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pA_x AND COMPETITIVE DRUG ANTAGONISM

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In a recent paper Guarino and Bovet (1949) have studied the use of the measure pA for expressing intensity of drug antagonism. pA_x has been defined (Schild, 1947) as the negative logarithm of the molar concentration of an antagonist which reduces the effect of a multiple dose, x, of a stimulant drug to that of a single dose. Guarino and Bovet have pointed out that this definition does not specify concentration of active drug, and therefore by implication assumes that pA is independent of the initial concentration of active drug used. In studying the antagonism between acetylcholine and flaxedil* on the frog's rectus abdominis, however, they found that pA was not entirely independent of the initial concentration of acetylcholine used. For instance, an increase in the concentration of acetylcholine of 1.5 log units produced a decrease of pA_2 of 0.53 units. Guarino and Bovet then proceeded to evolve a new formula for the quantitative relations of antagonistic drugs whereby pA would be dependent on the concentration of active drug in competitive antagonism.

This interesting paper raises several issues connected with the use of pA and the definition of competitive drug antagonism. I propose to discuss the following points:

1. Conditions under which pA is independent of concentration of active drug.
2. Quantitative relations of antagonistic drugs.
3. The practical use of pA.

Dependence of pA on Height of Contraction. Types of Concentration-Action Curves

It is well known that concentration-action curves plotted on a logarithmic scale, first in the absence of an antagonist and then in the presence of antagonist, are frequently parallel. This experimental fact forms the basis for Gaddum's formula (1937) for drug antagonism and it is also at the basis of the pA measure, since parallelism on a log abscissa implies that pA is independent of height of contraction (and hence of concentration of active drug). A simple way of finding whether pA is applicable is thus to draw concentration-action curves on a log scale with and without antagonist. This method may also be used to determine pA values.

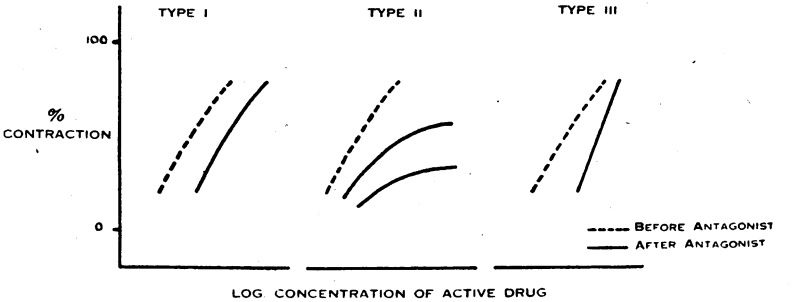

FIG. 1.—Three types of concentration-action curves.

Concentration-action curves have also been described, however, which are not parallel when plotted on a logarithmic scale, and where pA in consequence is dependent on the height of contraction. Amongst these, two types emerge. One type (type II of Fig. 1) has been described by Zadina (1947) with the following two characteristic properties: the concentration-action curves become progressively flatter and their maxima decline as the concentration of antagonistic drug is increased. Examples are: histamine-harmine on guinea-pig ileum (Zadina, 1947), acetylcholine-magnesium on guinea-pig ileum (Zadina and Kriz, 1948), acetylcholine octyltrimethylammonium on rat's intestine (Clark and Raventos, 1937). Particularly in Zadina's careful experiments it is perfectly clear that not only the slopes but also the maxima of the curves are decreased. These results are presumably due to some sort of non-competitive antagonism, since the effects of the antagonist cannot be completely

* 1:2:3—Tri-β-diethylaminoethoxybenzene triethiodide.

reversed. Such curves might occur, for example, if a constant number of contractile elements (or "receptors") were put out of action by a given concentration of antagonist. This would have the effect of decreasing both slopes and maxima.

Lastly there are curves of type III (Fig. 1), for example Guarino and Bovet's curves obtained with acetylcholine-flaxedil on the frog's rectus; here the concentration-action curve with antagonist is steeper than without. It was in order to account for these results that Guarino and Bovet suggested a new formula for competitive drug antagonism.

Quantitative Relations of Competitive Antagonists

Gaddum (1937) and Guarino and Bovet (1949) have suggested formulae to express quantitatively the relations of antagonistic drugs competing for receptors. The two formulae are incompatible; it can be shown, in fact, that according to one of them pA is independent of concentration of active drug, whilst according to the other it increases as the negative logarithm of concentration of active drug. Since the matter is of general interest I shall try, in the following, to point out the differences underlying these two formulations of competitive antagonism.

