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ANALYSIS OF APPARENT CO-OPERATIVITY IN THE CATECHOLAMINE-STIMULATED LIPOLYSIS OF RAT ADIPOSE TISSUE

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Abstract—(1) The methods available for assessment of the regulatory features of dose–response relationships that do not conform to the Hill equation are considered, and the sensitisation index S_i introduced. (2) The value of determining S_i is assessed using lipolytic dose–response relationships yielded by β-adrenergic agonists both alone and in the presence of their specific antagonists [D. M. F. Cooper and J. I. Davies, *Biochem. Pharmac.* **31**, 721 (1982)]. (3) It is shown that where the Hill equation is fitted to relationships between L-noradrenaline concentration and lipolysis, the high value obtained for the Hill coefficient (1.69) is artefactual. (4) By fitting a rational quadratic model and evaluating S_i, a solution is obtained ($S_{i(max)}$, 1.02) which is free of this artefact. (5) The relationship between the results obtained by analysis of dose–response curves and direct binding of catecholamine analogues to membrane receptors is discussed.

Co-operative activity transitions in response to minor alternations in the concentrations of effector molecules are a central behavioural feature of regulatory enzymes. Consequently a number of diagnostic tests for the detection of such co-operative behaviour have been described (for review, see [1]). They range from those that are purely descriptive and independent of kinetic models (e.g. activity ratio) to those that are based on parameters of a specific kinetic model (e.g. Hill coefficient) and which are therefore applicable only where the particular model appears to be valid.

The multiple enzyme events initiated by hormones in their target cells lead to dose–response relationships that are in kinetic terms more complex than those observed with individual enzymes. It follows therefore that in analysing the kinetics of hormone action there are circumstances under which a wider range of kinetic models will warrant consideration. In dealing with such situations the sensitisation index S_i introduced by Davies and Williams [2] which can be applied either empirically or non-empirically for both the detection and quantification of apparent co-operativity has been found to be very useful. In this paper it is used to analyse the relationship between the concentrations of catecholamine analogues (L-noradrenaline, propranolol) and the lipolytic response elicited in isolated fat cells, and to evaluate the various models applied elsewhere to these data [3].

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MATERIALS AND METHODS

Methods of data analysis

Selection and sub-classification of dose–response curves. Where an objective selection from among sets of dose–response curves was required, a variety of statistics were used. They included ΣCC and $K_{0.5}$, respectively the sum of the correlation coefficients that relate one curve to all other curves, and the hormone concentration that yields a half-maximal response, which were determined as described elsewhere [4]. In each instance, curve selection depended on whether or not the statistic used lay within certain specified limits.

An alternative approach to the elimination of complete dose–response curves was to exclude individual data values that were aberrant. These were detected by transforming each dose–response curve so that its mean value was 0 and its standard deviation was 1 (thus eliminating differences of scale among the dose–response curves), and then comparing each data point with the corresponding mean value derived from all of the relationships, using the programme Vector, described elsewhere [4]. Individual response values were eliminated from consideration if their deviations from the mean value exceeded specified limits.

The method used for the sub-classification of dose–response curves is described elsewhere [4].

No selection or sub-classification of dose–response curves was used to obtain the results presented except where specified.

Models. The analysis of dose–response curves was based on the following models in which (H) and (R) respresent the concentrations of two catecholamine

analogues and a, b, c, d, e, p, q, A, B, C, D, E, R and U are constants:

Model I

$$L = \frac{b[H] + c}{e[H] + 1}$$

Model II

$$L = \frac{b[H]^p + c}{e[H]^p + 1}$$

Model III

$$L = \frac{a[H]^2 + b[H] + c}{d[H]^2 + e[H] + 1}$$

Model II-II

$$L = \frac{b[H]^p + c + B[h]^q}{e[H]^p + 1 + E[h]^q}$$

Model III-III

$$L = \frac{a[H]^2 + (b + R[h])\,[H] + c + B[h] + A[h]^2}{d[H]^2 + (e + U[h])\,[H] + 1 + E[h] + D[h]^2}$$

Further details of these functions are given elsewhere [3].

Optimisation using the models I, II, III, II-II *and* III-III. Families of dose–response curves were subjected to the clustering transformation described elsewhere [4], and the composite relationship obtained in this way was optimised using models I, II, III, II-II and III-III [4].

