

## Perspectives in Pharmacology

# An Overview of Drug Combination Analysis with Isobolograms

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### 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; i.e., 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.

This communication is concerned with the analysis of combinations of two drugs that produce overtly similar effects that are measurable. Therefore, each drug is an agonist that displays dose dependence. 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 composed 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) 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 demonstrated that experimental results in this case could be mistaken for synergism or antagonism. That result (nonlinearity), which represents a de-

parture from the common use of isobolograms, prompted further attention to other situations of variable relative potency. However, the variability condition was not explicitly connected to Loewe’s concept of dose equivalence in the author’s previous review. In this review, we make this explicit by showing (for the first time) that the derivation leading to curved 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 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 nonadditive interactions.

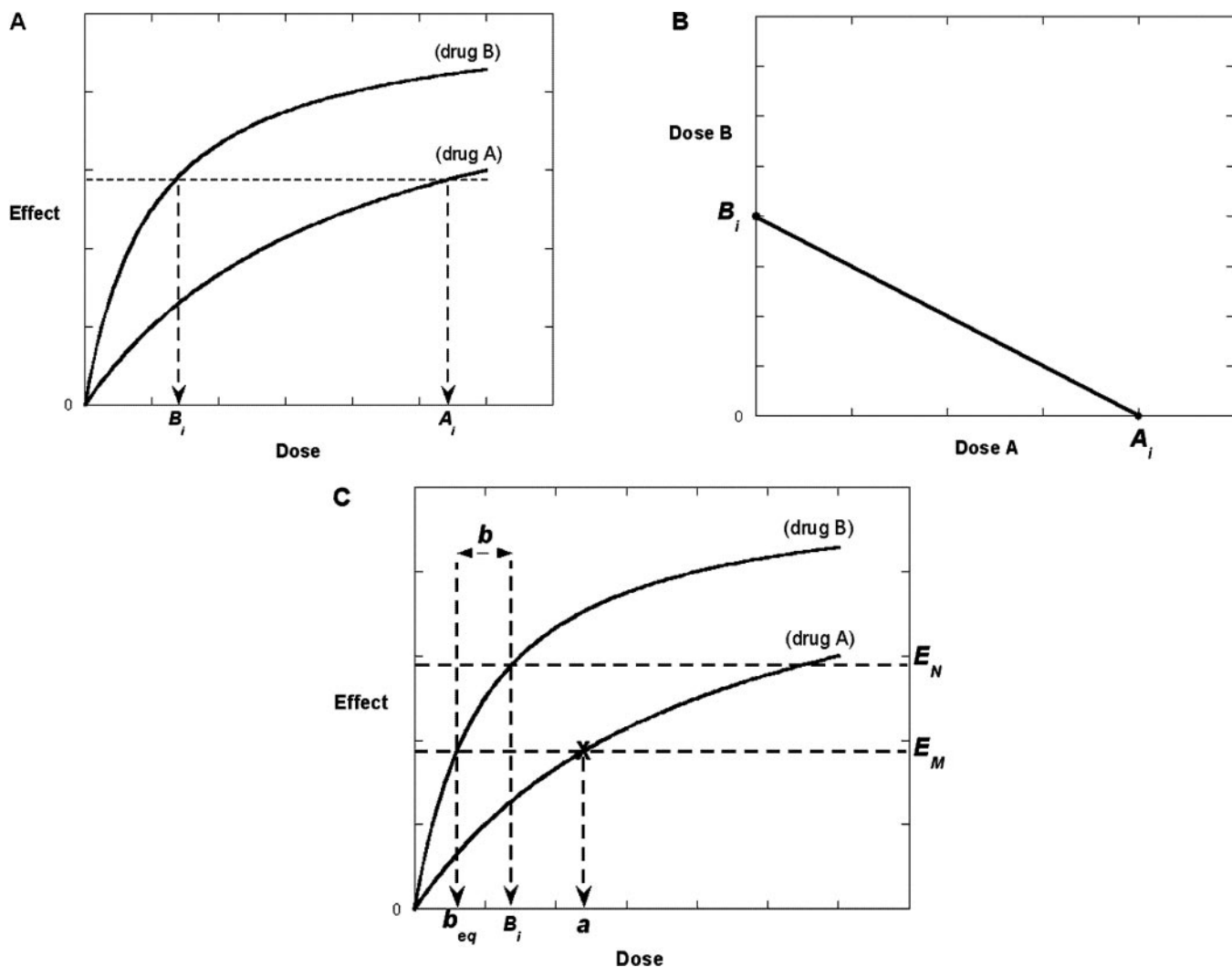
The theoretical basis for this graphical procedure, i.e., the assumptions regarding the individual drug dose effect data on which it is based and the consequent alterations on this graphical procedure under different assumptions, does not seem to be well known. Indeed, this important topic is not discussed 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 is reviewed here. A new analysis applicable to two full agonist combinations with a varying relative potency, a topic not previously discussed, is also presented. The principle aim is to predict the effect of the drug combination and thereby distinguish between exaggerated effects and those that are

