equal strength, i.e., the displacement of a single radioactive ligand molecule for each receptor type. Under these conditions, the relative receptor number is an important determinant of the overall ligand binding profile. Therefore, the data from such binding studies may be misleading, because they primarily yield information about the relative presence of receptors and not about their physiological importance. There are examples of tissues with heterogeneous receptor populations (as detected by ligand binding experiments) where the response to the endogenous agonist is primarily mediated by only one of the receptor subtypes. For example, ligand binding studies indicate the presence of large populations of β_1-adrenoceptors on rat adipocytes, whereas response is mediated by an atypical β-adrenoceptor with a different pharmacological profile (7, 8). It is estimated that, in the brown adipose tissue of the rat, most of the lipolytic response to the agonist BRL 37344 is mediated by only 15% of the total β-adrenoceptor mixture of β_1- and atypical adrenoceptors (9). In the rat vas deferens, there are equal proportions of

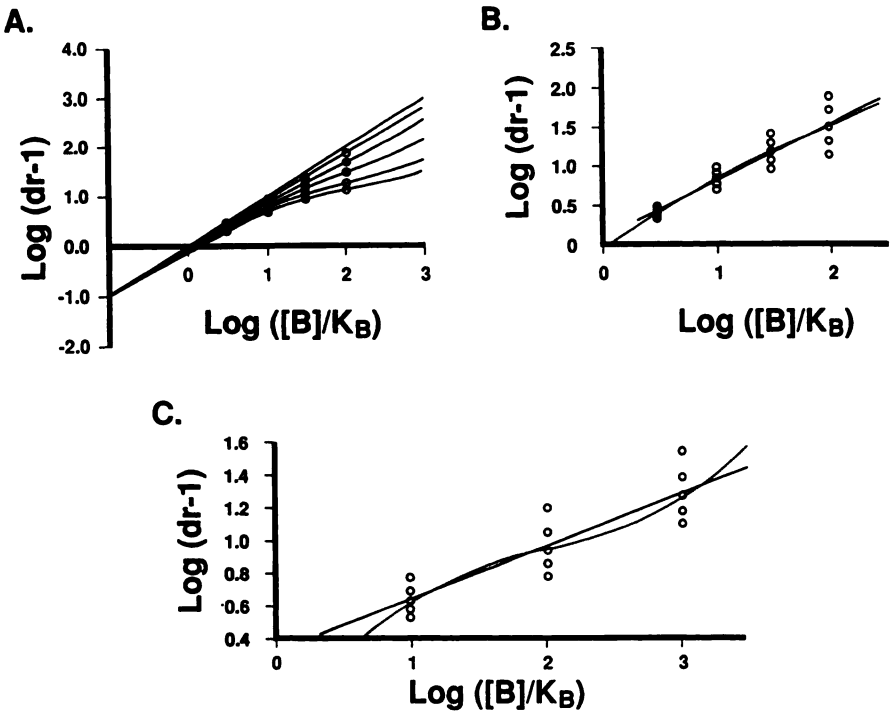

Fig. 6. Effect of variability in secondary receptor input on Schild regressions for two-receptor systems. A, Schild regressions for tissues with variable maximal secondary receptor input (μ). *Ordinates* and *abscissae*, as for Fig. 3A. Data points are for regressions for constant $\beta = 0.03$, $L = 0.3$, and variable μ (antagonist with a 1000 times greater affinity for receptor 1, compared with receptor 2). Points and calculated curves are for a single receptor (*straight line*) and a secondary receptor input of $\mu = 0.1$, 0.02, 0.03, 0.04, and 0.05. B, Data points from regression shown in A, with the best fit curved line to the model (····) and the best fit straight line by least squares regression (——). C, Schild regressions for tissues with secondary receptor input of variable sensitivity (L). Data points were calculated for a range of tissues with constant maximal secondary receptor input ($\mu = 0.2$), constant stimulus transduction efficiency ($\beta = 0.03$), and variable secondary receptor sensitivity ($L = 1$, 1.2, 1.5, 2, or 3). ····, Best fit curved line from the model; ——, best fit straight line, by the least squares method.

