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A KINETIC ANALYSIS OF THE ACTIONS OF L-NORADRENALINE AND ITS RELATED AGONISTS AND ANTAGONISTS ON *IN VITRO* LIPOLYSIS IN RAT ADIPOSE TISSUE

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Abstract—(1) Iterative non-linearising optimisation techniques have been used to fit three alternative models to relationships between β-adrenergic effector concentrations and the lipolytic response which they elicit in dispersed adipocytes derived from rat epididymal fat pads. (2) The models, which consist of a simple hyperbolic relationship, a Hill-type function and a rational quadratic formulation, were fitted to data obtained with agonists, 'partial' agonists and antagonists both alone and in combination. (3) Whereas the hyperbolic relationship was inadequate in all circumstances, the Hill-type function accommodated dose–response curves which exhibit no 'auto-inhibitory' hook or bell-shaped feature. However, the rational quadratic function could be satisfactorily fitted to the data whether or not the auto-inhibitory phase was apparent. (4) The mechanisms that govern the steepness of the dose–response relationships and their bell-shaped feature are discussed. Evidence is presented that the latter originates at the level of adenylate cyclase.

The response of the lipolytic system of adipose tissue to hormones has been intensively investigated [1–4], largely because the non-esterified fatty acid formed is a significant factor in many physiological and pathological conditions. Two deviations from the classical view of how hormone concentration is related to the evoked response have been widely observed to occur in this system. Firstly, the dose–response curve is biphasic: a bell-shaped or hooked relationship is obtained as the stimulatory phase gives way to an inhibitory phase at high hormone concentrations [5]. Secondly, the stimulatory phase has been considered to be nonhyperbolically related to the concentration of hormone present [6, 7].

In many hormone-responsive systems, models based on the Hill equation [8] which can accommodate sigmoidal dose–response relationships, have been considered useful functions on which to base optimisation studies [9–11]. However, Wenke and co-workers [6] preferred to use the empirical equation (1) to describe the action of the catecholamines on lipolysis:

$$L = \frac{a[H]^2 + c}{d[H]^2 + 1} \quad (1)$$

where $[H]$ and L represent the hormone concentration and the rate of lipolysis respectively, and b, d and e are constants.

In the previous paper, methods are described for increasing the definition of lipolytic dose–response relationships obtained in isolated fat cells so that they are rendered more amenable to optimisation [12]. In the present paper an evaluation is made of three different models in accommodating the co-operative and bell-shape features of the dose–response curves obtained with β-adrenergic agonists, antagonists and partial agonists both alone and in combination.

The form of the three models applied to agents acting alone were as follows.

Model I.

$$L = \frac{b[H] + c}{e[H] + 1} \quad (2)$$

This model has been traditionally used to accommodate simple 'saturating' relationships [13, 14]. It is also the form taken by the relationship between lipolysis and hormone concentration which was derived by the authors and their collaborators [15, 16] from a simple treatment of the kinetics of the enzymes of the lipolytic cascade.

Model II.

$$L = \frac{b[H]^p + c}{e[H]^p + 1} \quad (3)$$

where p is the power factor discussed by Parker and Waud [9]. As pointed out earlier, such forms of the Hill equation have been widely used in kinetic analysis where dose–response relationships are too steep to be accommodated by hyperbolic models such as model I. This model was applied to the lipolytic system of adipose tissue by Cooper *et al.* [17].

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Model III.

$$L = \frac{a[H]^2 + b[H] + c}{d[H]^2 + e[H] + 1} \quad (4)$$

Few attempts have been made in this field to accommodate hooked or bell-shaped dose–response curves. In enzymology, a variety of mechanisms proposed to account for such effector-activity profiles led to kinetic equations of the form represented by model III [18, 19].

Models based on the kinetics of enzymes of the lipolytic cascade, in which multiple binding of hormone–receptor complexes to adenylate cyclase is postulated, also take the form of model III [20]. Furthermore, Wenke and his associates [6] have used empirical models which are equivalent to model III, where $b = e = 0$ [see equation (1)].

The forms of the models applied to agents acting in combination were as follows:

Model I–I. Assuming that the kinetic expressions describing the response to two agents (H and h) acting alone is:

$$L = \frac{b[H] + c}{e[H] + 1} \qquad L = \frac{B[h] + C}{E[h] + 1} \quad (5)$$

then the simplest plausible composite expression is:

$$L = \frac{b[H] + c + B[h]}{e[H] + 1 + E[h]} \quad (6)$$

Thus, where $[H] = 0$ or $[h] = 0$, function (6) reverts to one of the equations (5). Expressions of a similar form are used in enzymology where interactions between substrates and other effectors are dealt with [21]. It also has the form taken by models based on the kinetics of the enzymes of the lipolytic cascade where two hormones compete for the same receptor associated with adenylate cyclase [20].

Model II–II. A similar argument to that used in relation to model I–I yields the following equation:

$$L = \frac{b[H]^p + c + B[h]^q}{e[H]^p + 1 + E[h]^q} \quad (7)$$

Loftfield and Eigner [22] proposed similar expressions as a basis for investigating the mode of interaction between substrate and non-substrate effectors in biological processes.

Model III–III. Where the actions of two agents (H and h) may be expressed as:

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

$$L = \frac{A[h]^2 + B[h] + c}{D[h]^2 + E[h] + 1} \quad (8)$$

then the simplest compatible composite expression for the interaction of the two agents is:

$$L = \frac{a[H]^2 + b[H] + c + B[h] + A[h]^2}{d[H]^2 + e[H] + 1 + E[h] + D[h]^2} \quad (9)$$

Davies and Williams [20], however, derived slightly more complex expressions based on a simple kinetic treatment of the enzymes of the lipolytic cascase where two hormones are considered to compete for the same receptor:

$$L = \frac{a[H]^2 + (b + R[h])[H] + c + B[h] + A[h]^2}{d[H]^2 + (e + U[h])[H] + 1 + E[h] + D[h]^2} \quad (10)$$

MATERIALS AND METHODS

Data clustering. Experimental dose–response relationships were subjected to the 'clustering' transformation described elsewhere [12] which provided the composite curves used in optimisation studies.