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



**Fig. 1.** A, dose-effect curves for drugs A and B that show equally effective doses  $A_i$  and  $B_i$  for a specified effect (often 50% of the maximum). B, the additive isobole for the specified effect is a straight line with intercepts  $A_i$  and  $B_i$  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 the  $b$  equivalent of  $a$  and  $b$  (arrow length) is  $B_i$ , the dose of drug B alone that yields the specified effect.

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. Thus, only doses in the nondecreasing portion of the graph of each drug 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 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 are denoted here in italics  $A_i$  and  $B_i$ , respectively. The diagonal line connecting intercepts A and B is termed the “additive” isobole and is commonly expressed in the equation

$$\frac{a}{A_i} + \frac{b}{B_i} = 1 \quad (1)$$

$$(0 \leq a \leq A_i, 0 \leq b \leq B_i)$$

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 was variable; 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." However, he provides no mathematical proof or other details 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 Fig. 1C 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  $b_{\text{eq}}$ . To reach the required dose  $B_i$ , an additional quantity, indicated by the arrow length  $b$ , must be added to  $b_{\text{eq}}$ . Thus,  $b + b_{\text{eq}} = B_i$ .

An examination of this graph shows that, as dose  $a$  increases, the quantity  $b$  must decrease; thus, the additive isobole, which plots  $b$  against  $a$ , decreases as  $a$  increases. If the parent curves have a constant potency ratio, i.e.,  $R = A/B$ , then  $b_{\text{eq}} = a/R$  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 eq. 1. That line is the common isobole that is graphed as a downward diagonal in the upper quadrant that is illustrated in Fig. 1B.

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

$$D_{\text{EM}}^{(\text{A})} + (D_{\text{EN}}^{(\text{B})} - D_{\text{EM}}^{(\text{B})}) = D_{\text{EN}}^{(\text{C})} \quad (2)$$

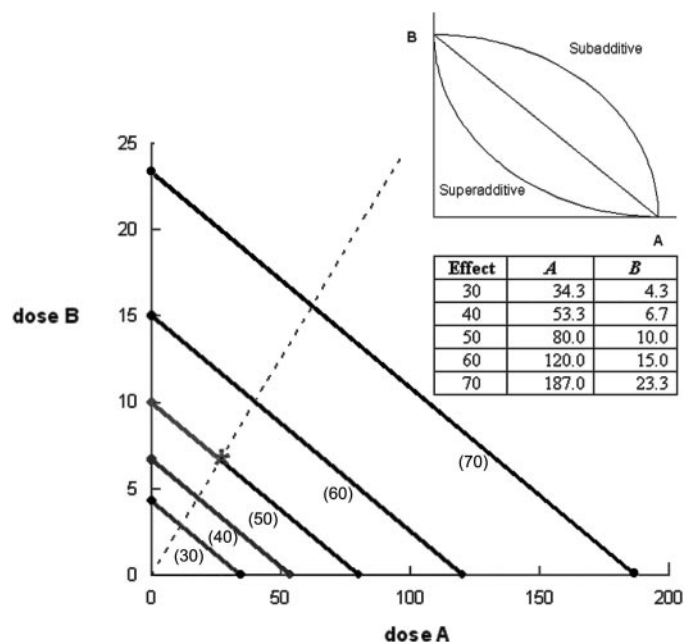
where  $D_{\text{EN}}^{(\text{C})}$  denotes the summed dose of the combination (Loewe, 1953). The graph of Fig. 1C, when attached to Loewe's notations, illustrates how he used the "concept of dose equivalence in arriving at the isobole of additivity." In particular, it is seen that the term in the parentheses 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.,  $a + a_{\text{eq}} = A_i$ , and because  $a_{\text{eq}} = bR$ , we get  $a + bR = A_i$ , which is  $a/A_i + b/B_i = 1$ .

