JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117

JPET #104117 PiP

An Overview of Drug Combination Analysis with Isobolograms

Ronald J. Tallarida

Temple University School of Medicine

Philadelphia, Pennsylvania

Perspectives in Pharmacology

Running title: Drug Combination Analysis

Address Correspondence to: Ronald J. Tallarida

Temple University School of Medicine

3420 N. Broad Street

Phila. PA 19140

Phone: 215-707-3243

FAX: 215-707-7068

Email: Ronald.tallarida@temple.edu

Number of text pages	17
Number of tables	0
Number of figures	4
Number of references	19
Number of words in Abstract	127
Number of words in Introduction	1588
Number of words (total text)	4795

ABBREVIATIONS: NMDA, N-methyl-D-aspartate; WIN 55212-2, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone.

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

Drugs given in combination may produce effects that are greater than or less than the effect predicted from their individual potencies. The historical basis for predicting the effect of a combination is based on the concept of dose equivalence, that is, an equally effective dose (*a*) of one will add to the dose (*b*) of the other in the combination situation. For drugs with a constant relative potency this leads to linear additive *isoboles* (*a*-*b* curves of constant effect), whereas a varying potency ratio produces nonlinear additive isoboles. Determination of the additive isobole is a necessary procedure for assessing both synergistic and antagonistic interactions of the combination. This review discusses both variable and constant relative potency situations and provides the mathematical formulas needed to distinguish these cases.

Introduction

This communication is concerned with the analysis of combinations of two drugs that produce overtly similar effects that are measurable. Each drug is therefore an agonist that displays dose-dependency. As studies of drug combinations have become more common there has emerged an increased use of the isobologram, a graph that was introduced many years ago (Loewe, 1927, 1928). That graph, constructed on a coordinate system comprised of the individual drug doses, commonly contains a straight "line of additivity" that is employed to distinguish additive from synergistic and antagonistic interactions. This graphical construction is based on the assumption of a constant relative potency.
In a previous review (Tallarida 2001) this author discussed the use and construction of the common (linear) isobole, the set of points (dose pairs) that give a specified effect magnitude. A subsequent study (Grabovsky and Tallarida, 2004) considered

combinations of a full and partial agonist, a situation that necessarily means a variable relative potency. That situation was shown to lead to nonlinear isoboles of additivity instead of the widely applied linear isobole, and demonstrates that experimental results in this case could be mistaken for synergism or antagonism. That result (nonlinearity), which represents a departure from the common use of isobolograms, prompted further attention to other situations of variable relative potency. The variability condition, however, was not explicitly connected to Loewe's concept of dose equivalence in the author's previous review. In this review, *we now make this explicit* by showing (for the first time) that the derivation leading to curves isoboles is consistent with the same concept of dose equivalence that was employed by Loewe. A further consequence of this concept is in its application to two full agonists with varying relative potency, a case that is shown here to lead to not just one, but to two, nonlinear, but *symmetric* isoboles. The demonstration (proof) of symmetry of this pair of isoboles, presented here for the first time, provides a new criterion for distinguishing between additive and non-additive interactions.

The theoretical basis for this graphical procedure, i.e., the assumptions regarding the individual drug dose-effect data on which it is based, and the consequent alterations on this graphical procedure under different assumptions, seems to be not well known. Indeed, this important topic is not discussed to any extent in our major textbooks of pharmacology. The theory that leads to either linear or nonlinear additive isoboles and its connection to the underlying assumptions, are reviewed here. Also presented is a new analysis applicable to two full agonist combinations with a varying relative potency, a topic not previously discussed. The principle aim is to predict the effect of the drug

combination and thereby distinguish between exaggerated effects and those that are expected. This expectation must have some well established basis. Toward that end the analysis of the action of a drug combination begins with the individual dose-effect data of the constituent drugs. This analysis requires that both drugs produce effects that increase with dose and, thus, only doses in the non-decreasing portion of each drug's graph are used in these calculations, i.e., there should be a unique dose for each effect level. (Thus, "inverted U" curves that may occur with higher doses of certain psychostimulant and other drugs must be restricted to lower dose combinations in the isobolar procedure described here.) From these dose-effect relations a particular effect that can be attained by each drug alone is selected. Very often this effect is 50% of the maximum, although any other effect that is reached by each can be used. The individual doses that produce the specified effect are determined from the dose-effect graphs (Fig 1A) and these doses are plotted as the axial points in a Cartesian coordinate plot termed the isobologram, a plot that was popularized by Loewe (1927,1928,1953) and shown in Fig. 1B.

In this plot each axis represents the dose of one of the drugs and the intercept values represent the doses of the individual agents that produce the specified effect. For drugs A and B these doses will here be denoted in italics, *A* and *B*, respectively. The diagonal line connecting intercepts *A* and *B* is termed the "additive" isobole and is commonly expressed in the equation

$$\frac{a}{A}+\frac{b}{B}=1 \qquad (1)$$

($0 \le a \le A,\ \ 0 \le b \le B$.)

All points (*a*,*b*) on this line segment represent dose pairs that give the specified effect. As we will see, the term "additive" is not based on the addition of effect magnitudes and,

thus, this invites the question, *"What is added?"* The answer to this will emerge as we proceed with the definitions and the underlying assumptions.

The basis of the *linear* isobole is rooted in the assumption of a constant potency ratio. It employs the concept of *dose equivalence*. This concept was actually described by Loewe (1953) who, unfortunately, used rather cumbersome symbols without much explanation and no mathematical derivation. We will expand on that concept and use another graph to further illustrate what Loewe's symbols mean and how his description leads to the necessary conclusion that *linear isoboles of additivity are based on a constant relative potency of the two drugs.* Such drug pairs were called "homodynamic" by Loewe. He used the term "heterodynamic" when the potency ratio is variable and, for such cases, he pointed out that the additive isoboles for such drug combinations are not the straight line diagonals. His actual description (Loewe, 1953) for heterodynamic drugs is "*The likelihood that the isobole coincides with the endpoint diagonal is minimal."* But he provides no mathematical proof or other detail to explain his statement

To illustrate the concept of dose equivalence and how dose-effect data are used to generate the additive isobole, we refer to the graphs shown in Figure 1(C) that show two dose response curves. The effect of interest is indicated by the upper horizontal line and, when referred to drug B, it corresponds to dose B_i. That reference dose B_i will determine the quantities, *a* and *b,* that are *additive* for this effect level. The quantity of drug A that is given, denoted *a*, produces its own effect (less than the selected effect and shown by the lower horizontal line); this dose is equieffective with a quantity of drug B that is denoted b_{eq} . In order to reach the required dose B_i an additional quantity, indicated by the arrow length *b*, must be *added* to b_{eq}. Thus, $b + b_{eq} = B_i$.