The following best-fit criteria (BF) were incorporated into the optimisation programmes as indicated in the text (cf. [3]):

$$\mathrm{BF}_{(1)}, \Sigma(1_i - L_i)^2 = \text{minimum},$$

$$\mathrm{BF}_{(2)}, \Sigma(1_i - L_i)^2/L = \text{minimum},$$

$$\mathrm{BF}_{(3)}, \Sigma(1_i - L_i)/L)^2 = \text{minimum},$$

$$\mathrm{BF}_{(4)}, \Sigma\sqrt{(1_i - L_i)^2} = \text{minimum}.$$

Determination of the sensitisation index S_i. Where S and R represent the stimulus and response respectively $(\delta R/R)/(\delta S/S)$ is the ratio of the fractional change in the response to the fractional change in the stimulus. The limiting value of this expression $(\mathrm{d}S/S)/(\mathrm{d}R/R)$ which is defined to be the sensitisation index S_i changes over the range of S values. For any expression capable of being differentiated, S_i can be evaluated explicitly as illustrated for the following:

Model I [3]

$$L = (b[H] + c)/(e[H] + 1)$$
$$R = L - c$$
$$S = [H]$$
$$\frac{\mathrm{d}R}{\mathrm{d}S} = (b - ce)/(e[H] + 1)^2$$

Therefore

$$S_i = \frac{\mathrm{d}R}{\mathrm{d}S} \cdot \frac{S}{R} = 1/(e[H] + 1) \quad (1)$$

It follows from equation (1) that when $[H] = 0$, $S_i = 1$.

Model II [3]

$$L = (b[H]^p + c)/(e[H]^p + 1)$$
$$R = L - c$$
$$S = [H]$$
$$\frac{\mathrm{d}R}{\mathrm{d}S} = (b - ce)p[H]^{p-1}/(e[H]^p + 1)^2$$

Therefore

$$S_i = p/(e[H]^p + 1) \quad (2)$$

It follows from equation (2) that when $[H] = 0$, $S_i = p$.

Model III [3]

$$L = (a[H]^2 + b[H] + c)/(d[H]^2 + e[H] + 1)$$
$$R = L - c$$
$$S = [H]$$
$$\frac{\mathrm{d}R}{\mathrm{d}S} = \frac{(ae - bd)[H]^2 + 2(a - cd)[H] + b - ce}{(d[H]^2 + e[H] + 1)^2}$$

Therefore

$$S_i = \frac{(ae - bd([H]^2 + 2(a - cd)[H] + b - ce}{(a - cd)[H]^3 + (bd - ac)[H]^2 + (a + be - cd - ce^2)[H] + b - ce} \quad (3)$$

Dose–response relationships (generally composite curves obtained by the clustering technique) were optimised using the models I, II or III as appropriate and the parameters determined in this way used to obtain plots relating S_i to the concentration of hormone by substitution into equations (1), (2) or (3).

Computing. Graphs were plotted using the library routine PLOTN and the plotter peripheral of the ICL 4130 device located in the computing laboratory at Bangor.

Experimental

The experimental data quoted here consist of products of optimisation studies performed on dose–response relationships yielded by L-noradrenaline both alone and in combination with the β-adrenergic antagonist propranolol (1-isopropylamino-3-(1-naphthyloxy)-2-propranol) in the lipolytic system of isolated adipocytes of rat epididymal fat pads. Details of the incubation procedures, the determination of glycerol release, the 'clustering' of results to obtain a composite curve from replicated experiments and optimisation of the resulting composite dose–response relationships, are described elsewhere [3, 4].

L-Propranolol was a gift from I.C.I. Pharmaceuticals, Alderly Park, U.K.