Gaddum's formula is based on the assumption that drug and antagonist compete for free receptors on the cell surface according to a simple mass action law, and that at equilibrium the number of drug-receptor combinations is equal to the number of drug-receptor dissociations and similarly for the antagonist. Contraction of the muscle is supposed to be proportional to the average number of receptors occupied by the active drug. With these assumptions a formula can be derived which relates percentage contraction y to concentration A of stimulant drug and B of antagonistic drug:

$$\frac{y}{100-y} = K_1 A = \frac{K_1 x A}{K_2 B_x + 1} \quad \ldots\ldots\ldots\ldots\ldots (1)$$

$$\therefore K_2 B_x = x - 1.$$

A similar equation is obtained by applying Langmuir's adsorption isotherm to the case of two gases competing for the same surface (Taylor, 1931).

Guarino and Bovet's formula is derived as follows:

Assuming concentration A of a drug to produce effect y, and concentration $2A$ to produce a bigger effect, what concentration of antagonist is required to reduce the effect of $2A$ to that of A? To simplify the argument it is assumed that the antagonist ("false drug") has the same affinity for receptors as the active ("true") drug has. The answer is that the concentration of false drug required is equal to that of true drug, namely $2B = 2A$, since in this way the chances of true drug reacting with receptors will be halved and hence its action will be reduced to that of A. Generalizing this argument, if the concentration of true drug is increased to xA, then the amount of false drug required to reduce this effect to that of A must be such that the total amount of true and false drug present in the solution is x times the amount of true drug present. This leads to the following general formula:

$$K_2 B_x = x(x-1)K_1 A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

where B_x is concentration of antagonist required to reduce the effect of concentration xA of stimulant drug to that of A.

In non-competitive antagonism this formula is assumed to reduce to simple proportionality, namely:

$$K_2 B_x = x K_1 A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

This formula, incidentally, was originally used by Clark (1926) to formulate the antagonism acetylcholine-atropine, K_1/K_2, depending on the effect produced.

That the three equations are incompatible may be shown by transforming each to give the antagonist ratio B_{x_2}/B_{x_1}, required to balance an increase of active drug from x_1-fold to x_2-fold.

From equation (1)

$$\frac{B_{x_2}}{B_{x_1}} = \frac{x_2 - 1}{x_1 - 1}.$$

From equation (2)

$$\frac{B_{x_2}}{B_{x_1}} = \frac{x_2(x_2-1)}{x_1(x_1-1)}$$

From equation (3)

$$\frac{B_{x_2}}{B_{x_1}} = \frac{x_2}{x_1}$$

In this way the applicability of one or the other equation may be tested. For example, in order to balance an increase of active drug from 2-fold to 10-fold the antagonistic drug would have to be increased 9 times, 45 times, or 5 times respectively, according to the three equations. Unfortunately, this test is not very fruitful, since all sorts of ratios seem to occur; for example, a B_{10}/B_2 ratio of 5 was found for acetylcholine-atropine, 10 for histamine-benadryl (Schild, 1947), and 21 for acetylcholine-flaxedil (Guarino and Bovet, 1949).

With regard to pA the following difference exists between equations (1) and (2). From (1), $B_x = x - 1/K_2$. Therefore B_x is not a function of A. Since $pA_x = -\log B_x$, it also is not a function of A. Hence whenever equation (1) applies pA_x must be independent of concentration of stimulant drug.

From (2) B_x is proportional to A. Therefore pA is inversely related to concentration of stimulant

drug. Thus an increase in the concentration of stimulant drug by one log unit should cause pA to diminish by one unit.

In Guarino and Bovet's experiments pA did not decrease to the extent required by their theory, but they regard equation (2) as a limiting case of perfect competitive antagonism which has not yet been shown to occur.

Guarino and Bovet's assumptions differ in two respects from the assumptions underlying, e.g., equation (1).

(i) It is assumed that if to a given concentration of true drug a quantity of false drug be added to increase total concentration x-fold, then the chances of true drug reacting with receptors would be reduced to $1/x$. By equation (1) this would only apply if all receptors were initially occupied. Otherwise the addition of antagonist would increase the total number of receptors occupied and the chances of reacting would be reduced to $1/x$ of the new total and not of the original.

(ii) It is assumed that reducing the chances of receptor combination to $1/x$ by false drug has the same effect as decreasing the concentration of true drug to $1/x$. By equation (1) this would apply approximately when a few receptors were occupied, but would not apply when most receptors were occupied. This may be illustrated as follows: Assume a sufficient concentration of true drug present to produce an almost maximal effect. If this concentration were halved the effect would be only slightly decreased since most receptors would still be occupied. If, however, an equivalent amount of false drug were added each receptor would have an equal chance of being occupied by one or the other and the effect would be halved.

