1980) from a carton nest in a fallen tree (Jadini Forest, Diani, Kenya). Both have been maintained in the laboratory at 27°C and 85 percent relative humidity since their arrival at Stony Brook. *Reticulitermes flavipes* (Kollar) colonies were obtained on campus and held under similar laboratory conditions.
10. After incubation, 20 termites were homogenized in 1 ml of methanol in a Microflex vial, and insoluble material was removed by centrifugation (2000 rev/min) followed by filtration through a Millipore HPLC sample clarification kit. Radioactivity was determined in samples of soluble and tissue-digested insoluble fractions. Of the recovered radioactivity (62 percent of total applied), 82 percent was in the soluble fraction, 6 percent was in the insoluble fraction, and 12 percent remained in the petri dish. The methanol was removed at reduced pressure, and the residue was dissolved in 2 ml of water buffered to *p*H 5.6 with 0.1 percent acetic acid and ammonium hydroxide, and a portion was injected onto a Whatman PXS 10/25 ODS reverse-phase column and eluted at 1.5 ml/min following a linear gradient to 100 percent methanol. Eluent was monitored continuously at 254 and 219 nm. Samples collected at 2-minute intervals were counted with the use of a Packard TriCarb, with quench corrections by automatic external standarization.
11. Glutathione and cysteine adducts were prepared by modifications (*8*) of the thiosilyl ether method [J. Rokach *et al.*, *Tetrahedron Lett.* **21**, 1485 (1980)].
12. Gas chromatography was performed on a Varian 3700 equipped with a 50-m glass capillary coated with Carbowax 20M (160°C) and a Vista CDS-401 data reduction system. An internal standard (*n*-hexadecane) was added to incubations to correct for injection variability and an external standard (*n*-pentadecane) was added to samples to check total recovery.
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Side-Effect Reduction by Use of Drugs That Bind to Separate but Equivalent Binding Sites

Abstract. *If two drugs cause the same molecular effect by binding to separate noncompetitive binding sites, therapy with a mixture of the two drugs can provide a given desired effect with a lower level of side effects than therapy with either drug alone.*

We recently demonstrated that batrachotoxin-activated sodium channels have at least two binding sites for local anesthetics and that binding of a local anesthetic at either or both sites prevents ions from passing through the channel. When we simultaneously applied two local anesthetics, each with a specificity for a separate site, we observed that the drugs acted synergistically to block the sodium channels (*1*). Synergism of two local anesthetics was also observed by Mrose and Ritchie (*2*). We have suggested the possible clinical usefulness of this effect (*1*).

This type of synergism is quite general, and corresponds to the case of cooperative noncompetitive inhibitors in enzyme kinetics (*3*). It occurs whenever (i) there are two receptor sites, binding to either or both of which causes the same molecular effect; (ii) drugs are available that have a specificity for each site; and (iii) the dissociation constant for the binding of each drug to its receptor does not depend on whether the other site is occupied.

Local anesthetics that are more potent in terms of desired effects also have stronger side effects (*4*). We considered the case in which both this generalization and cooperative noncompetitive inhibition apply and calculated the relative side effects for single-drug therapy and for two-drug therapy with additive side effects. We found that two-drug therapy results in weaker side effects. The ratio of side effects for two-drug therapy to side effects for single-drug therapy can be expressed as a function of the therapeutic ratio and the fraction of channels (or other molecules) that must be inhibited.

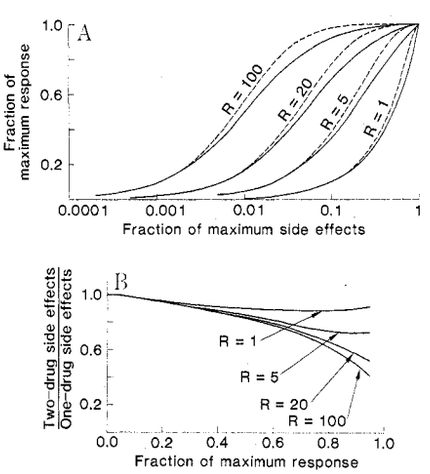

Fig. 1. (A) Side effect–response curves. The parameter R is the ratio of side-effect dissociation constant to desired-effect dissociation constant. For each R, the solid curve corresponds to one drug and the dashed curve corresponds to mixtures of two drugs that bind to separate but equivalent sites. (B) Side-effect ratio as a function of R and the fraction of the maximum response.