As indicated elsewhere [12], the scale of the clustered curves may not be representative of the dose–response relationships from which they are derived. Nevertheless, the regulatory features of the original curves remain intact. In order to compare directly dose–response relationships due to different effectors or combinations of effectors which were determined within the same experimental blocks (see Experimental), they were considered to comprise a single relationship for the purpose of clustering. This approach was used to obtain the dose–response curves for D- and L-phenylephrine, and the noradrenaline–dichloroisoproterenol and noradrenaline–propranolol combinations.

Parameter estimation for optimisation studies. The model-fitting procedures requires estimates of the parameters which were to be optimised.

Model I (equation 2). Estimates of the parameters b, c and e were calculated from observed values of BASL, MAXL and HMXH, which represent respectively the basal and maximal values taken by the lipolytic rate (L), and the $[H]$ value at which the increase in L above basal is half-maximal [i.e. (MAXL + BASL)/2]. c = BASL; e = 1/HMXH; and $b = e$.MAXL.

Model II (equation 3). c = BASL; $e = 1/(\text{HMXH})^p$; $b = e$.MAXL; and $p = 1.0$ or $p = 1.5$.

Model III (equation 4). The rational quadratic function may take one of several forms depending on the relative values of the parameters a, b, c, d and e. Continuous functions are obtained when the denominator ($d[H]^2 + e[H] + 1$) is positive at all positive values of $[H]$. These occur when $d > 0$ and $e > 0$ or when $d > 0$, $e < 0$ and $e^2 < 4d$. By contrast, discontinuous functions are encountered when the denominator is negative at some positive value of $[H]$. They occur therefore when $d < 0$ or when $d > 0$, $e < 0$ and $e^2 > 4d$.

Since in practice lipolysis is measureable over only a limited range of $[H]$ values, the dose–response relationships can be described by a number of the forms taken by the rational quadratic function, even though extrapolation of the latter would show many to be theoretically unrealistic. In these circumstances the model used as a basis for estimating the terms a, b, c, d and e can be crucial.

Generally, dose–response curves were considered to comprise a segment of a bell- or hook-shaped relationship in which a phase of positive response, after passing through a maximum (MAXL), enters a second phase in which lipolysis declines to an asymptotic value (ASML) at high concentrations of hormone. Furthermore, it was assumed that this asymptotic value of L is governed by the ratio of the

quadratic terms a/d and that the ratio b/e is a major influence on the maximum value taken by L. On this basis, crude estimates of the terms a, b, c, d and e were routinely obtained from values of ENDL, MAXL, ASML, MAXH and HMXH as follows: c = ENDL; in the lower range of $[H]$ values, $L \doteqdot (b[H] + c)/(e[H] + 1)$, and therefore $e \doteqdot 1/\text{HMXH}$ and $b \doteqdot e(\text{MAXL-ENDL})$. Since, where L = MAXL, $dL/d[H] = 0$, a and d can be evaluated:

$$ae[H]^2 + 2e[H] - bd[H]^2 + b - 2d[H] - e = 0$$

$$d = \frac{(ce - b)}{\text{MAXH}^2(\text{ASML} \,.\, e - b) + 2\text{MAXH}(\text{ASML} - c)}$$

$$a = \text{ASML} \,.\, d$$

Where evaluation of ASML required extrapolation beyond the experimental points, a number of alternative values were used to provide multiple estimates of a *and* d.

This method of parameter estimation makes precise assumptions concerning the contribution of each parameter to the form of the dose–response curve. As a result, the outcome of optimisation studies is prejudiced in favour of a particular form of the model, where alternative solutions may also exist. Therefore, in certain circumstances, especially where the priority was to describe dose–response relationships rather than to deduce the mechanisms involved, additional sets of parameter estimates were generated so as to reveal other minima, both continuous and discontinuous.

For agents acting in combination, estimates of the parameters were obtained as follows:

Model I–I (equation 6). This model was not employed where a hyperbolic function clearly could not describe the dose–response curve elicited by either one or both of the effectors when used alone (e.g. grossly bell-shaped relationships). Otherwise, these dose–response curves were generally used to provide parameter estimates as described for model I. The same procedure was used where the effector alone had no appreciable effect on basal lipolysis (e.g. propranolol), except that published K_D values were used to estimate HMXH.

Model II–II (equation 7). The parameters of this model were estimated in a manner analogous to that described for the model I–I, except that p and q were each empirically allocated values of 1.0 and 1.5.

Model III–III (equations 9 and 10). The procedures used to estimate the parameters of model I–I were also used to obtain those required for the model III–III. Routinely the parameters R and U (equation 10) were empirically evaluated as $R = U = 0$; but the effect of allocating alternative estimates was also assessed.

Model-fitting programmes. The various models were fitted to dose–response data using an iterative minimisation technique based on the method of Nelder and Mead [23]. It was found to be more readily applicable than an alternative routine based on the method of Peckham [24].

The discontinuous and therefore mechanistically unrealistic solutions to the rational quadratic model that were occasionally obtained could be avoided frequently by ensuring that the dose–response relationship (i.e. $[H]$ and L) was expressed in units such that the parameter estimates were all of a similar order of magnitude (this precaution also greatly increased the efficiency of the minimisation). Occasionally, the model was optimised using parameter estimates of high quality obtained in a preliminary application of the minimisation programme to experimental results supplemented (and sometimes extended) with simulated dose–response data.

The best-fit criteria. The four measures of the quality of model-fit quoted by Reich [25] were all used. Where 1_i is the response value elicited by one of the n hormone concentrations used and L_i is the solution of the function to be minimised at the same hormone concentration, then the best-fit criteria (referred to collectively as BF) were:

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

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

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

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

Iteration ceased when the convergence criteria of the minimisation technique was satisfied (BF = BF_{min}).

Perturbation analysis. On completion of the optimisation exercises, the solutions obtained were assessed by successively applying perturbations of +5% and −5% to each of the optimised parameters. The criterion used to determine the quality of model-fitting (BF) in the optimisation was then redetermined (BF*) in each instance and compared with BF_{min} as follows:

$$\text{PA} = 100\left(\frac{\text{BF}^*}{\text{BF}_{\text{min}}} - 1\right)$$

The outcome of perturbation analysis, which consists of two terms per model parameter is presented in the form:

$$\begin{bmatrix}\text{PA for } +5\% \text{ change in parameter value} \\ \text{PA for } -5\% \text{ change in parameter value}\end{bmatrix}$$

Approximately equal PA values for the positive and negative perturbations were taken to indicate true minima.