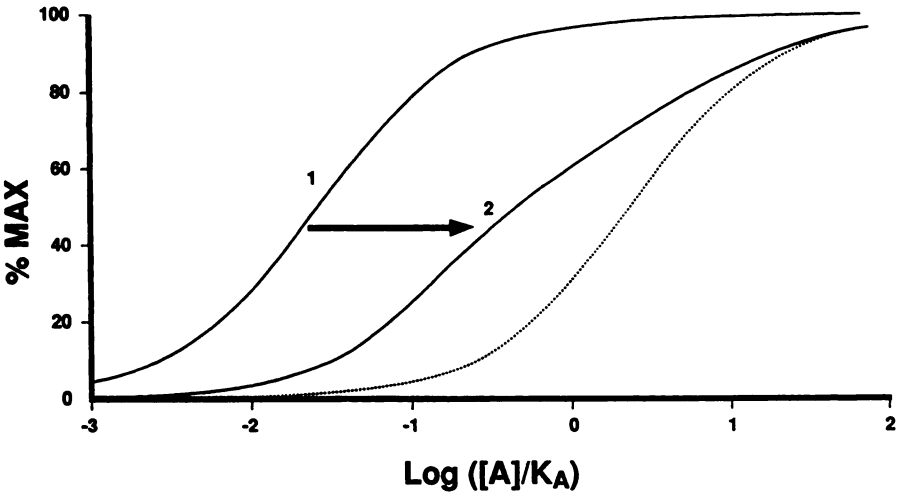

Fig. 7. Effects of a selective antagonist on the slopes of dose-response curves for an agonist activating two receptors in the same tissue. *Ordinates* and *abscissae*, as for Fig. 1. *Curve 1*, control dose-response curve in the absence of antagonist, for a two-receptor system of $\mu = 0.05$, $L = 0.5$, and $\beta = 0.03$. *Curve 2*, curve for the same agonist in the presence of an antagonist that has a 100 times greater affinity for receptor 1, compared with receptor 2. ····, predicted response for simple competitive antagonism of receptor 1 only.

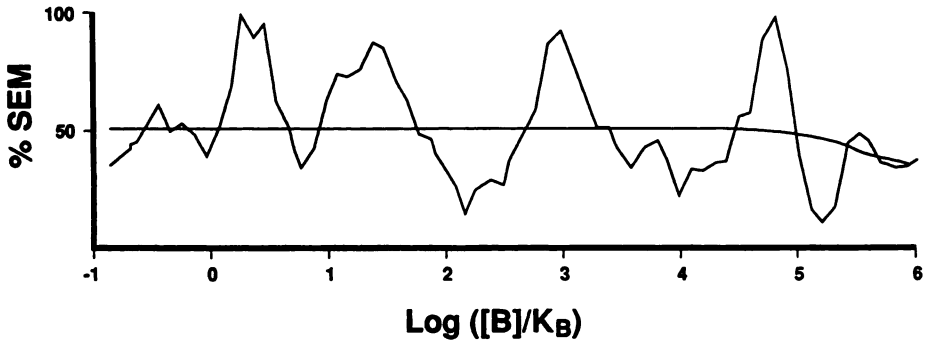

Fig. 8. Effects of a 20% random error in antagonist concentration on the standard error of ordinate values for a Schild regression. *Ordinates*, standard errors of the mean (*SEM*) for five replicate values of log (DR − 1), expressed as a percentage of the maximal standard error. *Abscissae*, logarithms of the molar concentration of antagonist expressed as a fraction of the equilibrium dissociation constant of the antagonist-receptor complex. Curves show the effects of 20% random error, including both raw data and smoothed data, to show the average trend.

α_{1a}- and α_{1b}-adrenoceptors, but only the α_{1a}-adrenoceptors mediate contractile response (10).

Schild analysis of the antagonism of agonists mediating responses in tissues theoretically has the advantage of emphasizing the physiologically relevant receptor. However, unlike in biochemical binding studies, the receptors mediating tissue response produce a stronger signal. Thus, the stimulus-response mechanisms of tissue provide an added discriminator for the detection of receptor heterogeneity. The antagonism of mixed receptor populations in tissues has been analyzed previously (11, 12). The present analysis differs from those studies in two ways. First, no attempts are made with this model to associate

