DRUG ANTAGONISM[1]

TABLE OF CONTENTS

THEORIES OF DRUG ANTAGONISM

J. H. GADDUM

Department of Pharmacology, University of Edinburgh, Scotland

Much has been written about the quantitative aspects of drug antagonism and many attempts have been made to account for the facts in terms of the generally accepted theory that most active drugs act by combining with specific receptors in the tissues. It will be necessary in this discussion to concentrate attention on certain aspects of the problem. Consider therefore the contraction of an isolated piece of plain muscle when the effect of an active drug (or agonist) is inhibited by an antagonist.

The effects are likely to depend on the intervals between the times when the two drugs are applied and the time when the measurement is made. Theories of antagonism are usually based on the assumption that equilibrium has been obtained, and it is illogical to apply them to the results of injecting drugs into whole animals, which are complicated by such factors as the distribution and fate of the drugs in the body and the fact that many tissues contribute to the effect. In experiments with isolated organs the tissue can be exposed to known concentrations of drugs for known times. It is convenient to use the short word "dose" in discussing the data, but since the volume of the bath is usually constant this is essentially the same thing as the concentration of the drug in the bath.

Dose-effect curves

If the effect (the size of the contraction) is plotted against log-dose, it is generally possible to fit an approximately S-shaped curve to the results. Two main theories have been proposed to account for the shape of these curves.

1) It has been suggested (50, 137) that the response of the whole tissue is the sum of the responses of a large number of small elements responding inde-

[1] This material was presented in a Symposium on Drug Antagonism at the XXth International Physiological Congress in Brussels, Belgium, July 30 to August 4, 1956. The editors of Pharmacological Reviews gratefully acknowledge the help given by Dr. J. H. Gaddum and Dr. H. O. Schild in preparing for publication the original presentations.

pendently to the drug, and the shape of the curve depends on the varying sensitivities of these elements. If this theory is true, the shape of the curve is the same as the shape of the curve connecting mortality with log-dose and, if the sensitivities are lognormally distributed, it can be converted into a straight line by plotting probits against log-dose. Variations of slope present no difficulty with this theory.

2) According to A. J. Clark (29, 30) the relation between dose and effect depends on an equilibrium between the rate at which drugs combine with receptors and the rate at which they disappear from receptors. According to this theory dose-effect curves are fundamentally the same thing as oxyhaemoglobin dissociation curves or the curves used by Langmuir to relate concentration to adsorption, or the titration curve of a buffer.

In its simplest form, when one molecule of drug combines with each receptor, this theory predicts the exact shape of the log-dose-effect curve. Curves which are too steep can perhaps be explained on the theory that several molecules of drug must combine with a receptor in order to affect it, or in order to make a stable combination with it. This type of theory has been successfully developed to explain the complex shape of oxyhaemoglobin dissociation curves (130). From the purely mathematical point of view curves which are too flat can be explained if one molecule of drug combines simultaneously with several receptors. This may be so with some drugs (*e.g.*, curare), but some very flat curves are difficult to explain without assuming some variation among the receptors (52). These curves can be converted into straight lines either by plotting logits against log-dose (20) or, when the reactions are monomolecular, by plotting the reciprocal of the effect against the reciprocal of the dose (27, 84). Their shape is so nearly identical with that predicted by theory 1) that it is unlikely that it will ever be possible to distinguish between these two theories by accurate measurement of the shapes of the curves. It is unlikely, however, that either theory is correct.

In its simplest form Clark's theory involves the assumption that the effect is proportional to the number of molecules of drug combined with the receptors. Recent experiments with antagonists, which have thrown doubt on this assumption, will be discussed later.

It would be convenient if there was a generally accepted way of expressing the slope of symmetrical log-dose-effect curves. If this is to be in general terms, it is essential to measure the maximum effect of large doses. The effects of smaller doses can then be calculated as percentages of this maximum effect and plotted against log dose (to base 10 for convenience). If either of the theories discussed above were known to be correct, the best way of expressing the slope would be based upon it. Since it is now fairly clear that neither of them is true, any simple way of expressing the slope is satisfactory, but it would be convenient if everyone used the same notation. One proposal is that the slope of the regression line of probits on log-dose should be used, merely for convenience of calculation and without theoretical implications. If the results do not fit this curve, the slope can be calculated (as Clark proposed) from the points corresponding to probits 4 and 6 (16% and 84%).

It would be interesting to hear more evidence about the slopes of dose-effect curves. Some workers have agreed with Clark that the slope is often that which is predicted by the mass laws applied to a monomolecular reaction, about 1.4 probits (27, 29, 143). On the other hand, there is also evidence that this is not always so.