Computing. The programme MINIM(A) was written in ALGOL 60. It was executed on either an ICL 4130 computer (University College of Wales) or through a MOD 1 link (located at the same address) to the CDC 7600 device at the Regional Computing Centre, University of Manchester. Both computers provided access to the Nottingham Algorithms Group (NAG) library which contained the iterative minimisation routines EO4CCA and EO4FAA, based on the methods of Nelder and Mead [23] and Peckham [24], respectively.

Experimental

L-Propranolol (1-isopropylamino-3-(1-naphthoxy) propal-2-ol) was a gift from I.C.I. Pharmaceuticals Ltd., Macclesfield, U.K. D- and L-phenylephrine hydrochlorides were donated by Winthrop Labora-

Table 1. Summary of dose–response data obtained using DL-dichloroisoproterenol, D- and L-phenylephrine and L-noradrenaline

Dichloroisoproterenol (DCI)			L-Phenylephrine and D-phenylephrine						L-Noradrenaline		
No. of curves: 7 ΣCC for each curve: (1) 5.48, (2) 4.74, (3) 6.08, (4) 6.10, (5) 6.03, (6) 6.10, (7) 6.14			No. of data sets 6 ΣCC for each set: 5.49, 5.57, 5.52, 5.23, 5.45, 5.33						No. of curves. 34 ΣCC for each curve: see [12]		
			L-Isomer			D-Isomer					
	Response			Response			Response			Response	
Hormone (μM)	Mean ± S E	Clustered ± S E.	Hormone (μM)	Mean ± S.E.	Clustered ± S.E	Hormone (μM)	Mean ± S.E	Clustered ± S.E.	Hormone (μM)	Mean ± S.E.	Clustered ± S.E.
0	3.16 ± 0 52	1 54 ± 0 05	0	4 69 ± 0.13	4,82 ± 0.16	0	4.78 ± 0 24	4 92 ± 0 20	0	2.11 ± 0.13	3 81 ± 0.27
0.0076	3.22 ± 0 50	1.58 ± 0 03	3.66	6 52 ± 0.74	5 86 ± 0.15	3.66	4.56 ± 0 22	4 85 ± 0 24	0.0078	2.34 ± 0 16	4.13 ± 0 23
0.0304	3.52 ± 0.41	1 81 ± 0.13	7.32	6 66 ± 1 47	5.67 ± 0.46	7.32	4.80 ± 0.24	4 97 ± 0.20	0.0156	2.50 ± 0.20	4.28 ± 0 22
0.122	3.74 ± 0.48	1.96 ± 0.08	14 6	11.05 ± 2 23	8.47 ± 0.49	14 6	4 84 ± 0.40	4.80 ± 0.11	0.0313	3.21 ± 0.32	5.28 ± 0.22
0.487	5.69 ± 0.62	3 27 ± 0.19	29 3	14.03 ± 2 98	10.58 ± 0.67	29.3	5.02 ± 0.27	5.05 ± 0 17	0 0625	3.59 ± 0.35	5.92 ± 0 29
1.95	10.09 ± 1 48	6 05 ± 0.27	58.6	17.09 ± 3 37	12.85 ± 0.40	58.6	5.39 ± 0.10	5.53 ± 0 33	0 125	5 47 ± 0 59	8.41 ± 0.37
7.80	13.24 ± 2 09	8.45 ± 0.56	117	19.40 ± 3.70	14 33 ± 0 64	117	5 06 ± 0.15	5 69 ± 0 50	0 25	8 25 ± 0 74	12 57 ± 0.50
31.25	13.63 ± 2 10	8.62 ± 0.45	234	19.36 ± 3 58	14 33 ± 0 86	234	8.67 ± 2.12	7 89 ± 0 55	0.50	10 39 ± 0.84	16 28 ± 0 63
125	7.97 ± 1.12	4.87 ± 0.62	469	19.63 ± 3.18	15 28 ± 0 74	469	10.98 ± 2.53	9 64 ± 0 47	1.00	12 04 ± 0.89	18 55 ± 0.61
500	3.64 ± 0.52	1.91 ± 0.10	937	21.94 ± 3.88	16.42 ± 0 78	937	12 51 ± 3.46	10 59 ± 0.68	2.00	11.82 ± 0.87	18 23 ± 0.55
			1875	20.13 ± 3 84	15.04 ± 0 34	1875	13 96 ± 2 97	11.11 ± 0.91			
			3750	17.45 ± 3.06	13.38 ± 0 50	3750	11 90 ± 2.54	10 28 ± 0.30			
			7500	13.59 ± 2.69	11.53 ± 1.02	7500	8 75 ± 1.78	7.80 ± 0.28			
			15000	13 08 ± 2.32	10 16 ± 0.19	15000	4.46 ± 0.11	4.68 ± 0 19			
			30000	9.16 ± 1.05	7.90 ± 0.26	30000	4.07 ± 0.10	4.32 ± 0 16			

Dose–response data relating the lipolytic response of adipocytes evoked by the indicated concentrations of each catecholamine analogue are presented before and after clustering as described elsewhere [12]. The dose–response curves elicited by the D- and L-phenylephrines were clustered simultaneously as described in the text Experimental details are described fully in [12]. The number of curves from which the mean (±S.E.) response were calculated and the ΣCC value for each curve are quoted

Units of the mean response data, μmole glycerol/g/hr

Table 2. Optimisation studies on the relationships between the concentrations of dichloroisoproterenol, D- and L-phenylephrines and L-noradrenaline, and adipocyte lipolytic activity