RESULTS

The criteria for application of the clustering technique

As indicated in an adjacent paper, one of the criteria which must be fulfilled in order to justify using the clustering technique to provide composite dose–response curves is that the individual curves should be related to one another as members of a single family [4]. The experimental findings that were

Table 1. Summary of noradrenaline dose–response data used in optimisation and sensitisation analysis

Noradrenaline concentration (μM)	(1) Curves eliminated: 0		(2) Curves eliminated: 5, 32		(3) Curves eliminated: 6, 13, 14, 32		(4) Curves eliminated: 4, 5, 6, 8, 9, 10, 11, 13, 14, 15, 16, 17, 22, 32, 33, 34		(5) Curves selected: 1, 3, 18, 23, 24, 25, 26, 27, 28, 29, 30, 31		(6) Curves selected: 7, 8, 9, 12, 20, 21, 33	
	Lipolysis		Lipolysis		Lipolysis		Lipolysis		Lipolysis		Lipolysis	
	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.
0	3.81	0.27	3.73	0.28	3.47	0.23	2.94	0.27	2.88	0.24	2.57	0.20
0.0078	4.13	0.23	4.05	0.23	3.83	0.19	3.40	0.18	3.33	0.16	2.60	0.14
0.0156	4.28	0.22	4.20	0.23	4.00	0.19	3.60	0.19	3.66	0.20	2.65	0.11
0.0313	5.28	0.22	5.22	0.23	5.08	0.22	4.80	0.29	5.12	0.35	3.07	0.20
0.0625	5.92	0.29	5.70	0.23	5.59	0.23	5.12	0.24	5.20	0.27	3.05	0.14
0.125	8.41	0.37	8.47	0.30	8.50	0.42	8.75	0.59	9.20	0.57	4.25	0.29
0.25	12.57	0.50	12.50	0.52	12.87	0.49	13.52	0.57	12.40	0.58	8.04	0.52
0.50	16.28	0.63	16.59	0.63	16.68	0.58	17.34	0.37	16.47	0.29	10.82	0.36
1.00	18.55	0.61	19.01	0.55	18.91	0.50	19.00	0.39	16.41	0.29	15.81	0.36
2.00	18.23	0.55	17.99	0.55	18.52	0.56	18.92	0.41	17.02	0.34	14.46	0.55
Model III solutions												
a	91.68		47.54		116.1		201.0		151.5		7.069	
b	38.51		39.45		38.78		37.22		52.36		16.00	
c	3.838		3.733		3.525		3.076		2.978		2.416	
d	5.442		3.242		6.686		11.05		9.570		1.025	
e	0.8510		0.5744		0.7861		0.6931		1.617		−0.3894	
$BF_{(2)}$	0.03950		0.05744		0.06541		0.1289		0.2672		0.2838	

Composite dose–response curves were obtained by averaging clustered data (for method, see [4]) from (1) 34 determinations of the relationship between the indicated concentrations of L-noradrenaline and lipolysis that are detailed elsewhere [4]. The composite relationships obtained after eliminating certain curves are also presented; (2) 32 curves with $K_{0.5}$ values in the range 0.240–0.654 μM; (3) 30 curves with ΣCC values (see Methods of data analysis) that exceed 25; (4) 18 curves with ΣCC values that exceed 30. The two further sets of data, (5) and (6), were obtained by elimination of 22 and 27 curves, respectively, using a stratification detection technique (see Methods of data analysis).

The composite curves were fitted using the rational quadratic function (model III) with the programme MINIM(A). The values of the model parameters and of the best-fit criteria $BF_{(2)}$ are shown.

Lipolysis is quoted in units of μmole glycerol/g/hr.