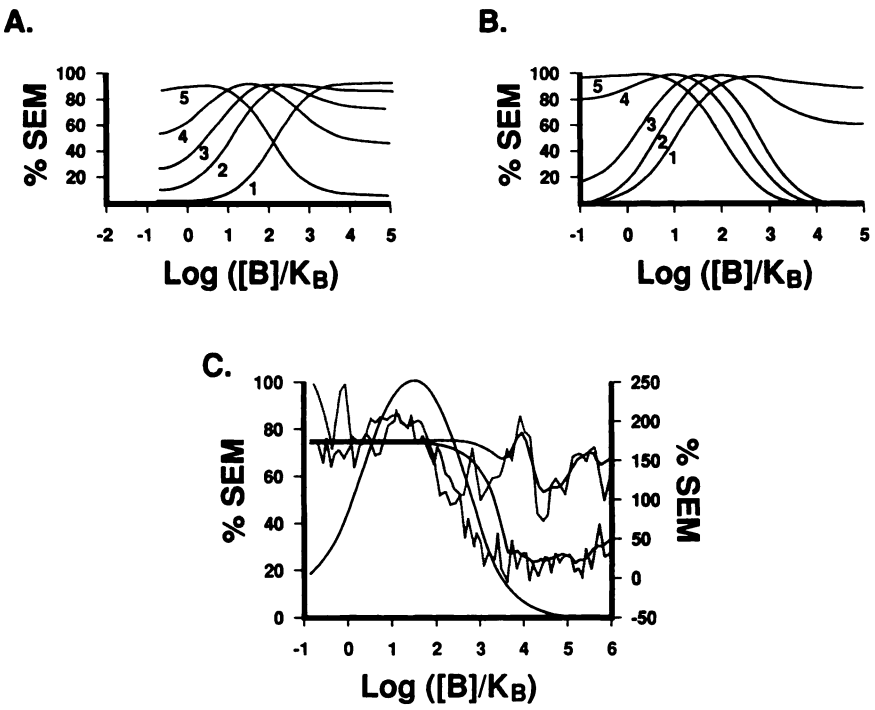

Fig. 9. Effects of variation in secondary receptor input, in two-receptor systems, on standard error of ordinate values for Schild regressions. *Ordinates* and *abscissae*, as for Fig. 9. A, Standard error for Schild regressions for a two-receptor system of $L = 1$ and $\beta = 0.01$, for an antagonist with 1000-fold selectivity for receptor 1. Curves were calculated for different maximal secondary receptor input; standard errors were generated by calculating log (DR − 1) values for five replicates, with differences in μ of −20%, −10%, 0%, 10%, and 20%. Curves are shown for $\mu = 0.1$ (*curve 1*), 1 (*curve 2*), 3 (*curve 3*), 30 (*curve 4*), and 100 (*curve 5*). B, Curves calculated for a fixed maximal input ($\mu = 1$) and variable sensitivity; $L = 0.03$ (*curve 1*), 0.1 (*curve 2*), 1 (*curve 3*), 3 (*curve 4*), or 10 (*curve 5*). C, Smooth bell-shaped curve for a 50% biological variation in μ, for a two-receptor system of $L = 1$, $\mu = 1$, $\beta = 0.03$, and $\tau = 1000$. *Ordinates* refer to *left axis*. *Right axis* refers to the standard error of this system with a 20% (*upper curve*) and and 30% (*lower curve*) random concentration error superimposed. Calculated and smoothed curves are shown.

the relative strength of the secondary receptor input with specific properties of agonists and/or tissues. Therefore, a given value of μ could arise from one or more of three sources, a high specific intrinsic efficacy of the agonist for the secondary receptor, a disproportionately larger number of secondary receptors, or an especially efficient stimulus-response cascade for the secondary receptor. An example of this latter effect is seen in rat adipocytes, with responses being mediated by a mixture of β_1- and atypical β-adrenoceptors. It was found that the atypical β-adrenoceptor agonist BRL 37344 was a partial agonist for adenylate cyclase, compared with responses to isoproterenol (which activated both β_1- and atypical β-adrenoceptors), but was 10 times more potent in promoting lipolysis. These data indicate that the transduction mechanism for the atypical β-adrenoceptors was considerably more efficient than that for β_1-adrenoceptors, i.e., atypical β-adrenoceptors are selectively amplified for tissue response (13).

Distinctions between efficacy, receptor number, and stimulus transduction cannot be made, because they translate into essentially the same general effect on receptor antagonism studies, namely, the magnitude of the secondary receptor input (μ). Difference in the "sensitivity" of the secondary receptor input (denoted by the factor L) could be caused by differences in the relative receptor number, the efficiency of the stimulus-response mechanisms, or differences in the relative affinity of the agonist for the two receptors. For these reasons, the present model treats the secondary receptor input operationally.

A second difference between this and previous studies is the attempt to generalize the model to a point where predictions can be made and criteria can be established to detect and study mixed receptor systems. The most commonly used criterion for detection of receptor heterogeneity in functional studies is the dependence of antagonist potency on the type of agonist used experimentally. Thus, if Schild regressions are different for a given antagonist when it is used to block the effects of different agonists, it can be assumed that those agonists produce responses by activating a mixed receptor population. This approach requires at least two selective agonists for either receptor type. This paper describes three additional experimental characteristics and/or manipulations of an experimental system that could be useful.

One hallmark of receptor heterogeneity would be a change in the slope and/or shape of the agonist dose-response curve during Schild analysis. This may occur because of the variation of the relative receptor inputs into the tissue response mechanisms as one of the receptors is selectively blocked more than the other. However, this could also occur in single-receptor systems, if secondary properties of the agonist are expressed at various points along the concentration axis as dose-response curves are shifted to the right.