	Parameters					
	a	*b*	*c*	*d*	*e*	
D,L-Dichloroisoproterenol						
Parameter values	-1.420×10^{-4}	4.684	1.581	4.160×10^{-3}	0.4026	($BF_{(1)} = 0.1710$)
Perturbation analysis	$\begin{bmatrix}1.816 \times 10^{-3}\\1.817 \times 10^{-3}\end{bmatrix}$	$\begin{bmatrix}286.1\\286.1\end{bmatrix}$	$\begin{bmatrix}17.97\\17.97\end{bmatrix}$	$\begin{bmatrix}19.99\\22.13\end{bmatrix}$	$\begin{bmatrix}117.1\\134.1\end{bmatrix}$	
Parameter values	7.966×10^{-4}	4.690	1.576	4.544×10^{-3}	0.3966	($BF_{(3)} = 9.61 \times 10^{-3}$)
Perturbation analysis	$\begin{bmatrix}0.1705\\0.1731\end{bmatrix}$	$\begin{bmatrix}115.2\\138.2\end{bmatrix}$	$\begin{bmatrix}83.86\\100.4\end{bmatrix}$	$\begin{bmatrix}29.34\\29.34\end{bmatrix}$	$\begin{bmatrix}36.04\\36.05\end{bmatrix}$	
L-Phenylephrine						
Parameter values	1.541×10^{-5}	0.5048	4.722	2.966×10^{-6}	2.962×10^{-2}	($BF_{(2)} = 0.3167$)
Perturbation analysis	$\begin{bmatrix}2.674\\2.775\end{bmatrix}$	$\begin{bmatrix}86.71\\94.71\end{bmatrix}$	$\begin{bmatrix}9.381\\10.100\end{bmatrix}$	$\begin{bmatrix}9.099\\9.641\end{bmatrix}$	$\begin{bmatrix}57.90\\62.60\end{bmatrix}$	
D-Phenylephrine						
Parameter values	5.424×10^{-7}	1.317×10^{-2}	4.597	2.513×10^{-7}	3.728×10^{-4}	($BF_{(2)} = 0.3365$)
Perturbation analysis	$\begin{bmatrix}1.806\\1.883\end{bmatrix}$	$\begin{bmatrix}22.23\\23.86\end{bmatrix}$	$\begin{bmatrix}24.56\\26.77\end{bmatrix}$	$\begin{bmatrix}12.49\\13.47\end{bmatrix}$	$\begin{bmatrix}1.819\\1.867\end{bmatrix}$	
L-Noradrenaline						
Parameter values	84.04	39.83	3.817	5.073	0.9076	($BF_{(1)} = 0.2928$)
Perturbation analysis	$\begin{bmatrix}370.7\\370.7\end{bmatrix}$	$\begin{bmatrix}141.2\\141.2\end{bmatrix}$	$\begin{bmatrix}75.14\\75.14\end{bmatrix}$	$\begin{bmatrix}396.5\\462.5\end{bmatrix}$	$\begin{bmatrix}16.50\\16.97\end{bmatrix}$	
Parameter values	91.68	38.51	3.838	5.442	0.8510	($BF_{(2)} = 3.950 \times 10^{-2}$)
Perturbation analysis	$\begin{bmatrix}160.7\\172.3\end{bmatrix}$	$\begin{bmatrix}69.32\\72.23\end{bmatrix}$	$\begin{bmatrix}105.5\\114.2\end{bmatrix}$	$\begin{bmatrix}178.5\\192.8\end{bmatrix}$	$\begin{bmatrix}6.558\\6.642\end{bmatrix}$	

The clustered dose–response data for the catecholamine analogues shown in Table 1 are fitted to the rational quadratic function

$$L = (a[H]^2 + b[H] + c)/(d[H]^2 + e[H] + 1)$$

using the optimisation procedure MINIM(A). The values of the model parameters are shown, together with the effect of perturbing each successively by 5% (see Methods) on the value of the indicated best-fit criteria $BF_{(1)}$, $BF_{(2)}$ or $BF_{(3)}$.

Table 3. The effect of simplifying model III on the solutions obtained from the dose–response data yielded by D,L-dichloroisoproterenol

Model	Coefficients a	b	c	d	e	$BF_{(1)}$
$y = \frac{ax^2 + bx + c}{dx^2 + ex + 1}$	-146×10^{-4}	4.68	1.58	0.00416	0.403	0.1718
$y = \frac{bx + c}{dx^2 + ex + 1}$		4.67	1.58	0.00419	0.401	0.1720
$y = \frac{ax^2 + c}{dx^2 + ex + 1}$	0.362		1.94	0.0767	−0.379	18.77
$y = \frac{ax^2 + bx + c}{ex + 1}$	−0.135	8.23	1.44		0.971	5.172
$y = \frac{ax^2 + bx + c}{dx^2 + 1}$	0.00949	1.16	2.11	0.00461		5.745

Model III and variants lacking one parameter were fitted to the clustered data presented in Table 1 using the best-fit criterion $BF_{(1)}$.

tories, Newcastle-upon-Tyne, U.K.; L-noradrenaline and dichloroisoproterenol hydrochloride were purchased from Sigma Chemical Co. (London, U.K.). The sources of other chemicals, the Wistar rats, and the methods of preparation and incubation of dispersed adipocytes were as described elsewhere [12]. All experiments performed were of a randomised block design: the results analysed were composite curves obtained by clustering (see earlier) individual dose–response relationships as indicated in the legends to the tables and figures.

RESULTS

The details of the dose–response relationships elicited by D,L-dichloroisoproterenol and D- and L-phenylephrine, which exhibit a grossly bell-shaped feature are shown in Table 1. Also included are the clustered data used for model optimisation.

When the rational quadratic function (model III, equation 4) was minimised using the data for D,L-dichloroisoproterenol, virtually the same solution was obtained irrespective of the best-fit criterion used (see Methods of data analysis). The result obtained using the 'least squares' criterion of fit (BF_1) is detailed in Table 2 and graphically represented in Fig. 1. The method appears to locate a well-characterised minimum as is confirmed by perturbation analysis of the model parameters.

Table 3 compares the adequacy of various simplified forms of model III in accommodating the dichloroisoproterenol dose–response curve. It appears that whereas the allocation ($a = 0$) is justified, all other parameters make a very significant contribution to the solution obtained.

The D- and L-phenylephrine dose–response curves shown in Table 1 were derived from the same experiments. Therefore, in order to avoid changing their relative dimensions during clustering, the two data sets were transformed as a single unit (see Methods of data analysis). It can be seen that the L-isomer elicits a considerably larger response. Although the half-maximal stimulatory response occurs at a lower concentration for the L- than the D-isomer (25 μM and 250 μM, respectively), their auto-inhibitory phase becomes apparent at similar concentrations ($K_{0.5}$, 10 mM approx).