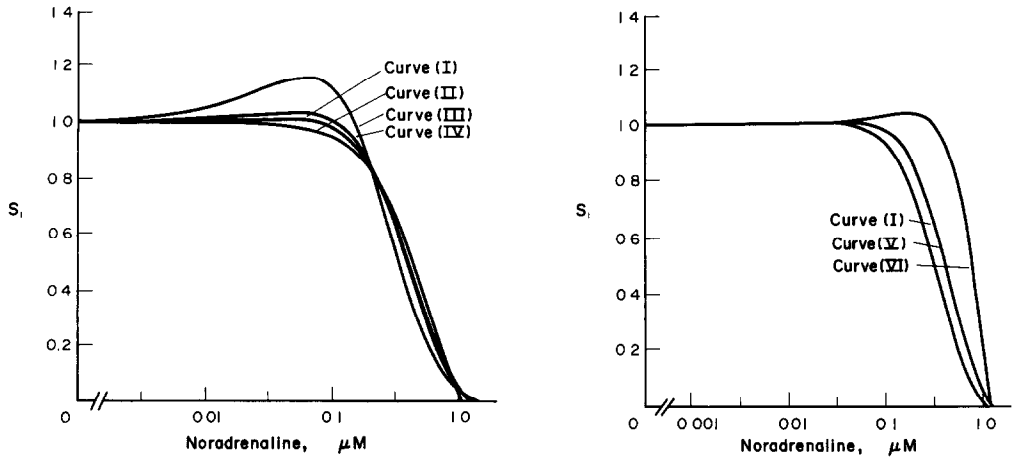

Fig. 1. The relationship between the sensitisation index (S_i) and noradrenaline concentration derived from variously selected dose–response curves. The clustered dose–response data were fitted to model III as described in Table 1. The parameters of model III were then used to calculate S_i values as described in Methods of data analysis. The data utilised include the various selections described in Table 1: (I) all 34 curves, (II) 32 curves with $K_{0.5}$ values in the range 0.240–0.654 μM, (III) 30 curves with ΣCC values that exceed 25, (IV) 18 curves with ΣCC values that exceed 30; (V) and (VI) are sets of data selected by a curve stratification detection technique (see Methods of data analysis).

presented as evidence that this criterion was fulfilled by the lipolytic dose–response curves elicited by L-noradrenaline are further supported by studies involving the evaluation of S_i.

It is established that artefacts can be introduced when individual dose–response curves that are displaced with respect to one another along the hormone-concentration axis are combined to provide a composite relationship [5]. The extent to which this form of distortion existed in a composite relationship obtained from thirty-four noradrenaline-derived dose–response curves by clustering (as described elsewhere [4]) was assessed by comparing the S_i plots obtained from composite curves derived from the thirty-four curves and various selections from these curves (Table 1). The selections (1)–(4) were based on the statistics $K_{0.5}$ and ΣCC (see Methods of data analysis): from the total curves, they yielded families of 18–32 curves. All of the curve sets were then optimised using model III (which was shown elsewhere to provide a suitable function for the purpose [3]) and the parameters determined in this way used to obtain the S_i plots shown in Fig. 1. It can be seen that the composite curves obtained from these selections by clustering share the regulatory properties of that derived from the complete set of dose–response curves even though the S_i values of 1.03 in the latter rises to 1.10 in the 18-curve selection.

In the most discriminatory analysis undertaken, the curve selection used was based on a curve classification method (see Methods of data analysis) which was designed to detect stratification within families of dose–response curves. Although no such stratification was detected, a slight degree of affinity was detected within two groups of curves consisting

Table 2. Comparison of the optimisations achieved by various models with a progressive exclusion of data in the high-dose range to the lipolytic response evoked by noradrenaline

Model	Number of data pairs	Coefficients a	b	c	d	e	n	Derived terms $(a - cd)e$	$(b - ce)d$	$(b - cd)e$	$BF_{(1)}$
Model I	10		83.43	3.30		3.89					4.98
	9		67.98	3.49		2.77					1.56
	8		55.62	3.65		1.82					0.65
	7		38.57	3.83		0.28					0.15
Model II	10		268.2	4.16		14.11	1.69				0.9554
	9		165.4	4.03		8.11	1.46				0.3201
	8		137.3	3.98		6.43	1.39				0.3004
	7		35.3	3.81		0.117	0.974				0.1477
Model III	10	84.04	39.83	3.817	5.033	0.908		59.0	18.3	33.0	0.2928
	9	229.8	36.70	3.891	11.71	1.890		348	34.4	55.5	0.2064
	8	−48.54	30.69	3.840	0.675	−1.471		75.2	24.5	−53.5	0.1474
	7	−14.19	34.77	3.840	0.713	−0.474		8.02	26.1	−17.3	0.1477

The clustered dose–response data for L-noradrenaline described in Table 1(1) were fitted using models I, II and III by applying the programme MINIM(A) and the quality of fit criterion $BF_{(1)}$ (cf. [3]). The number of data points utilised in the optimisation was decreased progressively from 10 to 7. For model III, the terms $(a - cd)e$, $(b - ce)e$ were calculated from the fitted parameters.