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

An examination of this graph shows that as dose *a* increases the quantity *b* must decrease and, thus, the additive isobole, which plots *b* against *a*, decreases as *a* increases. If the parent curves *have a constant potency ratio R = A/B,* then $b_{eq} = a/R$ and, thus, $b + a/R = B_i$. Division by B_i yields $b/B_i + a/RB_i = 1$ and, since $RB_i = A_i$, we get $b/B_i + a/A_i = 1$, which is the straight line equation (1). That line is the common isobole that is graphed as a downward diagonal in the upper quadrant that is illustrated in Fig. 1(B).

In Loewe's notation, $b_{eq} = D_{E_M}^{(B)}$; $B_i = D_{E_N}^{(B)}$; $a = D_{E_M}^{(A)}$ and, thus, his expression for $a + b$, is the left-hand side of the equation he presented in the following way:

$$D_{E_M}^{(A)} + \left(D_{E_N}^{(B)} - D_{E_M}^{(B)}\right) = D_{E_N}^{(C)} \qquad (2)$$

where $D_{E_N}^{(C)}$ denotes the summed dose of the combination (Loewe, 1953).

The graph of Fig. 1(C), when attached to Loewe's notations, illustrates how he used the *concept of dose equivalence in arriving at the isobole of additivity*. In particular, it is seen that the term in parentheses above is equivalent to *b* (the arrow length). In this derivation of the equation of the linear isobole of additivity we expressed the dose of drug A as an equivalent of drug B. The same equation is obtained if we express the dose of drug B as an equivalent of drug A, i.e., $a + a_{eq} = A_i$, and since $a_{eq} = bR$, we get $a + bR = A_i$ which is, $a/A_i + b/B_i = 1$.

The additive isobole of figure 1(B) consists of dose pairs (*a*,*b*) and may be viewed as follows: In the absence of drug A the needed dose of drug B for a specified

effect is B_i. When drug A is present in dose *a*, the amount of drug B is reduced to quantity *b*. If $a = A_i$, then the reduction of drug B is total, i.e., no quantity of drug B is needed. This way of viewing additivity will prove useful in our subsequent discussion. Isoboles of additivity (in cases of constant relative potency) lead to parallel lines, one for each effect level (Fig. 2). Every additive dose pair (*a,b*) lies on one and only one such isobole.

The interest in isoboles is mainly to establish a basis for classifying drug interactions. A dose pair (*a,b*) on the line is expected to produce the specified effect. In that regard actual testing of combinations may reveal an exaggerated effect of the dose combination. Stated differently, lesser doses (*a,b*) may achieve the specified effect. When such a combination is plotted on the same axes as the additive isobole the point will be in the upper quadrant but *below* the additive line. Conversely, if the combination results in a reduced effect, then greater quantities of drug A and B are needed to get the effect and the point (*a,b*) will appear above the additive line. Each of these situations is illustrated in Fig. 2 (inset) where the terms superadditive and subadditive are used to describe these cases. The set of superadditive points gives rise to upward concavity whereas the subadditive set shows downward concavity.

Some Examples. Numerous drug combinations producing a myriad of effects have been reported. These represent diverse endpoints, with most based on measurement of effect at a single time, for compounds that act through mechanisms known or presumed to be non competitive. All showed dose dependency so that the individual dose-effect relations allowed assessment of equally effective doses from which isobolar analysis was used.

An early example is given by Gessner and Cabana (1970) who described the hypnotic effect of chloral hydrate and alcohol using the righting reflex as the effect measure. Locomotion was the endpoint in studies in Holtzman's laboratory (Kimmel et al, 1997) that examined combinations of cocaine and buprenorphine. Pasternak's group has been concerned with *mu* opioids in combination (Bolan et al, 2002) and Field *et al* (2002) looked at combinations of gabapentin and NK1 antagonists in a model of neuropathic pain. Neostigmine was shown to interact synergistically with nonsteroidal anti-inflammatory drugs (Miranda et al, 2002; Tallarida, 2002). Especially interesting are cases in which one of the two compounds lacks efficacy but its presence enhances the effect of the active compound. An example of that situation was evident in work in Porreca's laboratory that examined opioid delta receptor agonists with morphine (Horan et al, 1992). A more recent study (Tallarida et al, 2003) demonstrated that glucosamine, which lacks efficacy in the mouse abdominal constriction test, significantly enhanced the antinociceptive activity of both ibuprofen and ketoprofen (Tallarida et al, 2003). Numerous other studies have proceeded to analyze combinations with isobolograms. An especially interesting application is that in which the same drug is given at two different sites (Raffa et al, 2000) thereby demonstrating site-site additivity or synergism. The basis for this application is also the concept of dose equivalence, i.e., the potency at one site has its equivalent value at the other site. Synergistic interactions have also been examined for enantiomers of an active compound, viz, tramadol (Raffa et al, 1993). This insightful study showed that the (+) and (-) enantiomers of tramadol each independently produced centrally mediated antinociception in a standard test of antinociception in mice.

The racemic compound was found to be more potent than the additive potency predicted from the enantiomers.

Independent Action

The use of combinations of drugs is quite common in pharmacological (and clinical) use, and among the most common are research protocols that use competitive agents, i.e., agonists and antagonists that act on a common receptor. Competing drugs or chemicals are not suitable for the *usual* isobolographic analysis because that analysis requires independent action. For example, dose equivalence as employed in isobolographic analysis matches dose that are equally effective when tested individually and assumes that an equally effective dose of one can substitute for the other when the two are simultaneously present. But that substitution would not apply when the agents compete. In that competitive situation mass action binding quantitates the reduction in the binding of one due to the other. If one compound is present its receptor-bound concentration, denoted by [*AR*], is a function of its concentration *A* , the receptor concentration R_t and the drug-receptor dissociation constant *K* according to $[AR] = [A][R_t] / ([A] + K)$. (Other binding relations are more complex and require a curve-fitting parameter *p* as an exponent on *A*.) A competitive compound in concentration *B* and having dissociation constant K_B reduces the binding to $[AR]'$ and this relation, first proposed by Gaddum (1937) is given by

$$[AR]' = \frac{[A][R_t]}{[A] + K(1 + [B]/K_B)} \qquad (3)$$

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

The above equation is the basis of Schild analysis that has been extensively used for competitive agonist-antagonist pairs and leads to pA_2 values (Arunlakshana & Schild, 1959; Tallarida et al, 1979). Less well-known is the relation that applies when three competing agents are simultaneously present where the bound concentration is now denoted $[AR]''$ and is given by

$$[AR]'' = \frac{[A][R_t]}{[A] + K\left(1 + [B]/K_B + [C]/K_C\right)} \qquad (4)$$

In equation (4) [*C*] is the concentration of the second competitor and K_C is its dissociation constant. (See appendix). The reduction in [*AR*] due to competition makes clear why competing agonists are not used in the usual isobolographic analysis.

