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USE OF THE LOGISTIC FUNCTION FOR THE CALCULATION OF DOSE-RATIOS AND POTENCY RATIOS

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1 Dose-ratios and potency ratios, obtained with the guinea-pig isolated ileum by calculating matching concentrations from a least-squares fit using a logistic relationship between response and dose, differ from those obtained with the more usual assumption that response is a linear function of log dose over a limited range.

2 The differences are not due to the mathematical treatment of the results but arise from changes in sensitivity associated with the production of very high or very low responses.

3 With this preparation there is no advantage in avoiding the linear transformation and fitting the results to the complete dose-response curve though this might not apply to results obtained from other tissues with less variable sensitivity.

Introduction

Modern computers make it possible to fit results to many complex expressions directly and thus avoid transforming the results into functions which are linearly related. In the calculation of Michaelis constants from values of substrate concentration and reaction rate, Colquhoun (1971) has shown that the direct fit to the hyperbolic relationship is more accurate as it avoids the bias associated with linear transformations, particularly with the reciprocal-plot method of Lineweaver & Burk (1934).

In most pharmacological tests giving graded responses, such as a contraction of a piece of smooth muscle, it is usual to assume that the response is linearly related to log dose over the middle range of contractions. This linear transformation therefore makes it possible to use only simple algebra for calculating concentrations producing matching responses, such as in bioassay or in the calculation of dose-ratios produced by antagonists or the potency ratios of different agonists. In fact the relationship between log dose and response is usually sigmoid and results can satisfactorily be fitted to the logistic function:

$$\text{response} = M \frac{A^p}{A^p + K^p}$$

where A is the concentration of drug, M is the maximal response, and K and p are empirical constants (Parker & Waud, 1971). These authors have described how results may be fitted to this expression for calculating matching responses produced by agonists and partial agonists (or irreversibly blocked agonists) and how these may be fitted to the adsorption isotherm for the calculation of partial agonist (or agonist) affinity constants; it is clear that this procedure should be more accurate than a linear transformation with reciprocals (Barlow, Scott & Stephenson, 1967).

Waud & Parker (1971) have described procedures for fitting sets of results to curves having common values of M and p (i.e. the same maximum response and 'slope') which therefore make it possible to calculate dose-ratios or potency ratios (from the ratios of the values of K). It seemed, therefore, worthwhile investigating whether these were different from values obtained in the usual way, assuming a linear relationship between response and log dose. Ideally the estimations of dose-ratios or potency ratios are null methods, the concentrations being adjusted so that the responses are identical, but in practice the control and test responses invariably differ and it is necessary to make some assumption about the relationship between dose and response in order to calculate matching concentrations. If the difference is of any size, it might be possible that more accurate results would be obtained by fitting the results direct to the logistic function.

Methods

Experiments were made at 37° C with the guinea-pig isolated ileum suspended in aerated

Tyrode solution, containing hexamethonium (2.67×10^{-4} M). Responses were recorded isotonically and the agonist, carbachol, was allowed to act for 30 s and applied once every 90 seconds.

In the first group of experiments dose-ratios were measured for 10^{-6} M phenylacetyltropine methiodide (Abramson, Barlow, Franks & Pearson, 1974). In one set the agonist was tested at two concentrations which produced contractions roughly between 25 and 75% of the maximum, exactly as described previously (Abramson, Barlow, Mustafa & Stephenson, 1969; Edinburgh Staff, 1970). In the second set the agonist was tested in additional concentrations above and below those used in the first set, making four concentrations in all. The order in which they were tested was based on a latin square but because of the way in which the programmer operated the latin square applied to four pairs of concentrations (of the six combinations possible), rather than to the four concentrations themselves.

The mean responses (of five or more observations) to the four concentrations before and after the antagonist were fitted to a logistic expression with common values of M and p and the ratio of the values of K gave the dose-ratio. The mathematical basis for the computation is given by Waud & Parker (1971) and a series of programmes, written in Fortran II, was used to implement the calculations with a PDP 8I digital computer. These required starting estimates of K and p which were then altered until the changes in K were less than 1 in 200.

In the second group of experiments responses were obtained with carbachol, as in the first group, and then a second solution of carbachol was

Table 1

A		*Mean dose-ratio*	*Range*	*s.e.*	n
1		11.6	8.8-15.8	1.1	7
2		9.59	6.5-12.7	0.8	7
3		9.58	6.3-11.9	0.8	7
B		*Mean potency*	*Range*	*s.e.*	n
Standard solution tested first					
1		1.16	1.01-1.29	0.04	6
2		1.39	1.20-1.60	0.06	6
3		1.44	1.31-1.61	0.05	6
Standard solution tested second					
1		1.17	1.02-1.36	0.06	5
2		1.06	0.92-1.14	0.04	5
3		1.00	0.93-1.14	0.04	5
Test conc. increased relative to standard; standard solution tested first					
1		1.05	0.90-1.24	0.05	5
2		1.25	1.08-1.45	0.05	5
3		1.29	0.99-1.61	0.13	5
Pooled results:	1	1.13	0.90-1.36	0.03	16
	2	1.23	0.92-1.60	0.05	16
	3	1.21	0.93-1.61	0.06	16

(A) Dose-ratios produced by phenylacetyltropine methiodide, 10^{-6} M, and (B) potency ratios of carbachol solutions.