The effect of an antagonist

It is generally found that even when the effect of a given dose of an agonist has been completely suppressed, effects can still be obtained by increasing the dose. It has been suggested that such antagonisms should be called "surmountable" (55). This term describes the facts without any implications about the mechanism involved.

So long as the effect is not completely suppressed dose-effect curves may be determined both in the absence and in the presence of the antagonist and known antagonisms may be divided into three types according to the results obtained when this is done (133). In the commonest type the log-dose-effect curve in the presence of the antagonist is parallel to the corresponding curve in its absence. In one of the other two types it is steeper, and in the other it is less steep. Parallel curves have been studied by numerous authors (29, 30, 50).

The effect of the antagonist is conveniently expressed in terms of the ratio of the dose of the agonist causing an effect in its presence to the dose causing the same effect in its absence. When the log-dose-effect curves are parallel, this ratio is constant, and equal to the antilogarithm of the distance between the curves. It may be called the "dose-ratio" (55).

When the curves are not parallel the dose-ratio varies. It may then sometimes be desirable to adopt the arbitrary convention that it should be measured at the points corresponding to 50% of the maximum effect, and if this maximum is lessened by the antagonist the measurement should be made at the point corresponding to 50% of this smaller maximum (133).

The dose-ratio generally increases with the time of exposure and eventually reaches an approximately constant value, but it may continue to increase over long periods and it is sometimes convenient to adopt a standard time of exposure (say 14 min or 60 min) in order to avoid the errors inherent in very long experiments.

When the antagonist is removed from the bath its effect often disappears again, but sometimes this is a very slow process. The time relations of the rise and fall of the dose-ratio are generally the same. If the effect develops slowly it also disappears slowly. The effects of some antagonists, however, appear to be almost completely irreversible (49).

The activity of an antagonist

It is often possible to determine the conditions for a given standard effect and to plot the doses of the two drugs which just produce this effect against one another. Graphs obtained in this way have been called isobols by Loewe (86), who has written much about their properties.

If the dose-ratio is plotted against the dose of antagonist, the resulting curves

are the same shape as isobols, though the vertical scale is different. The dose-ratio corresponding to zero dose is unity and the line slopes upwards from this point. Sometimes it is a straight line (50), but this is not always so. Schild's (131) pA_2 and pA_{10} are calculated from the doses corresponding to dose ratios of 2 and 10. Measurements of these quantities have given remarkably constant results in different laboratories and provide the most satisfactory method of measuring the activity of antagonists. When the isobol is linear $pA_2 - pA_{10} = \log 9$.

In screening tests it is often convenient to use all the antagonists in the same concentration, and to increase the dose of agonist until a standard effect is obtained. This technique does not give a satisfactory estimate of pA_2 or pA_{10}, which can only be obtained by several trials with different concentrations. The results are frequently expressed as the ratio of the dose of one drug to the dose of the other (55). This method cannot give constant results when the dose-ratio is small unless the dose of agonist is corrected by subtracting the effective dose in the absence of antagonist. Even when this correction is made, however, the method is not ideal because its estimation involves errors due to variations of the sensitivity of the tissue to both drugs.

A simple method which avoids this fault is to calculate the slope of the line connecting dose-ratio and dose from the expression

$$K = (\text{dose-ratio} - 1)/B$$

where B is the concentration of the antagonist.

This definition is independent of all theories, but when the antagonism is competitive K is likely to be equal to the affinity constant of the antagonist-receptor complex. This symbol K was used in this sense by A. J. Clark. Other workers use the same symbol to denote the dissociation constant, which is the reciprocal of the affinity constant. The affinity constant has the dimensions of $(\text{concentration})^{-1}$, and increases when the activity of a drug increases. When the isobol is linear $pA_2 = \log K$.

It sometimes happens that when the dose of antagonist is sufficiently high the tissue does not give a maximum response even to the highest possible dose of agonist. Some receptors appear to be completely blocked, or destroyed, and the antagonism may be said to be unsurmountable (55). In this case the isobol eventually becomes vertical; the ratio of the dose of agonist to that of antagonist and the index K both cease to be even approximately constant and become infinite.

Theories of antagonism

Antagonism may doubtless be due to a number of different mechanisms.

1. Independent antagonism. This occurs when drugs have opposite but independent effects. For example, carbachol causes contraction of the rat uterus and this effect is inhibited by adrenaline, acting presumably on different receptors; papaverine depresses smooth muscle and antagonizes the effects of

various agonists unspecifically. Both of these may be called independent antagonists. They can generally be identified because the same antagonist is effective against various types of agonist, but this test is not infallible since one antagonist may combine with several different kinds of receptor.