Parallel Isoboles

Many dose-effect relations are well described by an equation of the form $E = E_{max}\, A / (A + A_{50})$ where *A* is the dose, E_{max} is the maximum effect and A_{50} is a constant numerically equal to the dose that yields an effect = $E_{max}/2$. Two agonist drugs (A and B) of this kind, a "homodynamic pair", will necessarily have a constant potency ratio and, thus, give rise to linear isoboles of additivity as illustrated in Fig. 2. In this diagram A_{50} = 80 and B_{50} = 10, and this produces the additive isobole labeled "50" in the figure. The additive isoboles for several other effects are also shown and the inset shows the *A*,*B* values (intercepts) that define each isobole. The dose ratio *R* = 8. The graph also shows the intersections of a radial line representing a fixed ratio combination, in this

example one unit of B to four of A. Each intersection of this radial line with an isobole gives the point (dose combination) that is expected to produce the effect of that isobole.

The effect of a combination, if plotted against the dose pair in a three dimensional Cartesian coordinate system with doses in the X-Y plane, produces a surface whose height above each point (dose pair) in the plane represents the effect *E*. This kind of plot (response surface) was generated for the combination of morphine and clonidine in a previous publication (Tallarida, et al, 1999). The equation for an additive response surface gives *E* as a function of (*a*,*b*) and follows easily from either's dose-effect equations, e.g., $E = E_{max} B / (B + B_{50})$, and substitution of $B = b + a/R$ for the dose pair (*a*,*b*). It should be emphasized that the linear isobole follows from two main assumptions, viz., that the two agents do not compete for the same receptor and have a constant relative potency. That linear plot, the additive isobole, is also described as a case of zero interaction.

Calculating the Additive Effect of a Combination

In this case in which isoboles are linear the effect of any given dose combination (*a*,*b*) may be calculated from this dose pair and the potency ratio *R* (dose A/dose B) by the simultaneous solution of equations, $a/A+b/B = 1$ and $R = A/B$. This leads to either *A* or *B* ($A = a + bR$ or $B = b + a/R$) and each of these defines the effect level by insertion into its respective dose-effect equation, $E = E_{max} A/(A + A_{50})$ or $E = E_{max} B /(B + B_{50})$. The expressions for *A* and *B* further illustrate that linear isoboles are based on dose equivalence, i.e., *bR* is the equivalent for drug A and *a/R* is the equivalent for drug B as previously mentioned. Every dose pair lies on one and only one additive isobole and

therefore this calculation leads to the predicted (additive) effect. For example, the data used in Fig. 2 show that $R = 8$ and, thus, a dose combination such as (50, 15) is seen to fall somewhere between the effect levels 60 and 70 (Fig. 2). To determine the actual effect level, we illustrate with the A-equivalent, $A = 50 + (15)(8) = 170$. Inserting this value into drug A's dose-effect equation yields $E = 100\ (170) / (\ 170 + 80) = 68$.

Statistics and the Linear Isobole

When an additive isobole has been determined one can calculate the variance of the estimated value of *b* ("on the line") for a given dose *a* of drug A. The specified effect level, denoted E_i, leads to individual dose estimates A_i and B_i for drugs A and B, respectively. Because A_i and B_i are independent and presumed to be normally distributed, the variance of *b* can be approximated from these values and the individual variances, $V(A_i)$ and $V(B_i)$, according to the following formula

$$V(b) = V(B_i) + a^2\left[\frac{B_i^{\,2}}{A_i^{\,2}}\left(\frac{V(B_i)}{B_i^{\,2}} + \frac{V(A_i)}{A_i^{\,2}}\right)\right] - 2a\frac{V(B_i)}{A_i} \qquad (5)$$

This calculation of variance will be illustrated here for data derived from the combination of morphine and clonidine, administered intrathecally to mice, and tested in the 55°C tail immersion test of antinociception (Tallarida et al, 1999). Those data were well fitted to hyperbolic equations with the same maximum effect and, thus, a constant potency ratio (= 1.55) with ED_{50} values (micrograms) for morphine SO_4 (drug A) and clonidine HCl (drug B), and their respective variances, as follows: $A_i = 5.86$, $V(A_i) = 0.27$; $B_i = 3.79$, $V(B_i) = 0.61$. The estimate of $V(b)$ for $a = 3.0$, calculated from the above equation, is

0.175. For statistical analyses applicable to other experimental designs, see Tallarida (2000) pp 65-66.

Dose-Effect Curves with Different Maxima

When the dose effect relations of the individual agents have different maxima (whether experimentally determined or obtained from curve fitting) the potency ratio (R) is not constant. In this case the b equivalent (a/R) does not lead to the familiar relation, $a/A_i + b/B_i = 1$, since this equation must hold for all combinations ($a.b$) that lie in the upper quadrant bounded by A_i and B_i. But in this case, as the combination point (a,b) changes the dose ratio also changes. The drug with higher efficacy will be here denoted as drug B. The basis for defining additivity in this case is that *combinations that contain sufficiently large doses of drug B should attain its maximum effect.* Thus, we view the combination as one in which drug A contributes some equivalent of drug B. In contrast to the previous (constant potency ratio) case, the drug B equivalent of dose a is now a more complicated function of a. We illustrate for two agonists with the individual hyperbolic dose-effect equations in which the respective maxima are different. Because these are different we avoid using the common "Emax" and, instead, denote these by E_b and E_c where $E_b > E_c$, $E = E_b B / (B + B_{50})$ and $E = E_c A / (A + A_c)$. In this case B_{50} is the dose of drug B that yields one half its maximum effect (½ E_b) and A_c is the dose of drug A that yields one half its maximum (½ E_c). These relations and the concept of dose equivalence lead to the following equation derived by Grabovsky and Tallarida (2004) for the additive isobole:

$$b = B_i - \frac{B_{50}}{\frac{E_b}{E_c}\left(1+\frac{A_c}{a}\right)-1} \qquad (6)$$