The additive isobole of Fig. 1B 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, the 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 noncompetitive. All showed dose dependence 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



**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 subadditive and superadditive isoboles.

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 whose 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 pharmacologically (and clinically), 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 doses 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. However, that substitution would not apply when the agents compete. In that competitive situation, mass action binding quantitates the reduction in the binding of one because of 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

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

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 and 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

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

In eq. 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 maximal 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 dose-effect equation; 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; therefore, this calculation leads to the predicted (additive) effect. For example, the data used in Fig. 2 show that  $R = 8$ ; thus, a dose combination, such as 50 and 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 that  $A = 50 + (15)(8) = 170$ . 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

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

This calculation of variance is 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 maximal 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).

**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,  $a/A_i + b/B_i = 1$ , because 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 is 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 maximal 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  $E_{max}$  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 maximal effect ( $1/2 E_b$ ), and  $A_c$  is the dose of drug A that yields one-half its maximum ( $1/2 E_c$ ). These relations and the concept of dose equivalence lead to the following equation derived by Grabovsky and Tallarida (2004) for the additive isobole:

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

Here  $B_i$  is the dose of drug B (alone) that gives the specified effect. It is seen from eq. 6 that, in the special case in which the maxima are the same so that  $A_c$  is also a 50% dose, we get the familiar case given by eq. 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 eq. 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, eq. 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 (10–50%) for two drugs whose dose-effect relations are given by  $E = 100 B/(B + 10)$  and  $E =$

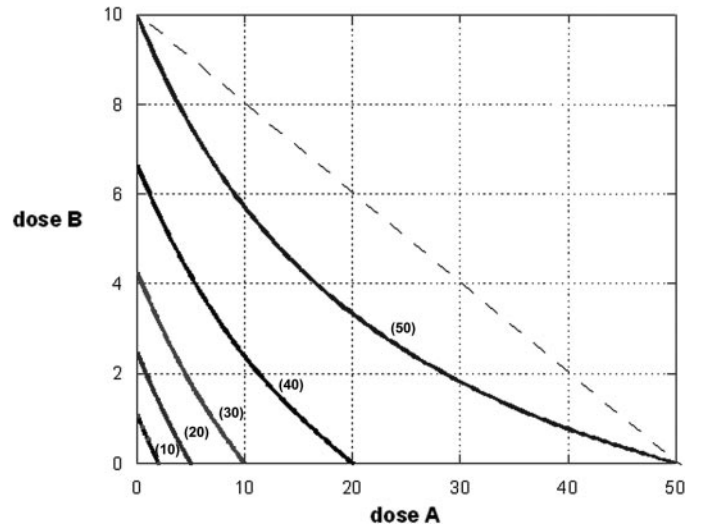


Fig. 3. Additive isoboles for combinations of a full and a partial agonist at several different effect levels are illustrated. See text.

$60 A/(A + 10)$ . For each effect  $E_i$  of interest, the quantity  $B_i$  is calculated from drug B's equation, and that quantity is used in eq. 6. For example, for effect level 50, eq. 6 leads to  $b = 10 - 10/[1.667 \times (1 + 10/a) - 1]$ , 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 (WIN 55212-2) 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 fitted 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 eq. 6 becomes generalized to eq. 7 given below. From this equation, a set of additive isoboles were obtained for the specified effect levels (temperature drop in °C).

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

**Full Agonists with a Variable Potency Ratio.** A most interesting case is that in which both agonists attain the maximal effect but are described by nonparallel log dose-effect curves. This situation means that the Hill coefficients  $p$  and  $q$ , which, 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 maximal effect). However, in this case, each agent attains the maximum when it acts alone so that the term  $A_c$  in eq. 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; 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). However, 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 eq. 1. However, it is clear that the dose equivalence concept in this case leads to two different isoboles that are given by the pair of eqs. 8 and 9 below. This situation can now be dealt with as shown here. The two isoboles result from effect  $E_i$  in which the individually effective doses are  $A_i$  and  $B_i$ . However, these curves do 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 eq. 8