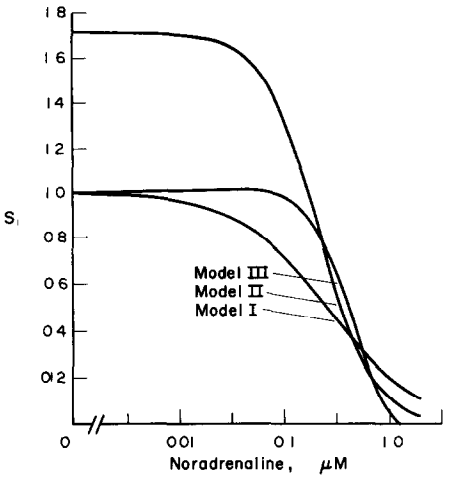

Fig. 2. Comparison of the sensitisation index (S_i) plots obtained from various models of the relationship between noradrenaline concentration and lipolytic activity. The clustered dose–response data for noradrenaline described in Table 1 was fitted using models I, II and III, with the programme MINIM(A) and the best fit criterion $BF_{(1)}$. The details of the various solutions are in Table 2. From the various parameters, the sensitisation index was calculated for each model in the indicated noradrenaline concentration range as described in Methods of data analysis.

of 7 and 12 individual relationships (Table 1, selections (5) and (6)). The difference between these two groups (which appear to represent the two opposite extremes among the 34-curve set) are apparent in their corresponding S_i plots (Fig. 1). Nevertheless, the maximum values of S_i (1.03 and 1.12 for the 7- and 12-curve clusters, respectively) are comparable with that obtained for the 34-curve cluster (1.02).

S_i *curves derived from the product of optimisation studies on noradrenaline-induced dose–response curves: their implications for the regulatory characteristics of the system*

Table 2 summarises the results of optimisation studies using models I, II and III (see Methods of data analysis) on the clustered composite relationship shown in Table 1 (data set (1)) which was derived from thirty-four noradrenaline dose–response curves [4]. The values of certain terms calculated from the parameters of model III are also shown. Of these $(a - cd)$ and $(b - ce)$ correspond to the parameters i and j respectively of the form of the rational quadratic model analysed by Ferdinand [6]. He showed that for model III to exhibit both positive co-operativity and the auto-inhibitory feature, a hook effect, the following condition must be fulfilled:

$$(a - cd)e < (b - ce)d; (a - cd)e > (b - ce)e$$

By substituting the parameter values relating to the complete dose–response relationship quoted in Table 2 into equations (1), (2) and (3) (in Methods of data analysis), the value of S_i corresponding to models I, II and III respectively can be plotted against noradrenaline concentration as shown in Fig. 2. The S_i value derived from model I necessarily approaches unity at low noradrenaline concentration, from which it declines as the hormone concentration is increased. As can be seen from equation (2), the value of S_i at low concentrations approaches p, the Hill coefficient. The value of p indicated in Fig. 1 is therefore 1.69. When the noradrenaline concentration is low, the S_i value implied by the product of optimisation using model III approximates 1.0, but as the hormone concentration increases, the value of S_i rises to a maximum of 1.1 before declining steeply.

It could be shown that the discrepancy between the degree of co-operativity implied in the analyses based on models II and III was due to the inadequacy of the former function in accommodating the experimental dose–response relationship. The parameters of the two models were re-determined using the same experimental dose–response curve as for Fig. 1, but with the data points corresponding to the highest noradrenaline concentration successively excluded (see Table 2). The implications of these parameter values for S_i were then determined by