A second possible characteristic of mixed receptor systems is a pattern of heteroscedastic errors in the Schild analysis. In single-receptor systems, differences in tissue sensitivity due to variations in stimulus-response mechanisms and/or receptor densities would have no effect on the dose ratios produced by simple competitive antagonists. Similarly, random errors in the independent variable of antagonist concentration would produce a corresponding error, in the ordinate values of Schild regressions, of uniform magnitude along the antagonist concentration axis. The same is not true of mixed receptor systems. Differences in the sensitivity of tissues due to variation in stimulus-response characteristics and/or relative receptor densities would cause differences in the relative receptor mixture of input stimuli, with a corresponding difference in dose ratios. Because this varies with antagonist concentration, these differences vary in magnitude along the antagonist concentration axis. Experimentally, these would be perceived as "errors" (variability in measurement); thus, the error would be dependent on the concentration of antagonist (it would be heteroscedastic).

Perhaps the most striking feature of these calculations is the dependence of antagonist potency on the total sensitivity of

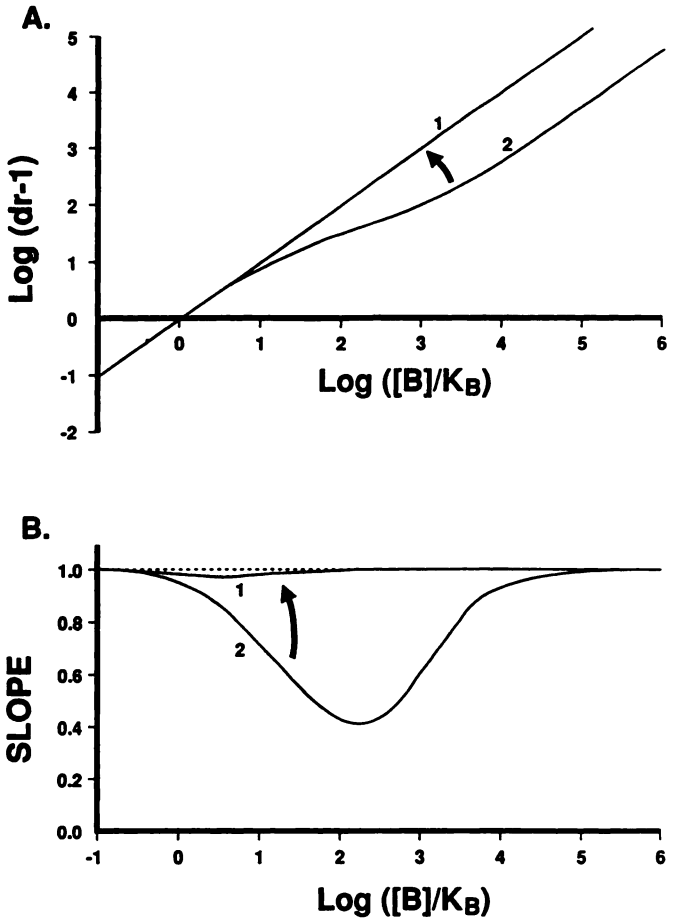

Fig. 10. Effect of diminishing transducer efficiency on a Schild regression for a two-receptor system. *Ordinates* and *abscissae*, as for Fig. 3, A and C, for A and B, respectively. A, Calculations for a selective antagonist ($\theta = 1000$) in a tissue with secondary receptor stimulus input of $\mu = 0.05$ and $L = 0.5$. *Curve 2*, curved regression for tissue with a transducer of efficiency of $\beta = 0.03$. *Curve 1*, regression for the same system when the transducer efficiency has been reduced 10-fold ($\beta = 0.3$). B, Slopes of the Schild regressions shown in A.

the tissue to agonists. This approach is based on the assumption that tissue amplification systems often produce an effective receptor reserve for agonists, in that a very low receptor occupancy would be sufficient to produce the maximal response. Therefore, in such systems a very low secondary receptor input could be sufficiently amplified to contribute significantly to tissue response, and this input would be expressed in the Schild analysis. If the tissue amplification were altered, then the relative contribution of that secondary receptor input would also be altered, with a corresponding difference in the Schild analysis. No differences in antagonist potency would be expected in a single-receptor system unless secondary effects of the agonist and/or antagonist were operative in one of the tissue states. Therefore, a change in the Schild regression with a change in the total sensitivity of the tissue would suggest that a heterogeneous population of receptors were mediating the tissue response to the agonist.

In general, functional studies offer theoretical advantages for the detection of receptor heterogeneity of physiologically relevant receptors. The behavior of Schild regressions and dose-response curves can be used to detect receptor heterogeneity under certain circumstances. Unfortunately, these approaches do not suggest obvious methods to quantify relative antagonist potency on various receptor subtypes in such systems.

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