Although mixed-drug therapy with the intent of reducing side effects while maintaining maximum desired effect has been common medical practice for some time, it generally has not been employed for the case in which the side effects are additive. In this report we explicitly consider this case.

For two cooperative noncompetitive inhibitors, the fraction of states that have at least one drug bound is (*3*)

$$F_2 = \frac{(D_1/K_1) + (D_2/K_2) + (D_1D_2/K_1K_2)}{1 + (D_1/K_1) + (D_2/K_2) + (D_1D_2/K_1K_2)} \quad (1)$$

where D_1 and D_2 are the drug concentrations at the receptor sites (which we will refer to as dosage) and K_1 and K_2 are the respective dissociation constants.

Mathematically, the synergism between the two drugs occurs because the cross-term in Eq. 1 increases F_2. Using the example of drugs that block sodium channels, we can give a more physical explanation for the synergism. After application of the first drug, addition of a second drug not only blocks additional channels (as would increased dosage of the first drug), it doubly blocks some channels that would otherwise be singly blocked. Since there is a lower probability for a channel blocked by two drugs to open than there is for a channel blocked by one drug, the equilibrium is driven toward more closed channels. At low dosages of both drugs, the fraction of channels with both sites occupied is small, so there is relatively little synergism. As the dosages increase, the relative fraction of doubly blocked channels increases, as does the synergism.

Equation 1 represents the dose-response curve for two drugs. If only one drug is present, D_2 is zero and Eq. 1 reduces to the dose-response curve for one drug binding to a single site:

$$F_1 = D_1/(K_1 + D_1) \quad (2)$$

Comparison of Eqs. 1 and 2 indicates that only when the two dissociation constants are approximately equal does administration of a mixture of two drugs result in a lower total dosage for a given desired effect. Furthermore, reduction of dosage per se is only important for expensive drugs. A more important advantage of synergism is the reduction of

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unwanted side effects. Although the type and cause of side effects are diverse, we will consider a simple model in the hope that it will provide some insight into the advantages of two-drug therapy for more complex cases. We consider the case (*4*) in which drugs exhibit their side effects by binding to side-effect sites whose dissociation constants (K_S) are proportional to the dissociation constants for their desired effects (K_D):

$$K_S = R\,K_D \quad (3)$$

The parameter R is a measure of how much higher the affinity of the drug is for its desired receptor than for its side-effect receptor. In general, large R corresponds to large therapeutic index.

In our model we assume that the side effects of the two drugs are based on equilibrium binding, are additive, and are not synergistic. This corresponds to a case, for example, in which (i) the desired effect is to block sodium channels, which have two equivalent binding sites, and (ii) the side effects are caused by interactions of both drugs with the same receptor of a different channel.

With the above assumptions, the fraction of states related to the side effects that have a drug bound, F_{S2}, can be found from Eq. 1 by eliminating the cross-terms D_1D_2/K_1K_2 and by substituting RK for each K. The result is

$$F_{S2} = \frac{(D_1/K_1) + (D_2/K_2)}{R + (D_1/K_1) + (D_2/K_2)} \quad (4)$$

Since we want to obtain a desired value of F_2 with a minimum value of F_{S2}, regardless of dosage, we combine Eqs. 1 and 4 to obtain F_2 as a function of F_{S2} and R. In general, F_2 is also a function of the relative dosages of the two drugs. It can be shown that in order to maximize the ratio of desired effect to side effect, the two drugs should be administered in proportion to their dissociation constants. For this mixture, F_2 can be expressed as a function of F_{S2} and R only:

$$F_2 = \frac{(1/4R^2 - R)F_{S2}^2 + R\,F_{S2}}{(1/4R^2 - R + 1)F_{S2}^2 + (R - 2)F_{S2} + 1} \quad (5)$$