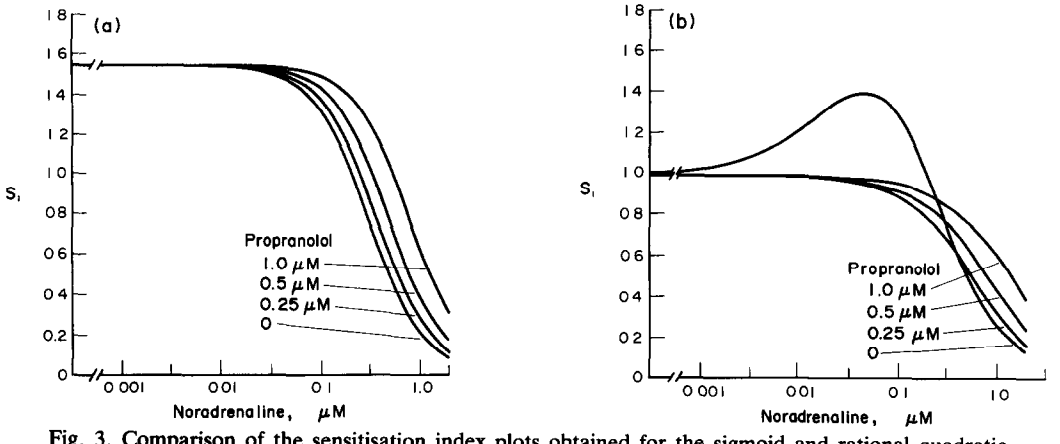

Fig. 3. Comparison of the sensitisation index plots obtained for the sigmoid and rational quadratic models of the relationship between noradrenaline concentration and lipolytic activity obtained in the presence of various concentrations of propranolol. The clustered dose–response data relating L-noradrenaline concentration to lipolysis in the presence of 0, 0.25, 0.5, and 1.0 μM L-propranolol were fitted using models II-II (a) and III-III (b) with the programme MINIM(A) and the best-fit criteria $BF_{(1)}$ as detailed elsewhere (see [3]). From the fitted parameters, the values of S_i were calculated as described in Methods of data analysis.

plotting (cf. Fig. 1). The parameters of model III yield values of $S_{i(max)}$ that remain relatively stable (in the range 1.00–1.11) regardless of the number of data points utilised, even though certain of the model parameters vary considerably. By contrast, however, in the case of model II, the S_i values at low hormone concentrations (which reflect the Hill coefficients) declined from 1.69 to 0.97 as the number of data points is reduced.

The results imply that the degree of co-operativity in the relationship between noradrenaline concentration and the lipolytic response is slight and that the contrary view suggested by the results obtained in the study using the Hill equation is an artefact caused by the application of an inappropriate model.

S_i *curves showing the effect of propranolol on the dose–response curves elicited by noradrenaline*

Elsewhere composite dose–response curves were obtained by clustering replicated determinations of the effect of various concentrations of the β-adrenergic antagonist propranolol on the lipolytic response of fat cells to L-noradrenaline [3]. Optimisation studies using the sigmoid and rational quadratic functions (models II-II and III-III) yielded solutions [3], which were used to obtain the S_i curves shown in Figs. 3(a) and (b), respectively. In these instances, where the autoinhibitory feature of the dose–response relationships is not apparent, both analyses lead to rather similar conclusions. Model II-II yielded an $S_{i(max)}$ of 1.54 (equal to p, as yielded by optimisation, [3]). Although the solution obtained using model III-III can be criticised because a large number of model parameters are evaluated using a limited range of experimental data (see [3]), the S_i analysis shows that the effect of the β-adrenergic antagonist is not necessarily limited to displacing the range of noradrenaline concentrations to which the lipolytic system is responsive.

DISCUSSION

At present virtually the only routinely evaluated parameter of the dose–response relationships elicited by hormones and drugs which provides any insight into the regulatory properties of the interaction of these substances in their target cells is the Hill coefficient. Clearly, the severely limited information that this single parameter can convey, cannot be obtained in situations where the Hill equation is not applicable. These circumstances are encountered in the analysis of the lipolytic response to catecholamine agonists, where bell-shaped titrations are generally observed [3]. In this situation, the sensitization index S_i, the definition of which is analogous to that of the 'sensitivity coefficient' and 'intrinsic sensitivity' introduced by Kacser and Burns [7] and Crabtree and Newsholme [8], respectively, can be directly evaluated from experimental dose–response data plotted over the entire effective hormone concentration range. From plots of the instantaneous value of S_i versus the concentration of ligand, the range over which the latter is critical in dictating the magnitude of the response can be precisely specified.