It is, in fact, commonly found that antagonists which act specifically on one type of receptor in low concentrations appear to act on other types of receptor when the concentration is increased. It is, however, very important in all experiments on antagonism to determine by experiment whether the antagonism is specific, by control tests with other agonists.

The theory that a given pair of antagonists is acting independently cannot at present be excluded by quantitative studies, since little is known about the quantitative relations between the effective doses of independent antagonists.

2. Antagonism by neutralization. In this type of antagonism the two drugs combine with one another to form an inactive compound. One well known example is provided by compounds containing *SH* groups which antagonize mercury and arsenic by combining with them to form an inactive compound. The antagonism between calcium and citrate in their effects on the frog heart (104) is another example.

Some writers appear to believe that this type of antagonism provides information of the nature of the receptors in the tissue. For example, Fildes (43) argued that mercury salts must act on bacteria by combining with *SH* groups because its action was inhibited by providing an excess of *SH* compounds. His conclusion was probably correct, but this evidence does not prove it. Goodman and Gilman (61, p. 847) used a similar argument in connection with the action of mercurial diuretics. If this kind of argument were applied to the experiments with calcium it would lead to the conclusion that the receptors in the tissue were citrate groups. Such antagonists merely decrease the amount of free agonist and provide no information that could not be obtained by simply diluting it.

The quantitative theory of this type of antagonism was discussed by Gaddum (53). The argument in the simplest case is as follows:

Let A, A_1 and B be the molar concentrations of total agonist, free agonist and total antagonist respectively.

Then the concentration of inactive compound $= (A - A_1)$

and the concentration of free antagonist $= B - (A - A_1)$

For equilibrium $A - A_1 = KA_1(B - A + A_1)$

or
$$A = A_1 + \frac{KA_1}{1 + KA_1} B$$

where K is the affinity constant, which is the reciprocal of the dissociation constant.

The isobol showing the conditions which produce the effect corresponding to the concentration A_1 is given by making A_1 constant, and is linear. When $B = 0$, $A = A_1$; the dose-ratio is therefore A/A_1.

If the antagonist is very active (for example, a drug which makes a very insoluble precipitate with A), or if the tissue is very insensitive, then KA_1 is large compared with 1 and $A = A_1 + B$. In these circumstances the amount neutralized is constant and equal to B and the antagonist makes the log-dose-effect curve steeper.

If the antagonist is not very active in relation to the sensitivity of the tissue, so that a large excess is needed, KA_1 can be neglected in comparison with 1, and $A/A_1 = 1 + BK$. For any given value of B the dose-ratio A/A_1 is then constant and the log-dose-effect curves are parallel. In this case antagonism by neutralization may be expected to produce results indistinguishable from those produced by competition.

3. Non-competitive antagonism. In this case the two drugs combine with different parts of the receptor mechanism so that the presence of either does not exclude the other, but when the antagonist is present the agonist is ineffective.

The quantitative relationships have been discussed by Chen and Russell (27), Schild (134), and Furchgott (49). This theory predicts that the proportion of the receptors inactivated by any given dose of antagonist will be independent of the dose of agonist. Furchgott (49) points out that if the response of the tissue is proportional to the number of receptors activated, all responses, including the maximum response, will therefore be reduced in a constant proportion. The log-dose-effect curve will become flatter, but its position will not change. These conditions are seldom if ever fulfilled in practice. Many examples are known in which the maximum effect in the presence of the antagonist is less than the maximum effect before, but this change is generally associated with an increase of the dose-ratio; the antagonist not only lowers the maximum, but it also shifts the log-dose-effect curve to the right.

4. Competitive reversible antagonism. According to Clark's theory in its simplest form the two drugs compete directly for the receptors and the effect is directly proportional to the amount of agonist which is combined with the receptors.

The results predicted by a simple application of the mass laws were given by Gaddum (51, 53) in terms of the final concentrations ($A + B$) of the drugs in the bath. They are contained in the formula

$$K_1 A^{n_1} = \frac{a}{1 - a} (1 + K_2 B^{n_2})$$

where K_1 and K_2 are the affinity constants of the two drugs for the receptors, a is the proportion of receptors occupied by the agonist and n_1 and n_2 are the numbers of molecules of agonist and antagonist combining with each receptor and are generally taken as unity.

In practice the data usually refer to the total amounts of the drugs added to the bath and allowance may have to be made (*e.g.*, with small baths) for loss of free drug due to combination with the receptors; appropriate formulae have been discussed by Goldstein (60).