When the clustered data obtained using the phenylephrines were analysed as described for dichloroisoproterenol, the consistency of the solutions to model III was found to be comparable (Table 2 and Fig. 2).

The composite dose–response relationship elicited by L-noradrenaline is shown in Table 1: further

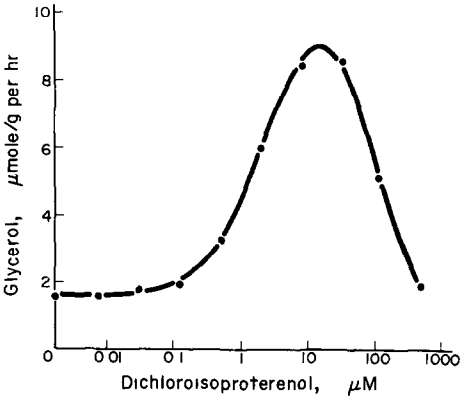

Fig. 1. Optimisation of the relationship between dichloroisoproterenol concentration and lipolytic activity using the rational quadratic function. The clustered dose–response data for dichloroisoproterenol described in Table 1 were fitted using the programme MINIM(A) and the best fit criterion $BF_{(1)}$ (see Methods of data analysis). Parameter values and other details of the solution are shown in Table 2. Clustered data points (●) and the curve generated from the fitted parameter values (——) are shown.

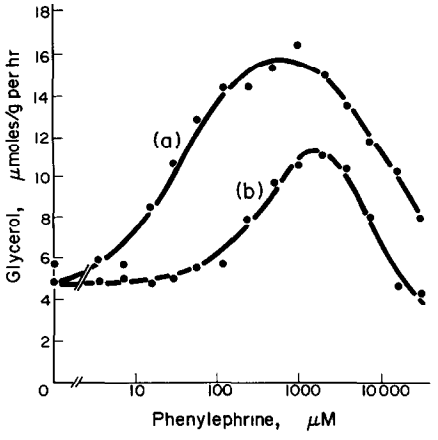

Fig. 2. Optimisation of the lipolytic response to phenylephrine stereoisomers with the rational quadratic model. The clustered dose–response data for (a) L-phenylephrine and (b) D-phenylephrine, which are detailed in Table 1, were fitted using model III with the programme MINIM(A) and the best-fit criterion, $BF_{(2)}$. The clustered data points are shown (●) together with the curve calculated from the fitted parameters. The parameter values and other details of the solution are shown in Table 2.

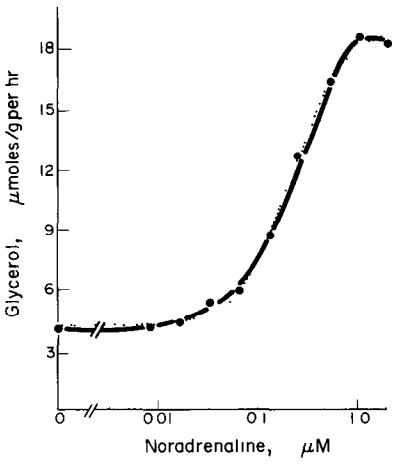

Fig. 3. Fitting models II and III to the lipolytic response evoked by L-noradrenaline. The clustered data for L-noradrenaline described in Table 1 were fitted using the programme MINIM(A) and the best-fit criterion $BF_{(2)}$ (see Methods of data analysis) with (a) model II and (b) model III. Clustered data points (●) and the curves generated from the parameter values obtained (which are detailed in Table 2) are shown: model II (....); model III (——).

details of the untransformed data are presented elsewhere [12]. Since the bell-shaped feature is only marginally evident, this dose–response curve can be regarded as less complete than those of dichloroisoproterenol and the phenylephrines. The accommodation of the clustered curve by models I and II was therefore compared with that by model III (Table 4). Whereas model I is clearly inadequate (see [17]), Fig. 3 shows that model II is comparable with model III in several respects, but distinctly inferior where the noradrenaline concentration is high and the 'autoinhibitory' hook effect is detectable. Model III is satisfactory throughout: the parameter perturbation analysis shown in Table 2 indicates that the solution represents a well characterised minimum. No alternative solutions were revealed when a wide range of parameter estimates were used to initiate the minimisation programme MINIM(A).

Table 4. Comparison of the outcome of optimisation studies on the relationship between adipocyte lipolysis and noradrenaline concentration, using models I, II and III

Term	Model I	Model II	Model III
a			91.68
b	73.30	208.6	38.51
c	3.531	4.054	3.838
d			5.442
e	3.304	10.80	0.8510
p		1.547	
ENDL	3.531	4-054	3.838
MAXL	22.19	19.32	18.48
$BF_{(2)}$	0.4212	0.1124	0.03950

The clustered dose–response data for L-noradrenaline (from Table 1) were fitted to models I, II and III using the programme MINIM(A) and the best-fit criterion $BF_{(2)}$.
Model I $L = (b[H] + c)/(e[H] + 1)$
Model II $L = (b[H]^p + c)/(e[H]^p + 1)$
Model III $L = (a[H]^2 + b[H] + c)/(d[H]^2 + e[H] + 1)$

Table 5 compares the accommodation of the noradrenaline dose–response relationship by various simplified forms of model III. Whereas allocating zero values to any one of the parameters of the model diminishes its adequacy in describing the experimental curve, a number of the simplified forms are of comparable quality to model II. One of these, in which $b = e = 0$, is essentially that used by Wenke and co-workers [6] in studies of the effects of catecholamines on adipose tissue lipolysis.

The relationship between models I, II, and III is clarified in the analysis shown in Table 6 where a comparison is made of the effect of progressively discarding data points from the noradrenaline dose–response curve from the higher-dose region on the outcome of model I, model II and model III optimisation. The limiting value of the 'least squares' criterion of model-fitting diminishes steeply for models I and II until, where seven data-points remain, the model-fitting is comparable with that of model III. In the case of the latter, the values taken by its parameters change drastically as the curve becomes less complete. However, the effect of the changes is almost entirely on the way in which the relationship extrapolates: as shown elsewhere [26], the description of the dose–response curve itself remains uniformly good.