Here B_i is the dose of drug B (alone) that gives the specified effect. It is seen from equation (6) that in the special case in which the maxima are the same so that A_c is also a fifty percent dose, we get the familiar case given by equation (1), viz., $b = B_i - a/R$ where R is the constant potency ratio A_c / B_{50}. However, the general result is given by equation (6) and this equation is applied to data when one of the two agents is a partial agonist in the test situation. In contrast to the straight line isoboles of additivity, equation (6) leads to curved isoboles. To illustrate the use of this equation we show the graphs in Fig. 3 containing additive isoboles for several effects (10% to 50%) for two drugs whose dose-effect relations are given by $E = 100\ B/(B + 10)$ and $E = 60\ A/(A + 10)$. For each effect E_i of interest the quantity B_i is calculated from drug B's equation and that quantity is used in equation (6). For example, for effect level 50, equation (6) leads to $b = 10 - 10 / [\ 1.667\ (1 + 10/a) - 1\ ]$ which is one of the several curves shown in Fig. 3. The broken line in that figure also illustrates the (usual) diagonal isobole for the 50 % effect and is inserted here to illustrate the departure from linearity of the true additive curve. It is especially noteworthy that effects greater than 60 are not attained by drug A. For such a large effect the isobole of additivity has no intercept on the abscissa but, instead, is a hyperbolic arc that decreases toward a horizontal asymptote (Grabovsky and Tallarida, 2004).

Dose-effect curves for drugs with different maxima and the corresponding isobole combination are exemplified by dextromethorphan (an NMDA antagonist) along with a cannabinoid agonist (WIN55212-2) in producing temperature depression in rats (Rawls et

al, 2002) and analyzed in Grabovsky and Tallarida (2004). The curves were not well described by simple hyperbolas; instead they were fitted to $E = E_b B^p/(B^p + B_{50}{}^p)$ for WIN55212-2 with $E_b = 4.17$, $p = 1.73$ and $B_{50} = 3.99$ and to $E = E_c A^q/(A^q + A_C{}^q)$ with $E_C = 1.58$, $q = 1.92$ and $A_C = 65.8$ for dextromethorphan. In this case in which "Hill coefficients" (p and q) are needed for the dose-effect equations the isobole of additivity of equation (6) becomes generalized to equation (7) given below. From this equation a set of additive isoboles were obtained for the specified effect levels (temperature drop in degrees C.)

$$b = B_i - \frac{B_{50}}{\left[\frac{E_b}{E_c}\left(1+\frac{A_c{}^q}{a^q}\right)-1\right]^{1/p}} \qquad (7)$$

Full Agonists with a Variable Potency Ratio

A most interesting case is that in which both agonists attain the maximum effect but are described by non-parallel log dose-effect curves. This situation means that the Hill coefficients, p and q, that respectively describe their dose-effect relations are different. This, too, is a situation of a variable potency ratio but, in contrast to the case of a full and a partial agonist, there is no obvious basis for distinguishing whether drug A is contributing to drug B or vice versa. (Recall, that the criterion for the full and partial agonist combination is that sufficiently large dose combinations should produce the maximum effect.) In this case, however, each agent attains the maximum when it acts alone so that the term A_c in equation (7) is an A_{50}. One might assume that the agent with the greater potency should be the standard and that the other compound is contributing an

equivalent to it, but this need not be the case. In the absence of a clear answer or known mechanism the use of dose equivalence leads to not one, but to two possible isoboles of additivity, depending on how the concept of dose equivalence is applied. In other words, does one convert dose (*a*) of drug A to an equivalent of drug B, or make the conversion from dose (*b*) of drug B to an equivalent of drug A? As mentioned previously, the basis for the usual linear isobole is that the relative potency is constant so that either conversion leads to a unique isobole of additivity. The linear isobole of additivity is not an empirical fact, but is derived from the dose equivalence concept and, thus, the previous case of a full and partial agonist produced a consistent generalization that led to a single (curved) isobole of additivity, a result that was recognized by Loewe (1953). But, in the case considered now, when both are full agonists with a variable potency ratio, the standard method (including past works by this author) has been to apply the linear isobole given by equation (1). But it is clear that the dose equivalence concept in this case leads to two different isoboles that are given by the pair of equations (8 and 9) below. This situation can now be dealt with as we show here. The two isoboles result from effect E_i in which the individually effective doses are A_i and B_i. These curves do, however, *possess a symmetry that allows one to detect departures from additivity*. This is illustrated with an example (Fig. 4) that follows from the two isobole relations, the first by converting dose *a* into its equivalent of drug B to give equation (8)

$$b = B_{50}\left(\frac{A_i - a}{A_{50}}\right)^{q/p} \qquad (8)$$

and the second by converting dose *b* into its equivalent of drug A to give

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

$$b = B_i - \frac{B_{50}}{\left(\frac{A_{50}}{a}\right)^{q/p}} \qquad (9)$$

The curves resulting from equations (8) and (9) are seen to have *symmetry with respect to the point* (A_i /2, B_i /2). (See appendix). For the case illustrated in the graph of figure 4, these are 50% isoboles of additivity with, B_{50} = 10 and p = 1.2, while A_{50} = 80 and q = 0.7. The symmetry provides a criterion for distinguishing departures from additivity, viz. a combination with $a = A_i/2$ (in this case 40) would require that the b quantity to be significantly less than that shown by point S for synergism, whereas for sub-additivity the quantity b should be significantly greater than that shown by point T. Prior to the recognition of this symmetry (described here for the first time) the only alternative was to use the straight line isobole of additivity. This detection of symmetry, however, has now provided a basis for distinguishing non-additive interactions. The complexity of the relations in equations (6)-(9) precludes the determination of an exact variance estimate for b. An approximation may be made for the standard error by employing a statistical technique known as the "delta method" (See, for example, Cassella and Burger (2002), p.243.