On the simple theory, when $n_1 = n_2 = 1$

1) the isobol, obtained by plotting the dose of one drug against the dose of the other for a given effect is linear. For large dose-ratios the concentrations of the two drugs are proportional to one another. In these respects the tissues behave just as if the drugs were combining with one another. In both cases it follows from the fact that the isobol is straight that $pA_2 - pA_{10} = \log 9$;

2) the dose-ratio is constant and the log-dose-effect curves are parallel. In this respect the drugs behave as if they were combining with one another and there was a large excess of antagonist.

When these conditions are fulfilled the conclusion is sometimes drawn that the antagonism is competitive (97, 138), but this conclusion is not justified without further evidence. Antagonism by neutralization would be expected to produce similar effects; even unspecific antagonism is not necessarily excluded.

Chen and Russell (27) suggest that the type of antagonism should be determined by the method of Lineweaver and Burk (84). The reciprocal of the effect is plotted against the reciprocal of the dose. If Clark's theory in its simplest form is true ($n_1 = 1$), the points lie on straight lines. If the straight lines determined in the presence and absence of the antagonist intersect on the line corresponding to infinite dose, the antagonism is said to be competitive. This is, however, merely another way of showing that the same maximum effect is obtained in the presence and absence of the antagonist. This condition may be fulfilled in any type of antagonism except the non-competitive type.

The theory of competition is attractive and some writers have accepted it uncritically, but recent work has revealed various facts which cannot be explained by this theory in its simplest form. Some of these facts suggest modifications of the theory, and others suggest that other forms of antagonism may occur. The facts are as follows:

(a) The slope of the log-dose-effect curve is often significantly different from that predicted by the simplest theory. Variations of slope have been attributed to variations in the number of molecules combining with each receptor, but this explanation is not entirely satisfactory.

(b) The presence of the antagonist may alter the slope.

(c) The maximum effect in the presence of the antagonist may be less than the maximum effect in its absence.

(d) The effects of some antagonists (*e.g.*, ergotamine acting on the adrenaline receptors in rabbit uterus (50)) develop slowly and disappear slowly when the drug is removed from the bath. The effects of dibenamine and allied drugs containing a chloroethylamine group (108) are almost irreversible and give particularly striking results. In experiments with such drugs it is possible to reduce the effect of a particular dose of agonist to indetectable proportions and yet obtain a maximum effect with a larger dose of agonist. A simple competitive theory would suggest that when the effect is abolished the receptors are all either destroyed, or occupied by an antagonist with an action lasting several hours. The larger dose of agonist, on the other hand, may act in a few seconds and the theory suggests that it does this by occupying receptors vacated by the antagonist during this time. The results with ergotamine have been explained (*cf.* 49) on the theory that the slow changes represent the uptake of the drug

from the bath into the "biophase" and that the competition at the receptors is a rapid process. The theories discussed in the next paragraphs make this theory unnecessary.

(e) Some drugs may produce a small effect themselves and yet block the effects of other drugs. Quantitative studies with such drugs led Stephenson (139, 141) to suggest that

(i) The effect of an agonist depends not only on its affinity for the receptors, but also on its ability to produce an effect when combined. This idea has been developed independently by Ariëns (4, 8) who speaks of the affinity and the intrinsic activity of drugs. According to this theory a drug may antagonize other drugs by occupying nearly all the receptors and yet produce a small effect itself.

(ii) The effect is not proportional to the number of receptors activated and a maximum effect may be produced when this proportion is small. This is the theory of spare receptors. The nature of the relationship between the effect and the number of active receptors is unknown, but, whatever it may be, this change in the theory does not affect the shape of the isobols, the value of the dose-ratio or pA_2, or even the parallelism of the log-dose-effect curves.

The original theory of competition explained the shape of one type of dose-effect curve. The new theory does not explain the shape of any dose-effect curves, but it preserves most of the old theory and explains the new facts. According to it, the reason that maximum effects can be produced even when most of the receptors are blocked is that only a small proportion of the receptors is in any case necessary for a maximum effect. So long as sufficient receptors remain free the shape of the log-dose-effect curve remains the same, but eventually with high concentrations of antagonist the proportion of free receptors becomes so small that the original maximum effect is not produced even by very large doses of agonist.

AFFINITY, INTRINSIC ACTIVITY AND DRUG INTERACTIONS

E. J. ARIËNS, J. M. VAN ROSSUM AND A. M. SIMONIS

Pharmacological Institute of the University of Nijmegen, Netherlands

TABLE OF CONTENTS