An analysis of the effect of β-adrenergic antagonists on the lipolytic response to L-noradrenaline was undertaken: blocks of experimental results obtained in the presence of various concentrations of the β-blockers were clustered as a single unit (see Methods of data analysis). The results obtained by analysing individually the dose–response curves elicited by noradrenaline in the presence of various concentrations of propranolol (see Table 7) using

Table 5. The effect of simplifying model III on the solutions obtained from dose–response data yielded by L-noradrenaline

Model	Coefficients a	b	c	d	e	$BF_{(1)}$
$y=\frac{ax^2+bx+c}{dx^2+ex+1}$	84.04	39.83	3.817	5.033	0.908	0.293
(1)						
$y=\frac{bx+c}{dx^2+ex+1}$		55.44	3.618	0.517	1.623	0.703
$y=\frac{ax^2+c}{dx^2+ex+1}$	479.8		4.35	25.59	0.2829	1.384
$y=\frac{ax^2+bx+c}{ex+1}$	−9.20	58.56	3.58		1.81	0.870
$y=\frac{ax^2+bx+c}{dx^2+1}$	145.8	23.62	4.053	8.477		0.609
(2)						
$y=\frac{c}{dx^2+ex+1}$			6-634	0.3540	−1.023	65.61
$y=\frac{bx+c}{dx^2+1}$		29.12	4.275	0.655		6.664
$y=\frac{ax^2+c}{ex+1}$	−16.19		5.449		−1.828	76.73
$y=\frac{ax^2+c}{dx^2+1}$	540.1		4.354	23.66		1.393
$y=\frac{bx+c}{ex+1}$		83.45	3.295		3.891	4.975
$y=ax^2+bx+c$	−9.38	25.4	4.62			13.28

Forms of model III, (1) lacking one term, and (2) lacking two terms as indicated, were fitted to the clustered data described in Table 1 using the programme MINIM(A) and the best-fit criterion $BF_{(1)}$.

models II and III are shown in Table 8. It is apparent that the two models are similarly effective in accommodating the experimental curves (which do not exhibit a bell-shape feature). These optima can be usefully compared with those obtained when all four dose–response curves were fitted simultaneously using models II–II and III–III (Table 9). The results obtained with the more complex models appear to be realistic in that the associated sum of the squared residuals is comparable with the total accumulated when fitting the same dose–response curves individually. Furthermore, in several of the solutions obtained using models II–II and III–III, the parameter values were similar to those obtained when the corresponding models II and III were applied to data relating to a single ligand (Table 8). The solutions obtained using the 11-term version of model III–III are shown in Fig. 4.

Table 6. Comparison of the solutions obtained by fitting various models to noradrenaline-derived dose–response relationships with progressive removal of data from the higher dose range

Model	No. of data pairs	Coefficients a	b	c	d	e	p	$BF_{(1)}$
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		0.280		0.15
II	10		268.2	4.16		14.11	1.69	0.96
	9		165.4	4.03		8.11	1.46	0.32
	8		137.3	3.98		6.43	1.39	0.30
	7		35.27	3.81		0.117	0.974	0.15
III	10	84.04	39.83	3.82	5.033	0.908		0.29
	9	229.8	36.70	3.89	11.71	2.89		0.21
	8	48.54	30.69	3.84	0.675	1.47		0.15
	7	14.19	34.77	3.84	0.713	0.474		0.15

The clustered dose–response data for L-noradrenaline (from Table 1) were fitted using models I, II and III in the programme MINIM(A). The number of data points which comprised each curve, the values taken by the various parameters and the respective best-fit criteria $BF_{(1)}$ are shown for each model.

Table 7. Summary of dose–response data obtained in the combined presence of L-noradrenaline and its β-adrenergic antagonists

(A) L-Noradrenaline (*H*) and L-propranolol (*h*)

No. of data sets: 10
ΣCC for each set: 8.76, 8.44, 8.67, 8.39, 8.87, 7.88, 8.37, 8.29, 8.20, 8.33

Lipolysis

(*H*)	(*h*) = 0		(*h*) = 0.25 μM		(*h*) = 0.5 μM		(*h*) = 1.0 μM	
(μM)	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.
0	2.88	0.22	2.87	0.20	2.81	0.19	2.85	0.21
0.077	3.89	0.20	3.53	0.17	3.24	0.17	3.20	0.19
0.116	4.57	0.24	3.92	0.22	3.69	0.23	3.28	0.18
0.75	5.46	0.34	5.06	0.25	4.09	0.19	3.45	0.16
0.262	7.66	0.49	6.45	0.43	5.94	0.45	4.32	0.25
0.394	8.26	0.41	7.49	0.46	7.36	0.53	5.22	0.28
0.591	10.15	0.43	9.20	0.67	8.33	0.63	6.56	0.32
0.888	10.34	0.53	10.06	0.37	9.98	0.72	7.54	0.46
1.33	12.12	0.52	11.73	0.35	10.43	0.48	9.96	0.64
2.00	12.31	0.81	11.77	0.70	10.90	0.49	9.66	0.51

(B) D, L-Dichloroisoproterenol (*H*) and L-noradrenaline (*h*)

No. of data sets: 2
ΣCC for each set: 1.93, 1.93

Lipolysis

(*H*)	(*h*) = 0		(*h*) = 0.0078 μM		(*h*) = 0.0313 μM		(*h*) = 0.125 μM		(*h*) = 0.50 μM		(*h*) = 2.000 μM	
(μM)	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.	Cluster	S.E.
0	1.43	0.07	1.70	0.15	2.57	0.03	8.14	0.63	12.81	0.65	12.81	1.15
0.0304	2.01	0.37	1.62	0.01	2,73	0.00	6.58	0.60	13.49	0.34	12.75	0.86
0.122	1.85	0.05	2.16	0.33	2.97	0.07	5.98	0.46	13.68	1.14	12.55	0.21
0.487	3.30	0.30	3.60	0.00	3.70	0.37	10.69	0.15	11.90	0.25	13.16	0.24
1.95	6.64	0.44	5.44	0.01	7.54	0.20	6.91	1.75	12.67	0.29	12.71	0.21
7.81	9.64	0.09	10.64	0.79	7.09	1.26	7.03	0.28	9.65	0.83	12.77	0.71
31.3	8.95	0.06	8.58	1.60	7.74	0.14	8.64	0.15	8.91	0.17	8.71	1.03
125	3.71	0.02	4.11	0.38	4.81	0.29	2.63	0.11	3.64	0.49	4.62	0.34
500	1.63	0.05	1.55	0.09	1.63	0.04	1.53	0.01	1.55	0.02	1.45	0.05

Clustered (cf. [12]) dose–response data relating the lipolytic response evoked by combination of L-noradrenaline with (A) L-propranolol, and (B) D, L-dichloroisoproterenol are shown together with their associated standard errors (S.E.) The number of curves used in the clustering, and the ΣCC value associated with each are also shown.