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

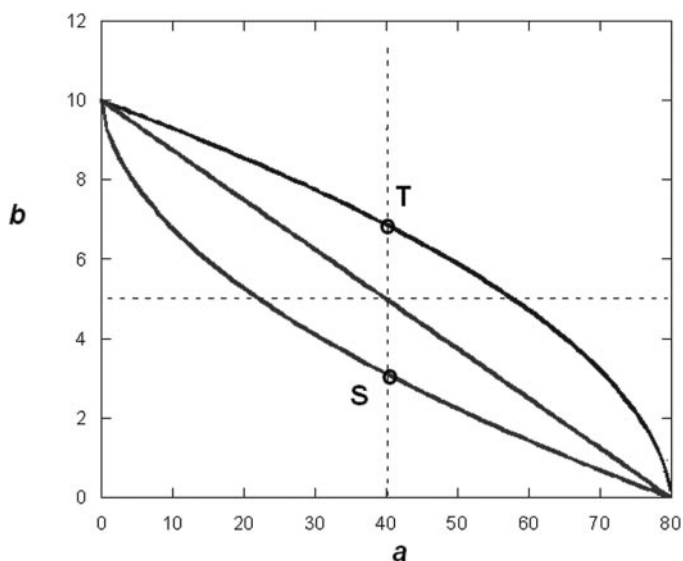
and the second by converting dose  $b$  into its equivalent of drug A to give

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

The curves resulting from eqs. 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 Fig. 4, these are 50% isoboles of additivity, with  $B_{50} = 10$  and  $p = 1.2$ , whereas  $A_{50} = 80$  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 subadditivity, the quantity  $b$  should be significantly greater than that shown by point T. Before 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 nonadditive interactions. The complexity of the relations in eqs. 6 to 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" (for example, see Cassella and Burger (2002).

**Summary.** In this review, as in Tallarida (2001), the emphasis is on the calculations that are needed to distinguish additive from nonadditive interactions. Measures of nonadditive 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 described 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 noncompetitive interactions. Synergism and the mechanism(s) responsible for this kind of interaction are especially interesting, because even a single drug or ligand exists in a sea of chemicals with which it might 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.

1. In summary, 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 superadditive and subadditive 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



**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 eqs. 8 and 9 are equal.

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 used, the additive isobole is still calculated using dose equivalence with the requirement that sufficiently large dose pairs should produce the maximal effect. This analysis leads to additive isoboles that are not straight lines. 5. When both drugs produce the maximal effect but are heterodynamic (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.

## APPENDIX

### 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

$$\begin{aligned} dx/dt &= k_1A(R_t - x - y - z) - k_2x \\ dy/dt &= m_1B(R_t - x - y - z) - m_2y \\ dz/dt &= l_1C(R_t - x - y - z) - l_2z \end{aligned} \quad (10)$$

where we have used  $k_i$ ,  $m_i$ , and  $l_i$  ( $i = 1$  and  $2$ ) 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:

$$\begin{bmatrix} \left(\frac{K}{A} + 1\right) & 1 & 1 \\ 1 & \left(\frac{M}{B} + 1\right) & 1 \\ 1 & 1 & \left(\frac{L}{C} + 1\right) \end{bmatrix} \cdot \begin{bmatrix} x \\ y \\ z \end{bmatrix} = \begin{bmatrix} R_t \\ R_t \\ R_t \end{bmatrix} \quad (11)$$

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 eq. 4.

$$[AR]'^r = \frac{[A][R_t]}{[A] + K\left(1 + \frac{[B]}{M} + \frac{[C]}{L}\right)} \quad (12)$$

### Symmetry

From eq. 8 (Fig. 4, top curve), we have  $b = B_{50}(A_1 - a/A_{50})^{q/p}$ , whereas eq. 9 (Fig. 4, lower curve) is given by  $b = B_i - B_{50}/(A_{50}/a)^{q/p}$ . To demonstrate the symmetry with re-

spect to  $(A_i/2, B_i/2)$ , we translated the coordinate axes to be centered at this point. Thus, we introduced variables  $x$  and  $y$  as follows:  $x = a - A_i/2$ , and  $y = b - B_i/2$ , and substituted them in eqs. 8 and 9. From eq. 8, this yields

$$y_U = -\frac{B_i}{2} + B_{50}\left(\frac{A_i/2 - x}{A_{50}}\right)^{q/p} \quad (13)$$

whereas eq. 9 becomes

$$y_L = \frac{B_i}{2} - B_{50}\left(\frac{x + A_i/2}{A_{50}}\right)^{q/p} \quad (14)$$

where the subscripts U and L are used to distinguish the two curves. It is determined that  $y_L(-x) = -y_U(x)$ , thereby demonstrating symmetry with respect to point  $(A_i/2, B_i/2)$ .

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