Summary

In this review, as in the previous *Perspectives* (Tallarida, 2001), the emphasis is on the calculations that are needed to distinguish additive from non-additive interactions. Measures of non-additive interactions have practical (potential clinical) value and are also an important first step in exploring the possible mechanisms responsible for the interaction. The possible mechanisms underlying synergistic and subadditive cases are

diverse and are not discussed here. Some are postulated in the works cited. In general, precise mechanisms are unknown. Various possible mechanisms that may apply to *in vivo* pharmacodynamic effects from a combination of two drugs have been assessed by Earp et al (2004) in models examined mathematically and by simulation. These investigators describe a turnover system applicable to indirect mechanisms and they examined both production and dissipation controlling processes under a number of conditions that include both competitive and non-competitive interaction. Synergism, and the mechanism(s) responsible for this kind of interaction, is especially interesting, for even a single drug or ligand exists in a sea of chemicals with which it may interact. Thus, the calculations that lead to measures of these interactions, when coupled to models of mechanism that are consistent with these measures, can further our understanding of the action of even a single drug. In summary:

1. When the two drugs exhibit a constant potency ratio in the production of the common effect that combination will produce a linear isobole of additivity that provides a basis for distinguishing super- and sub-additive interactions. **2**. Tests to determine whether the experimental point (dose combination, *a,b*) is "on the line" of additivity require an estimation of the variance of *b* on the line as well as the variance of *b* that is determined experimentally. Other experimental designs require similar tests of the significance of the difference. **3.** The additive isobole is based historically, and logically, on the concept of dose equivalence and is not an empirical fact. **4**. When one of the drugs is a partial agonist in the test adopted the additive isobole is still calculated using dose equivalence with the requirement that sufficiently large dose pairs should produce the maximum effect. This analysis leads to additive isoboles that are not straight lines. **5**. When both

drugs produce the maximum effect but are *heterodynami*c (dissimilar dose-effect curves) the isobole of additivity is not described by a single curve but is shown to be a region of the *a*-*b* plane that is bounded by two well-defined curves. In this case, a departure from additivity means that the experimental points are statistically outside this region.

References

Arunlakshana O and Schild HO (1959) Some quantitative use of drug antagonists. *Br J Pharmacol* 14:48-57.

Bolan EA, Tallarida RJ and Pasternak GW (2002) Synergy between mu receptor subtypes: Evidence for functional interaction among mu receptor subtypes. *J. Pharmacol Exp Ther* 303: 557-562.

Cassella G and Burger RL (2002) *Statistical Inference*, 2nd ed. Duxbury, Pacific Grove, CA.

Earp J, Krzyzanski W, Chakraborty A, Zamacona MK and Jusko WJ (2004) Assessment of drug interactions relevant to pharmacodynamic indirect response models. *J. Pharmacokin. Pharmacodyn.* 31: 345-380.

Field MK, Gonzalez MI, Tallarida RJ and Singh L (2002) Gabapentin and the NK1 antagonist CI-1021 act synergistically in two rat models of neuropathic pain. *J Pharmacol Exp Ther* 303: 730-735.

Gaddum, J (1937) The quantitative effects of antagonistic drugs. *J Physiol (London)* 89:7P-9P.

Gessner PK and Cabana BE (1970) A study of the hypnotic effects and the toxic effects chloral hydrate and ethanol. *J Pharmacol Exp Ther* 174:247-259.

Horan P, Tallarida RJ, Haaseth RC, Matsunaga TO, Hruby VJ and Porreca F (1992) Antinociceptive interactions of opioid delta receptor agonists with morphine in mice: Supra- and subadditivity. *Life Sci* 50:1535-1541.

Kimmel HL, Tallarida RJ and Holtzman SG (1997) Synergism between buprenorphine and cocaine on the rotational behavior of the nigrally-lesioned rat. *Psychopharmacol* 133: 372-378.

Loewe S (1927) Die Mischiarnei. *Klin Wochenschr* 6:1077-1085.

Loewe S (1928) Die quantitativen Probleme der Pharmakologie. *Ergebn Physiol* 27:47-187.

Loewe S (1953) The problem of synergism and antagonism of combined drugs. *Arzneimittelforschung* 3:285-290.

Miranda HF, Sierraita F and Pinardi G (2002) Neostigmine interactions with nonsteroidal anti-inflammatory drugs. *Br J Pharmacol* 135:1591-1597.

Raffa RB, Stone DJ, Jr and Tallarida, RJ. (2000) Discovery of 'self-synergistic' spinal/supraspinal antinoci-ception produced by acetaminophen (paracetamol). *J Pharmacol ExpTher* 295:291-294.

Rawls SM, Cowan A, Tallarida RJ, Geller EB and Adler MW (2002). NMDA antagonists and WIN 55212-2, a cannabinoid antagonist, interact to produce synergistic hypothermia. *J Pharmacol Exp Ther* 303: 395-402.

Tallarida RJ, Cowan A and Adler MW (1979) pA_2 and Receptor Differentiation: A Statistical Analysis of Competitive Antagonism. Mini-review *Life Sciences* 25: 637-654.

Tallarida RJ (2000) *Drug Synergism and Dose-Effect Data Analysis.* Chapman Hall, CRC, Boca Raton.

Tallarida RJ Cowan, A and Raffa RB (2003) Antinociceptive Synergy, Additivity, and Subadditivity with Combinations of Oral Glucosamine Plus Nonopioid Analgesics in Mice. *J Pharmacol Exp Ther* 307:699-704.

Tallarida RJ (2002) Commentary on neostigmine interactions with non steroidal anti-inflammatory drugs by Miranda *et al*. *Br J Pharmacol* 135: 1589-1590.

Footnotes

This work was supported by National Institutes of Health/NIDA Grant DA 09793 and by a Focused Giving Grant from Johnson & Johnson.

Legends for Figures

Fig 1. (A) Dose-effect curves for drugs A and B that show equally effective doses *A* and *B* for a specified effect (in this case 50% of the maximum). **(B)**The additive isobole for the specified effect is a straight line with intercepts *A* and *B* when the potency ratio is constant. **(C)** Values of the quantities *a* and *b* that constitute the additive isobole are graphically shown in relation to the individual dose-effect curves of the constituent drugs. The sum of *a* and *b* (arrow length) is B_i, the dose of drug B alone that yields the specified effect.

Fig. 2. Isoboles of additivity for several effects are shown and the axial intercepts *A* and *B* are tabulated. The broken line represents dose combinations in a fixed ratio and the intersection of this line with each isobole gives the dose combination that is expected to yield the specified effect, e.g., for effect 50, $a = 26.67$, $b = 6.67$. The inset illustrates typical sub-additive and super-additive isoboles.