Limits of the unclustered response data, μmole glycerol/g/hr.

Table 8. Comparison of the solutions obtained by optimising dose–response curves obtained with noradrenaline in the presence of propranolol using models II and III

Model	Propranolol concentration (μM)	Coefficients						
		a	b	c	d	e	n	$BF_{(4)}$
II	0		61.56	2.850		4.724	1.469	2.336
	0.25		58.54	2.870		4.748	1.622	1.645
	0.50		56.54	2.795		4.965	1.743	1.555
	1.0		21.32	2.850		1.981	1.643	1.891
III	0	14.35	14.85	2.878	1.506	0.2236		2.651
	0.25	4.604	17.27	2.829	0.561	0.7472		2.318
	0.5	24.40	32.52	2.810	189.7	61.38		1.942
	1.0	20.62	2.324	2.850	2.060	0.0378		1.818

The four L-noradrenaline dose–response curves obtained in the presence of the indicated L-propranolol concentrations (Table 1) were used independently for fitting with models II and III. The values taken by the best-fit criterion $BF_{(4)}$ are compared for each curve.

The clustered curve obtained from triplicated experimental data (Table 7) were used to provide the basis of a similar analysis of the interaction between L-noradrenaline and D,L-dichloroisoproterenol. Since the dose–response curves obtained exhibited a pronounced bell-shaped feature, only model III and its derivatives were used for the optimisations. As in many analyses of data with limited replications, the best-fit criterion $BF_{(4)}$ was used in order to accommodate the 'outliers' obviously present. It is apparent that the composite solution obtained using model III–III appeared to be at least superficially realistic as shown in Table 9 and Fig. 5.

DISCUSSION

The form of catecholamine-elicited dose–response curves

A previous statistical analysis of day-to-day differences in the lipolytic dose–response curve elicited by L-noredrenaline established that the systematic variation could be eliminated by 'clustering', to provide dose–response data suitable for analysis by model-fitting techniques [12]. The present assessment of hyperbolic, Hill-type and rational quadratic functions in expressing a number of related dose–response relationships was undertaken using data transformed in this way.

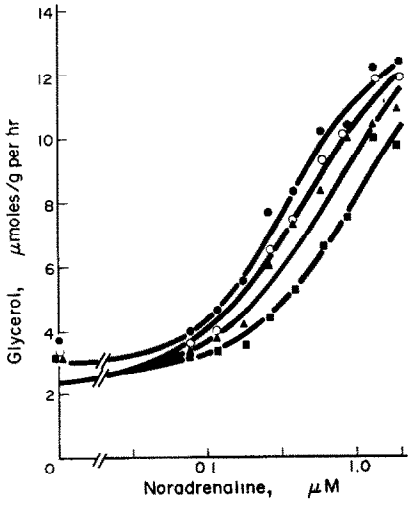

Fig. 4. Optimisation of the lipolytic response to combinations of noradrenaline with propranolol using model III–III. The clustered dose–response data for L-noradrenaline in the absence (●) and presence of 0.25 μm (○), 0.5 μM (▲) and 1.0 μM (■) L-propranolol described in Table 7 were fitted to the eleven-term version of model III–III using the programme MINIM(A) and the best-fit criterion $BF_{(4)}$. The data were treated as a composite block (see Methods of data analysis). The continuous lines show the solutions obtained using the fitted parameters. The parameter values and other details of the solutions are shown in Table 9.

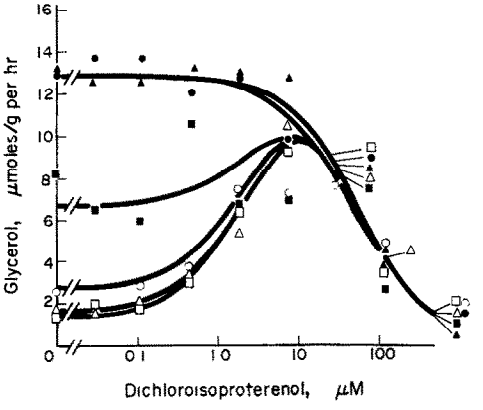

Fig. 5. Optimisation of the lipolytic response to combinations of dichloroisoproterenol with noradrenaline using model III–III. The clustered dose–response data for D,L-dichloroisoproterenol in the absence (□) and presence of 0.0078 μM (△), 0.0313 μM (○), 0.125 μM (■), 0.5 μM (●) and 2.0 μM (▲) L-noradrenaline described in Table 1(C) were fitted to the eleven-term version of model III–III using the programme MINIM(A) and the best-fit criterion $BF_{(4)}$. The data were treated as a composite block (see Methods of data analysis). The continuous lines show the relationship obtained with the fitted parameters. The parameter values and other details of the solution are shown in Table 9.

Table 9. Comparison of models II–II and III–III in accommodating the lipolytic response to noradrenaline in combination with either propranolol or dichloroisoproterenol