The elucidation of dose–response relationships reported in the present paper is based on the analysis, not of the experimental curves themselves, but of kinetic models derived from these curves. By comparing the S_i plots based on thirty-four dose–response curves obtained with L-noradrenaline alone and various selections from these curves, confirmation was obtained that the clustering technique was applicable in this situation (cf. [4]). Again, evidence was presented elsewhere [3] that, in expressing the relationship between the concentrations of catecholamine derivatives and the lipolytic response, the rational quadratic function (model III) is superior to the Hill function (model II). This is confirmed here in the analysis of the dose–response relationship obtained with L-noradrenaline, which shows that the value obtained for the Hill coefficient is grossly distorted by the bell-shaped feature elicited by high concentrations of the hormone. On elimination of the data points that reveal the hook effect, the Hill coefficient and maximum value of S_i decline from 1.69 to 0.97. This is the value taken by the latter when model III is applied, irrespective of the number of data points considered.

The lack of appreciable co-operativity in the dose–response curves is of some interest in relation to recent efforts to determine directly how the concentration of dihydroalprenolol influences the binding of this noradrenaline analogue to fat cell membrane preparations. By analysing their results in the context of the Hill equation, coefficients n_H of 0.65 and 0.70 were obtained by Williams *et al.* [9] and Malbon *et al.* [10] respectively. Although these findings were initially regarded as indicating negatively co-operative binding [9], it has been shown more recently that alternative explanations such as the existence of multiple binding-site populations are more probable [11]. It is conceivable, therefore, that the experimental findings derived from dose–response data relate specifically to just one of these receptors which is functionally linked to the lipolytic system.

Although it has been shown that analysis of dose–responsive relationships elicited by hormones is capable of providing relatively unambiguous information about the binding of these substances and their receptors, there are a number of ways in which the discrepancy between the degree of co-operativity observed in these studies of alprenol binding and of noradrenaline-stimulated lipolysis can be reconciled. For instance, the form taken by the latter is influenced by reactions of the lipolytic cascade other than the hormone-receptor interaction. Some reservations also derive from the interactions between noradrenaline and the serumalbumin of the fat cell incubation media. The protein appears to be partly responsible for the stability of noradrenaline demonstrated in these incubation media (D. M. F. Cooper and J. I. Davies, unpublished; cf. [12]). However, it also leads to a discrepancy between 'free' and 'total' noradrenaline concentrations in incubation media because of its binding capacity for the hormone [12, 13].

The artefacts that can be introduced by analysing composite dose–response relationships obtained by averaging individual relationships which are displaced with respect to one another along the dose-axis [5] would be expected to lower the value of the

Hill coefficient n_H rather than to raise it. The criticism that such an effect influences the value obtained in the present study has been largely met by analysing the composite curves obtained from various selections from among the dose–response curves elicited by noradrenaline. Figure 1 shows that when analysed these selected curves also showed a minimal degree of co-operativity.

The conclusion drawn from these experiments contrast with those of Rodbard [14] who analysed the relationship between steroidogenesis in the adrenal cortex and the concentration of adrenocorticotropin in *in vitro* incubations. This analysis, which was based entirely on the Hill equation, indicated that there was considerable positive co-operativity (n_H 1.41). However, the interpretation given to these results has since been challenged on the grounds that the extent of ACTH degradation in the system was correlated with the degree of apparent co-operativity [15], as indicated elsewhere, it is unlikely that such hormone degradation is a significant factor in the noradrenaline-stimulated adipocyte system [3].

Throughout the present study, the apparent co-operativity of noradrenaline dose–response curves was higher when the analysis was based on the sigmoid rather than the rational quadratic models. This was apparent in a study of dose–response curves obtained with noradrenaline in the presence of various concentrations of propranolol. Whereas the model II-II fitting yielded a p value of 1.54 (see [3]) which implies that $S_{i(max)}$ is also 1.54 (see Methods of data analysis), the $S_{i(max)}$ values derived from the model III-III vary in the range 1.00–1.43 (Fig 3).

We conclude from our study that the knowledge that exists of the regulatory properties of the hormone—and drug-induced dose–response relationships does not begin to match their practical and theoretical importance. Our aim has been to deal with the practical problems which are partly responsible for this situation. The virtue of the S_i statistic is that it is easy both to apply and to interpret. Indeed the maximum value of S_i has considerable affinity with the Hill coefficient n_H in that values greater, equal or less than unity have essentially the same significance. Although it has been used in connection with only one hormone-responsive system, it promises to be capable of far wider application.

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