Fig. 3. Additive isoboles for combinations of a full and a partial agonist at several different effect levels are illustrated. (See text).

Fig. 4. Isoboles of additivity for an effect equal to 50% of the maximum for two full agonists that have a variable potency ratio. In this situation the departure from additivity is demonstrated by values of *b* that lie outside the interval defined by the vertical segment ST. The diagonal straight line is drawn to enhance the recognition of symmetry and is, in fact, the additive isobole that results when exponents *p* and *q* in equations (8) and (9) are equal.

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

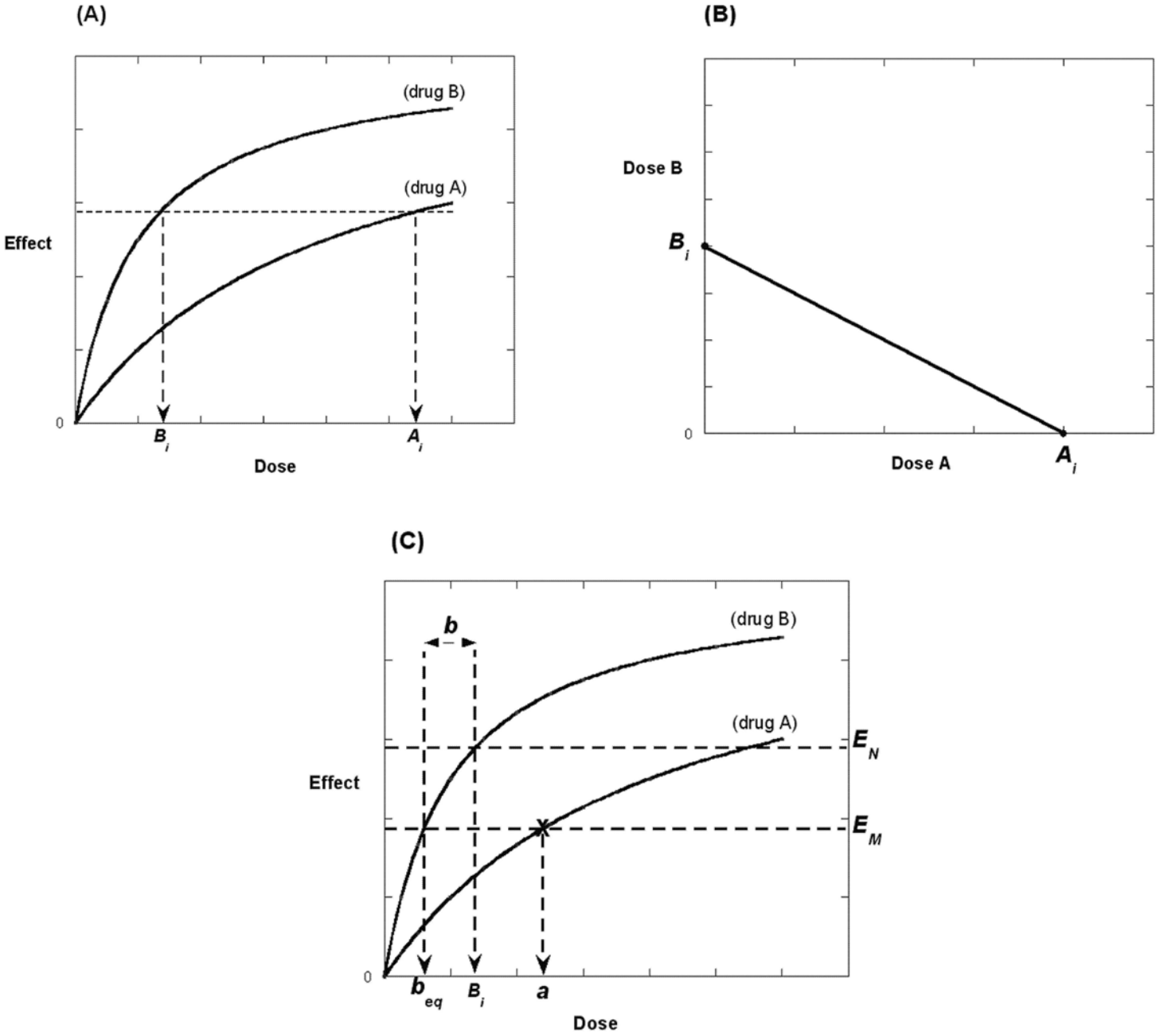
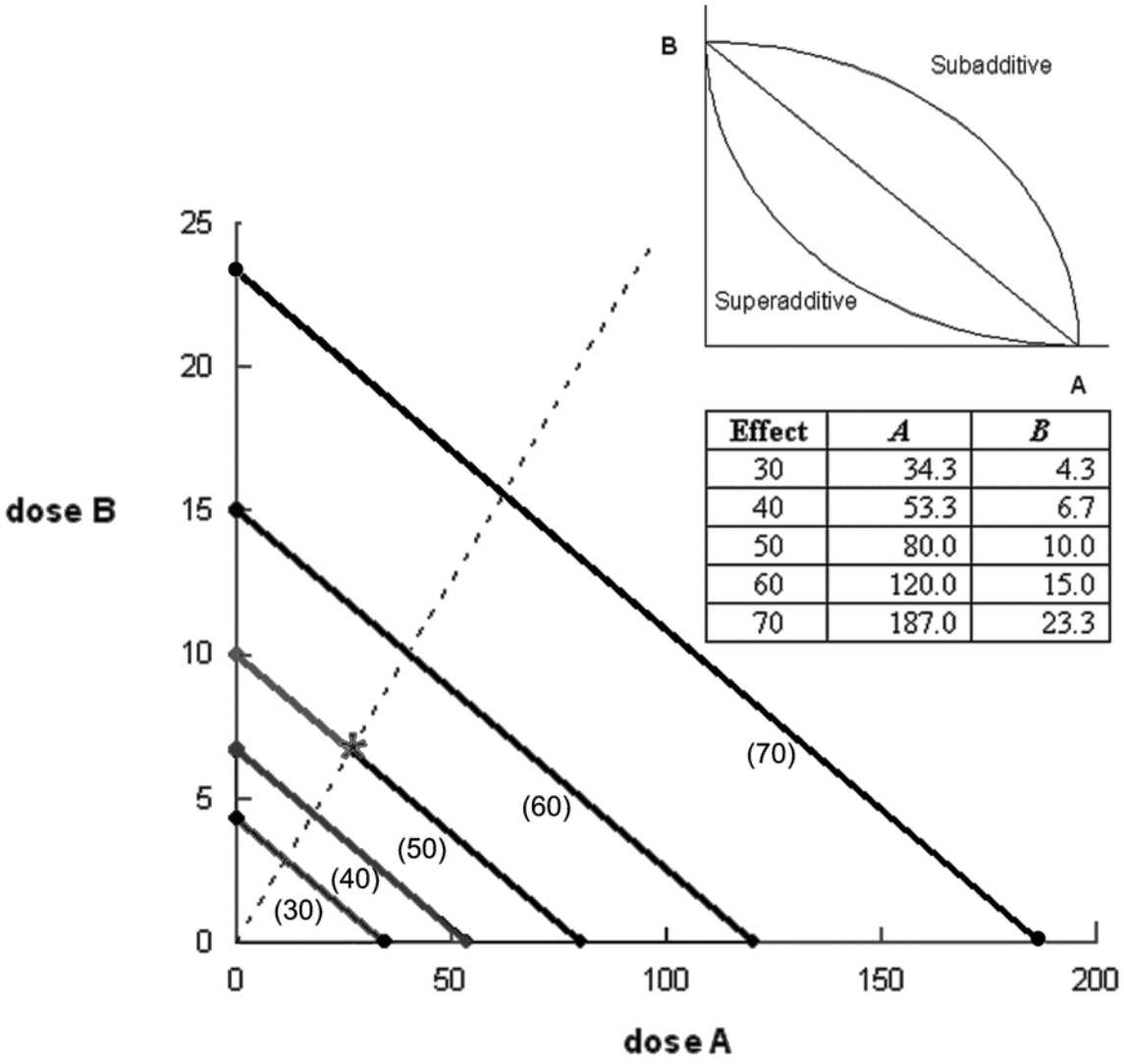


