Some quantitative uses of drug antagonists

O. Arunlakshana & H.O. Schild

Commentary by

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This is the final and most important of the three papers in which H.O. Schild expounded the experimental and theoretical basis for the study of the action of antagonists on isolated tissues (see also Schild 1947, 1949). It has become the most often cited article to have appeared in the Journal.

Though the paper has an important theoretical content, the primary aim was to examine and validate the various ways in which studies of drug antagonism, competitive and otherwise, could be useful in relation to the practical issues that concerned the pharmacologists of the day. Neither the radioligand binding technique nor methods for receptor isolation, let alone sequencing, were available. In fact the main means for the pharmacological identification of receptors was the use of antagonists, as the opening sentence of the paper notes. Schild regarded his work on drug antagonism as practical in its aim, and his interest in the quantification of antagonism was greatly strengthened when he became a consultant to Roche Products with whom he worked on the possible uses in asthma of antagonists acting on bronchial muscle.

The paper begins with a recapitulation of the applications of measurements of pA_x, the index of antagonism which Schild had earlier introduced (Schild, 1947). The theoretical basis for these applications is then considered. The starting point is the second and more general of two equations presented by J.H. Gaddum in a note on the quantitative effects of antagonistic drugs (Gaddum, 1937). Gaddum, in the tradition of A.J. Clark and other quantitative pharmacologists of the time, regarded the accurate quantitative description of drug action and antagonism of at least equal importance as establishing a molecular mechanism, though the latter was certainly an aim in the longer term. The first of Gaddum's equations gave the relation which should hold between the percentage receptor occupancy (y) by agonist and the concentration (C_1) of agonist when agonist and antagonist compete for a single population of binding sites:

$$K_1C_1=(1+K_2C_2)\frac{y}{100-y} \qquad (1)$$

Here K_1 and K_2 are affinity constants, and C_2 is the concentration of the reversible competitive antagonist[1]. This expression was (and still is) difficult to test without knowing the relationship between y and the response of the tissue. If direct proportionally was assumed, the equation was found to agree with the observed relationship between C_1, C_2 and y, *in the simplest case*, as Gaddum observed. However, he went on to note that a wider range of experimental results could be fitted using the more general expression

$$K_1C_1=(1+K_2C_2^n)\frac{y}{100-y} \qquad (2)$$

Gaddum pointed out that the physical interpretation of n was uncertain if n is less than 1. It can be added that even if n is greater than unity, and a whole number, it is quite improbable that, say, 2 molecules of antagonist have to combine with the receptor in a simultaneous trimolecular reaction.

It was probably the greater descriptive power of equation (2) that led Arunlakshana and Schild to take it as their starting point rather than the more physically interpretable equation (1). Making the crucial assumption that the same response elicited first in the absence and then in the presence of the antagonist corresponded to the same fraction of activated receptors, it followed from equation (2) that

$$\log (x-1)=\log K_2 - npA_x$$

[1]The terms and symbols used in this commentary are those of the original authors.

where x is the dose-ratio and pA_x is as defined by Schild (1947). Hence a plot of log (x-1) against pA_x should yield a straight line of slope -n. Further, by definition, the line intercepts the pA_x axis at a point corresponding to pA_2 *regardless of the value of n*.

If Gaddum's first equation is used, then, as Schild had shown in 1949, a simple expression follows:

$$x-1 = K_2 B_x \qquad (3)$$

Here B_x (equivalent to Gaddum's C_2) is the concentration of antagonist.

Hence

$$\log(x-1)=\log K_2 + \log B_x$$
$$=\log K_2 - pA_x$$

Thus for simple reversible competitive antagonism, a graph (a 'Schild plot') of log (x-1) against -log B (= pA_x) should provide a line with a slope of -1 (see Fig. 7). This test has been of the greatest importance in analysing the action of drug antagonists for it provides a means not only of quantifying the effectiveness of a given antagonist but also of determining whether the concentration dependence of its action is compatible with reversible competitive antagonism.

Though Arunlakshana & Schild were not the first to apply equation (3) to determine affinity constants for the combination of competitive antagonists with receptors (see van Maanen, 1950, Furchgott, 1955), it was this paper that established a practical and yet rigorous procedure for the study of competitive antagonism. A key feature is that dose-ratios are determined over as wide a range of antagonist concentrations as practicable. One of the most valuable applications of this approach has been the investigation of factors that cause deviations from the expected pattern of a linear Schild plot with a slope of unity. Such studies can provide a wealth of information about drug action (for an example see Black, Leff & Shankley, 1985).

Now some more general comments. Much of the experimental work described in the paper had been completed some years before its publication and had been done in a collaboration between H.O. Schild and a young graduate from Thailand, Dr. O. Arunlakshana. Schild had presented some of their results at the XXth International Congress of Physiology in 1956 (see *Pharmacological Reviews*, **9**, 211-268, 1957). There have been many subsequent developments but none requiring reconsideration of the fundamentals of the paper. It is straightforward to show that the equation (3) should apply equally to more complicated models of receptor action in which the receptor has to isomerise to an active form before a response occurs. Also it would hold for an agonist and an antagonist that bind to different domains of the receptor, provided that the binding of each was reversible and mutually exclusive.

It is interesting in retrospect that Arunlakshana & Schild did not choose to draw more attention to what was to become one of the main applications of their approach, namely, the quantification of the activity of new competitive antagonists. This was to transform drug development for several decades (see Black *et al.* 1972 for a landmark example). There has also been a great deal of discussion in later years of how best to analyse the effects of a competitive antagonist on the complete agonist concentration-response relationship of an isolated tissue. One issue to have received much attention is the overemphasis that can be placed on the control (i.e., pre-antagonist) concentration-response curve when calculating a set of dose-ratios at different antagonist concentrations: each will be affected by an error in the control curve. This had led to the development of computer-aided methods of analysis based on the simultaneous fitting of the whole data set (i.e., the concentration-response relationships with and without antagonist) to the Gaddum equations or variants of them (for references and a fuller discussion see e.g. Lew & Angus, 1995). Though this has clear advantages, it is interesting to observe in the current literature how often the simple analysis introduced by Arunlakshana & Schild continues to be found useful.

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