1. Experiments with two concentrations of agonist in each set and the results calculated assuming that response is linearly related to log dose. 2. Experiments with four concentrations of agonist in each set and the results calculated from fitting responses to a logistic expression by the method of least squares. 3. Results calculated as in 1 from the responses obtained with the two middle concentrations of agonist used in the experiments in 2.

The concentrations of the carbachol solutions were 1.00×10^{-1} M (standard stock solution) and 1.12×10^{-1} M ('test' stock solution): these were diluted as necessary.

tested. This solution was of known concentration, stored in the same conditions as the stock standard solution of carbachol (4° C), so estimates of its potency by the two methods could be compared and checked with the actual value.

Results

Different dose-ratios were obtained in the two sets (Table 1A). There was no means of knowing the correct value but the results in the second group of experiments (Table 1B) showed that the comparison using four concentrations was an overestimate of potency and in such a situation this would lead to an underestimate of the dose-ratio. When the order of testing the carbachol solutions was reversed, the comparison using four concentrations led to an underestimate of potency, suggesting that the sensitivity of the preparation increased during the experiment so that the solution tested second appeared relatively more active. When the concentrations of the test solution were slightly increased, so that their effects were consistently stronger than those of the standard, the potency ratio was again affected, with the comparison based on two concentrations now apparently an underestimate. These results show that the logistic fit using four concentrations of agonist gives less accurate estimates of potency than does the use of two concentrations and the assumption that response is linearly related to log dose.

Discussion

If the responses with the highest and lowest concentrations of carbachol in the experiments with four concentrations are ignored, an estimate of dose-ratio or potency ratio can be made in exactly the same way as in the experiments with two concentrations of agonist, assuming a linear relationship between response and log dose. These (marked 3 in Table 1) are very similar to the values obtained from the logistic fit of the results with all four concentrations (marked 2 in Table 1). It appears then that, in these experiments, the error arises from the inclusion of the high and low doses in the experimental design rather than from the way in which the results are treated mathematically. The use of concentrations of agonist producing a wide range of responses produces greater changes in sensitivity than when they are confined to the middle range.

In tests with an antagonist the difficulty of ensuring that recovery from its effects is complete usually makes it necessary to obtain separate sets of responses, as in this work, rather than to obtain interspersed responses as is usual in bioassay. In the latter situation the effects of changes in sensitivity should balance out, so the potency ratios obtained with a logistic fit of a wide range of responses should be less inaccurate than found here. With a tissue like the guinea-pig ileum, however, whose sensitivity is variable there would not appear to be any advantage in using a line-fitting procedure with a wide range of responses, even though such a procedure has been shown to be more accurate in other situations.

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References

ABRAMSON, F.B., BARLOW, R.B., FRANKS, FIONA M. & PEARSON, J.D.M. (1974). Relationships between chemical structure and affinity for postganglionic acetylcholine receptors of the guinea-pig ileum. *Br. J. Pharmac.*, **51**, 81-93.

ABRAMSON, F.B., BARLOW, R.B., MUSTAFA, M.G. & STEPHENSON, R.P. (1969). Relationships between chemical structure and affinity for acetylcholine receptors. *Br. J. Pharmac.*, **37**, 207-233.

BARLOW, R.B., SCOTT, N.C. & STEPHENSON, R.P. (1967). The affinity and efficacy of onium salts on the frog rectus abdominis. *Br. J. Pharmac.*, **31**, 188-196.

COLQUHOUN, D. (1971). In: *Lectures on Biostatistics.* pp. 257-272. Oxford: Clarendon Press.

EDINBURGH STAFF (1970). In: *Pharmacological Experiments on Isolated Preparations.* 2nd edition, pp. 26-28, 58-62. Edinburgh: Livingstone.

LINEWEAVER, H. & BURK, D. (1934). Determination of enzyme dissociation constants. *J. Amer. Chem. Soc.*, **56**, 658-666.

PARKER, R.B. & WAUD, D.R. (1971). Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. *J. Pharmac. exp. Ther.*, **177**, 1-12.

WAUD, D.R. & PARKER, R.B. (1971). Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. II. Competitive antagonists. *J. Pharmac. exp. Ther.*, **177**, 13-24.

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