FIG 1

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.



Effect	*A*	*B*
30	34.3	4.3
40	53.3	6.7
50	80.0	10.0
60	120.0	15.0
70	187.0	23.3

FIG 2

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

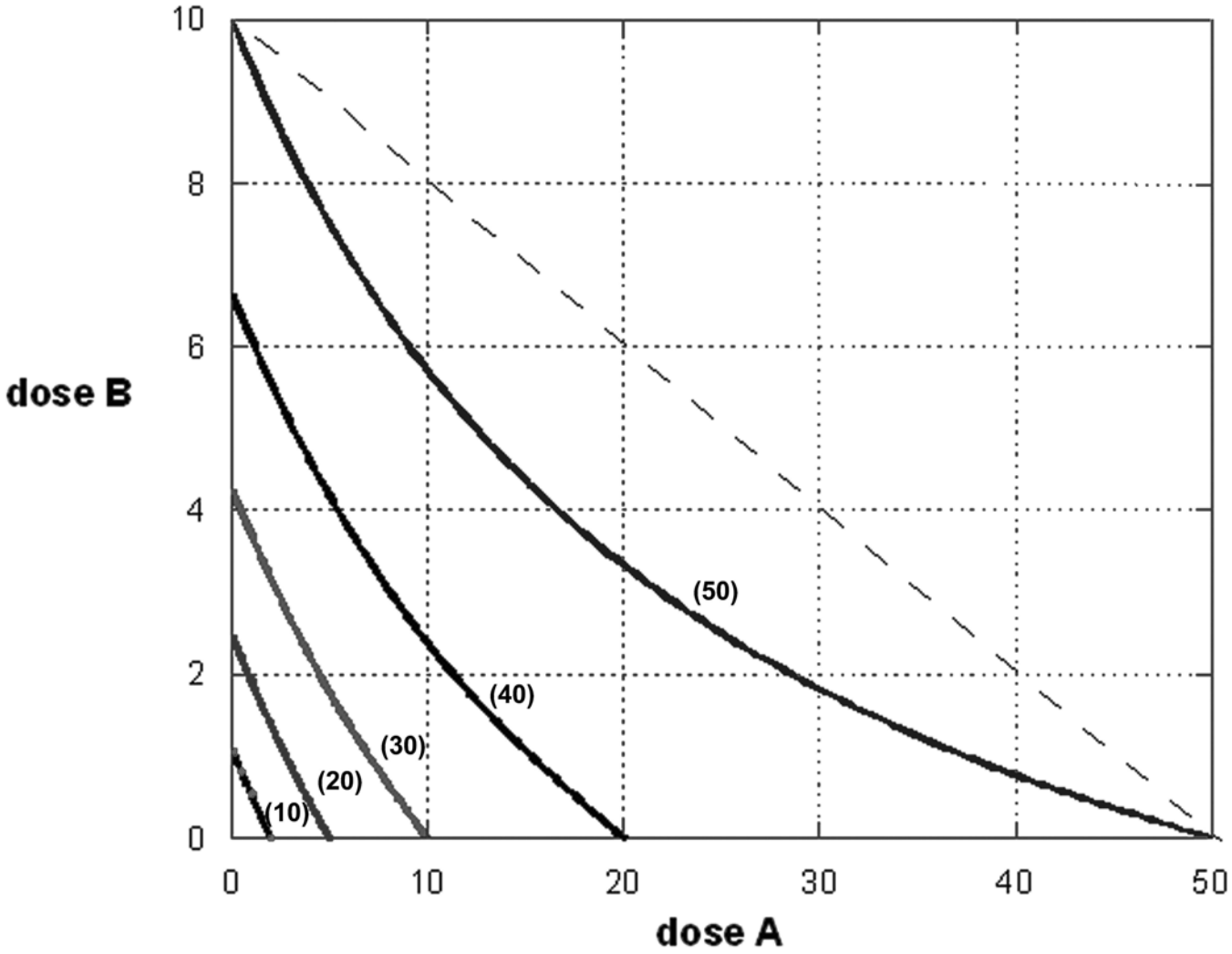


FIG 3

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

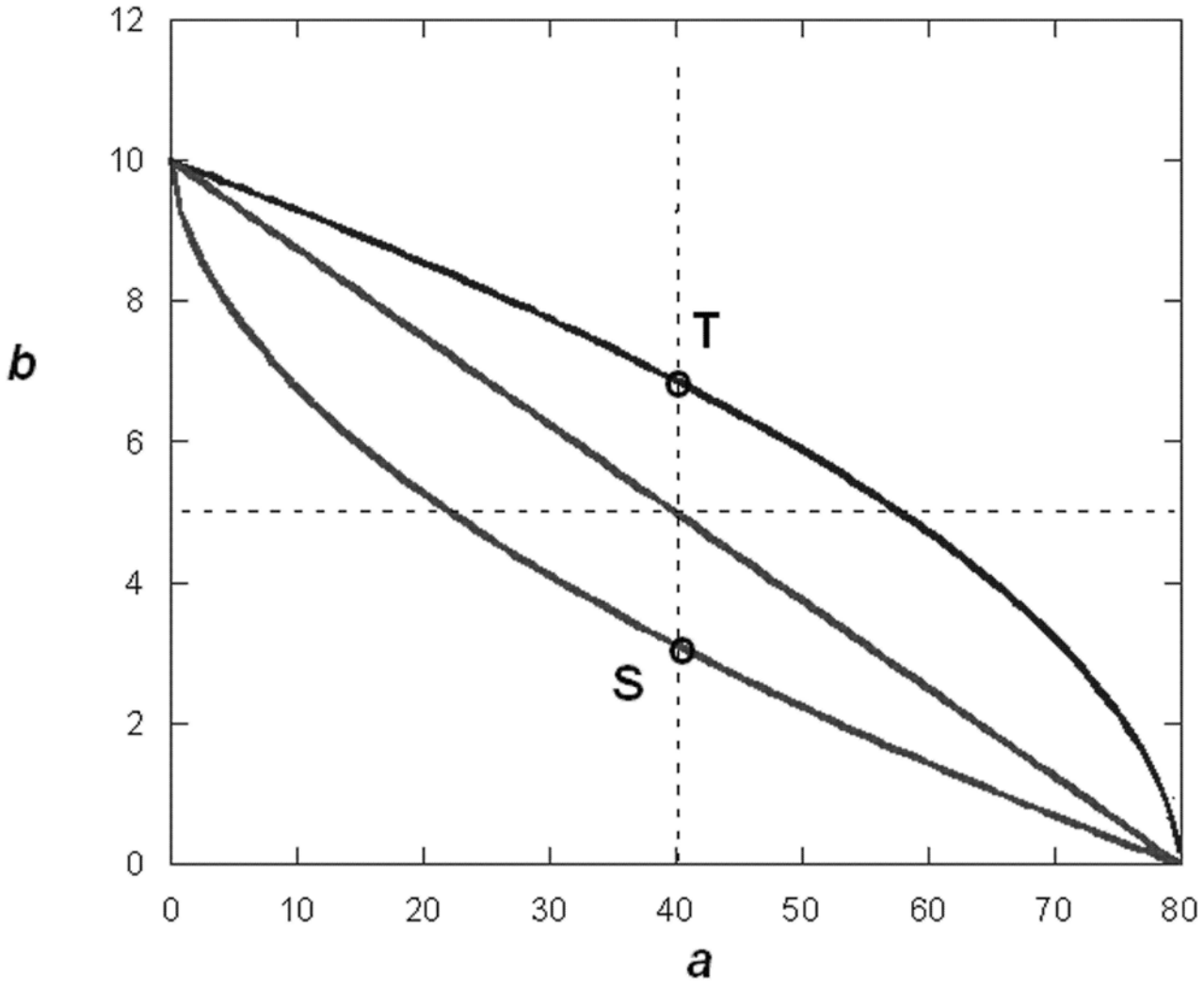


FIG 4

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

APPENDIX

1. Competition

For three ligands, A, B and C that interact with a common receptor we denote their bound concentrations $[AR]''$, $[BR]''$, and $[CR]''$ by *x, y and z*, respectively, for notational convenience in the equations that follow. Their binding rates are then the time derivatives, *dx/dt, dy/dt and dz/dt* , and are given by

$$dx/dt = k_1 A(R_t - x - y - z) - k_2 x$$
$$dy/dt = m_1 B(R_t - x - y - z) - m_2 y$$
$$dz/dt = l_1 C(R_t - x - y - z) - l_2 z$$

where we have used k_i, m_i and l_i (i=1,2) to denote forward (i =1) and reverse (i = 2) rate constants and R_t is the receptor concentration. Equating each derivative to zero (equilibrium) and denoting the dissociation constants by $K=k_2/k_1$, $M=m_2/m_1$, $L=l_2/l_1$, leads to the system, expressed in matrix form, given below:

$$\begin{bmatrix} \left(\frac{K}{A}+1\right) & 1 & 1 \\ 1 & \left(\frac{M}{B}+1\right) & 1 \\ 1 & 1 & \left(\frac{L}{C}+1\right) \end{bmatrix} . \begin{bmatrix} x \\ y \\ z \end{bmatrix} = \begin{bmatrix} R \\ R \\ R \end{bmatrix}$$