To obtain the comparable equation for a single drug, we can substitute RK for K in Eq. 5 to obtain the side-effect relation for one drug:

$$F_{S1} = (D_1/K_1)/(R + D_1/K_1) \quad (6)$$

We can combine Eqs. 2 and 6 to obtain F_1 as a function of F_{S1} and R for a single drug:

$$F_1 = (R\,F_{S1})/[(R - 1)F_{S1} + 1] \quad (7)$$

The two-drug side effect–response curve represented by Eq. 5 is compared with the one-drug side effect–response curve of Eq. 7 in Fig. 1A for several values of R. The reduction in the side effects of two-drug therapy is strongly dependent on both R and the fraction of states with at least one drug bound. This can be seen more explicitly in Fig. 1B, which shows the ratio of two-drug side effects to one-drug side effects corresponding to a given degree of binding and a given value of R. Figure 1B shows that side effects measured in molecular terms (such as fraction of channels blocked) can be reduced by as much as a factor of 2. However, Fig. 1B also shows that in order to obtain even a 20 percent reduction in side effects, it is necessary to block about half of the channels. It is not known what fraction of sites are blocked by drugs, but the large safety factor in number of available channels in axons suggests that blocking half of the available channels is a possible role for therapeutically useful drugs. The significance of a 20 percent reduction in side effects is difficult to evaluate, but there are two reasons why such a potential advantage should not be ignored. One is that this advantage can be obtained with no loss in efficacy. The other is that even a modest reduction in the number of side-effect channels blocked may provide a large physiological improvement.

Figure 1B only applies to cases in which side effects are additive. If the side effects are not only additive, but also synergistic (in the same way that the desired effects are synergistic), benefits will be smaller than indicated in Fig. 1B. On the other hand, if the side effects are neither synergistic nor additive, the benefits of two-drug therapy will be larger than indicated in Fig. 1B.

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Intracranial Self-Stimulation in 3-Day-Old Rat Pups

Abstract. *Three-day-old rat pups with electrodes directed at the medial forebrain bundle at the level of the lateral hypothalamic area were trained to push a paddle to receive electrical brain stimulation. Pups receiving stimulation that was contingent on lifting the paddle responded more frequently than did control pups and also learned a two-choice spatial discrimination task that was rewarded with brain stimulation. The experiments indicate that a neural substrate in the area of the medial forebrain bundle is involved in the central mediation of reinforcement in the rat pup.*

Although their neural development is not mature (*1*), during the first week of life rat pups can eat and drink independently (*2*), and they have been successfully trained in classical (*3*) and instrumental (*4*) conditioning experiments. One-day-old rat pups have learned an operant task to obtain oral injections of milk (*5*). Thus, under certain testing conditions, altricial rat pups exhibit motivated behaviors that are adultlike in complexity.

This behavioral complexity suggests that the central nervous system of the newborn rat contains elements for the representation of relationships among classes of stimuli, responses, and consequences. The central mediation of adult affective behavior has been investigated by electrical self-stimulation of the brain (*6*). We have developed methods that allow exploration of the infant brain by electrical self-stimulation and report that 3-day-old rat pups raise a paddle and solve a spatial discrimination task to receive electrical stimulation to the medial forebrain bundle.

Three-day-old Sprague-Dawley rat pups from our colony were removed from their litter, weighed (*7*), and anesthetized in ice. An ice bath, which was attached as a surgical stage to a modified Stoelting-Stellar stereotaxic device, maintained anesthesia throughout the electrode implantation. The pup's head was immobilized by gently securing it between concave clamps that fit snugly over the ears. To minimize the duration of anesthetization, a littermate served as a model for the construction of an acrylic crown around the electrode unit, which consisted of a monopolar electrode and

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