In conclusion Guarino and Bovet's assumptions do not appear to be based on mass action competition for receptors in the sense that drug or antagonist occupy a number of receptors for a finite time to the exclusion of each other. Langmuir's treatment is based on the assumption that after condensation of a molecule of the gas owing to adsorption on an " elementary space " a finite interval elapses before it escapes again from the surface. This notion gives a clear physical meaning to the term competition, which is absent in Guarino and Bovet's hypothesis.

The Practical Use of pA

Methods of measuring activity of antagonistic drugs may be divided into comparative and non-comparative methods and into methods in which the antagonist is injected first (preventive) and methods in which the active drug is injected first (curative). pA belongs to the group of non-comparative and preventive methods and in this way its field of usefulness is limited. For example, it is difficult to use a preventive method quantitatively in the whole animal since this requires producing a constant *concentration* of antagonist in the blood stream; on the other hand it is often possible to produce a constant *effect* with the active drug and reverse it by means of an antagonist (e.g., Ing, Dawes, and Wajda, 1945). The main disadvantage of pA is its variability, which is greater than that of an assay relying on a comparison with another antagonistic drug. For this reason comparative assays are likely to be preferred for routine work, but for the purposes of a general scale an absolute method and one in which the effect of time and concentration can be accurately assessed is preferable (Schild, 1947).

One way of decreasing variability is to define in great detail the conditions of the experiment. There is little purpose, however, in introducing arbitrary limitations which do not add to our information, unless some important systematic error is thereby avoided. For example, it is probably not necessary to choose a uniform strain of guinea-pigs since pA does not appear to vary systematically from one laboratory to another (cf. results of Reuse (1948) and Schild (1947)).

In an antagonist assay on an isolated preparation the following variables may determine the effect: experimental preparation, concentration of antagonistic drug, time of contact with antagonistic drug, concentration of active drug, effect produced by active drug alone, composition of Ringer's fluid, temperature, etc. In measurements of pA only the first two or three are defined. By implication the others may be assumed to introduce no important systematic variation.

In practice a compromise must be found between having too many arbitrary limitations and excessive variability. For example, in the method of Miller, Becker, and Tainter (1948) the concentration of active drug is fixed. This introduces an arbitrary limitation, but it certainly helps to reduce variability. In the case of pA neither concentration of active drug nor effect produced by active drug is defined, hence the assumption is that for practical purposes pA is independent of these variables. This is obviously not true when dealing with concentration-action relations of type II and III above, and I would suggest that in such cases pA be measured when the effect is 50 per cent of the maximal. In this way pA may be used as a practical measure of antagonistic activity even when it is not independent of concentration of active drug.

SUMMARY

1. Three types of concentration-action curves have been described when the effect of a drug on an isolated tissue is plotted against the logarithm of the dose, in the absence and presence of antagonist. Curves of type I remain parallel, those of type II become flatter, and those of type III steeper in the presence of antagonist. Type II is often associated with a lowering of the maximum contraction (Zadina). By definition pA is constant only when curves of type I occur.

2. Guarino and Bovet have recently shown that curves of type III may occur and have suggested a formula which would account for these curves. Gaddum had previously suggested a different formula for competitive drug antagonism, which accounts for curves of type I. The two formulae are contrasted and the conclusion is reached that Guarino and Bovet's formula implies a type of antagonism which does not involve the notion that drug and antagonist compete for and occupy the same receptors according to a law of mass action. The available experimental data are insufficient to distinguish between the two views.

3. Variability may be diminished by defining the height of contraction at which pA should be determined. With curves of type I it is unnecessary thus to define the effect, but in other cases a 50 per cent contraction may be chosen for determining pA.

REFERENCES

Clark, A. J. (1926). *J. Physiol.*, **61**, 547.
Clark, A. J., and Raventos, J. (1937). *Quart. J. exp. Physiol.*, **26**, 375.
Gaddum, J. H. (1937). *J. Physiol.*, **89**, 7*P*.
Guarino, S., and Bovet, D. (1949). *R.C. Ist. Sup. San.*, **12**, 215.
Ing, H. R., Dawes, G. S., and Wajda, I. (1945). *J. Pharmacol.*, **85**, 85.
Miller, L. C., Becker, T. J., and Tainter, M. L. (1948). *J. Pharmacol.*, **92**, 260.
Reusè, J. J. (1948). *Brit. J. Pharmacol.*, **3**, 174.
Schild, H. O. (1947). *Brit. J. Pharmacol.*, **2**, 189.
Taylor, H. S. (1931). *A Treatise on Physical Chemistry*, 2nd Ed., p. 1074. London : Macmillan.
Zadina, R. (1947). *Sbornik lekarsky*, **49**, 173.
Zadina, R., and Kriz, V. (1948). *C.R. Soc. Biol., Paris*, **142**, 1037.