The solution for bound ligand A is the solution for $x = [AR]''$ given below (with similar corresponding terms for *y* and *z*) and is seen to be the same as equation (4)

$$[AR]'' = \frac{[AR_t]}{[A] + K\left(1 + \frac{[B]}{M} + \frac{[C]}{L}\right)}$$

JPET Fast Forward. Published on May 2, 2006 as DOI: 10.1124/jpet.106.104117
This article has not been copyedited and formatted. The final version may differ from this version.

2. Symmetry

From equation (8), (Fig. 4 upper curve) we have

$b = B_{50}\left(\frac{A_i - a}{A_{50}}\right)^{q/p}$, whereas equation (9), (lower curve of Fig.4), is given by

$b = B_i - \frac{B_{50}}{\left(\frac{A_{50}}{a}\right)^{q/p}}$. In order to demonstrate the symmetry with respect to $A_i/2$, $B_i/2$ we translate the coordinate axes to be centered at this point. Thus, we introduce variables x and y as follows, $x = a - A_i/2$ and $y = b - B_i/2$, and substitute in equations (8) and (9). From (8) this yields

$$y_U = -\frac{B_i}{2} + B_{50}\left(\frac{A_i/2 - x}{A_{50}}\right)^{q/p} \qquad \text{(A-1)}$$

while (9) becomes

$$y_L = \frac{B_i}{2} - B_{50}\left(\frac{x + A_i/2}{A_{50}}\right)^{q/p} \qquad \text{(A-2)}$$

where the subscripts U and L are used to distinguish the two curves. It is seen that $y_L(-x) = -y_U(x)$, thereby demonstrating symmetry with respect to point $\left(\frac{A_i}{2}, \frac{B_i}{2}\right)$.