	(A) L-Noradrenaline (H) + L-propranolol (h)				(B) D, L-Dichloroisoproterenol (H) + L-noradrenaline (h)			
	Model II–II (equation 7)		Model III–III (equation 10)		Model III–III (equation 9)		Model III–III (equation 10)	
Parameter	Parameter value	Perturbation analyses	Parameter value	Perturbation analyses	Parameter value	Perturbation analyses	Parameter value	Perturbation analyses
a			226.3	$\begin{bmatrix}07.71\\17.00\end{bmatrix}$	0.003655	$\begin{bmatrix}0.07367\\0.09024\end{bmatrix}$	0.002074	$\begin{bmatrix}0.03735\\0.03558\end{bmatrix}$
b	73.72	$\begin{bmatrix}32.43\\31.46\end{bmatrix}$	17.71	$\begin{bmatrix}0.380\\1.151\end{bmatrix}$	5.745	$\begin{bmatrix}6.056\\2.836\end{bmatrix}$	5.493	$\begin{bmatrix}6.068\\4.064\end{bmatrix}$
c	2.880	$\begin{bmatrix}9.290\\5.405\end{bmatrix}$	2.880	$\begin{bmatrix}2.470\\2.309\end{bmatrix}$	1.249	$\begin{bmatrix}0.888\\1.011\end{bmatrix}$	1.264	$\begin{bmatrix}0.9925\\0.2041\end{bmatrix}$
d			16.75	$\begin{bmatrix}10.57\\5.59\end{bmatrix}$	0.008975	$\begin{bmatrix}0.725\\1.155\end{bmatrix}$	0.007609	$\begin{bmatrix}1.085\\0.565\end{bmatrix}$
e	5.911	$\begin{bmatrix}17.23\\18.45\end{bmatrix}$	4.496	$\begin{bmatrix}2.448\\0.098\end{bmatrix}$	0.4323	$\begin{bmatrix}0.112\\2.745\end{bmatrix}$	0.3996	$\begin{bmatrix}1.765\\1.883\end{bmatrix}$
A			139.9	$\begin{bmatrix}0.8150\\1.6990\end{bmatrix}$	95.24	$\begin{bmatrix}7.629\\8.153\end{bmatrix}$	94.03	$\begin{bmatrix}2.63\\10.42\end{bmatrix}$
B	8.738	$\begin{bmatrix}5.694\\3.511\end{bmatrix}$	19.1	$\begin{bmatrix}0.1247\\0.1244\end{bmatrix}$	43.70	$\begin{bmatrix}2.609\\2.944\end{bmatrix}$	48.91	$\begin{bmatrix}1.263\\2.475\end{bmatrix}$
D			53.50	$\begin{bmatrix}6.312\\1.651\end{bmatrix}$	8.395	$\begin{bmatrix}9.161\\9.290\end{bmatrix}$	8.265	$\begin{bmatrix}10.96\\7.71\end{bmatrix}$
E	3.138	$\begin{bmatrix}6.300\\9.346\end{bmatrix}$	13.20	$\begin{bmatrix}1.391\\0.565\end{bmatrix}$	1.126	$\begin{bmatrix}0.4597\\0.3796\end{bmatrix}$	1.518	$\begin{bmatrix}0.7284\\0.1631\end{bmatrix}$
R			700.5	$\begin{bmatrix}3.829\\11.60\end{bmatrix}$			0.004399	$\begin{bmatrix}7.0\times10^{5}\\111.0\times10^{5}\end{bmatrix}$
U			50.87	$\begin{bmatrix}5.099\\1.600\end{bmatrix}$			0.2802	$\begin{bmatrix}0.6307\\0.0233\end{bmatrix}$
p	1.541	$\begin{bmatrix}11.79\\16.90\end{bmatrix}$						
q	1.573	$\begin{bmatrix}1.015\\1.580\end{bmatrix}$						
$BF_{(4)}$	9.869		12.81		33.52		31.02	

The models were applied to the clustered data obtained for (A) L-noradrenaline together with a range of L-propranolol concentrations and (B) D, L-dichloroisoproterenol together with a range of L-noradrenaline concentrations. The parameter values for each model and the results obtained by performing perturbation analysis (cf. [23]) are shown together with the quality of fit criterion $BF_{(4)}$.

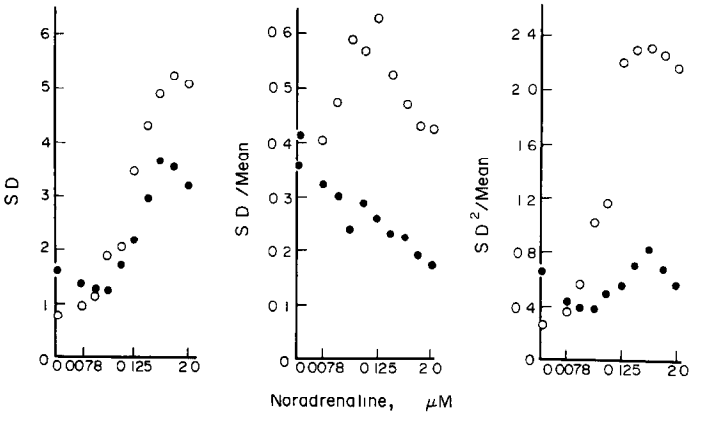

Fig. 6. The distribution of the error associated with the composite curve relating the concentration of noradrenaline to the lipolytic response. The data used were the thirty-four dose–response curves relating the rate of lipolysis to the concentration of L-noradrenaline that are detailed in Table 1. The standard deviations (S.D.) and square of the standard deviations (S.D.^{2}) associated with both the unclustered (○) and clustered (●) responses to each L-noradrenaline concentration were calculated and used as shown.

Throughout this study, a non-linearising minimisation technique was used for optimisation. Such procedures have been used previously to fit hyperbolic and Hill-type formulations [27–32]. When applied to the rational quadratic function, few difficulties were encountered, although particular attention to the choice of parameter estimates was required in order to provide discontinuous solutions with dose–response curves lacking pronounced auto-inhibitory phases. The procedures used should be applicable in numerous situations where optimisation using the rational quadratic function is appropriate [33–35].

Of the four 'best-fit' criteria investigated, the 'least squares' or 'maximum likelihood' criterion has been most widely used in published studies. It is strictly applicable only where the experimental error associated with each mean response value is normally distributed and is independent of the dimensions of the mean. Whereas the former condition may be met, this appears not to be true of the latter. Figure 6 shows that for the thirty-four dose–response curves relating the rate of lipolysis to the concentration of noradrenaline (as detailed in Table 1), the standard deviations (S.D.) are correlated with the corresponding mean values, although the range of S.D. is far more restricted for the clustered than for the untransformed data. Figure 6 also shows that S.D.^{2} is more nearly proportional than is S.D. to the mean response values, suggesting (as pointed out by Reich [25]) that his best-fit criterion $BF_{(2)}$ (i.e. $(1_i - L_i)^2/L_i = \min$) is most appropriate for the optimisation of these data (cf. [36]). However, since this conclusion is based on the analysis of only one set of experimental data, and since the range of S.D. values associated with this dose–response curve is limited, the least squares criterion was also used frequently in our investigation.

The inadequacy of hyperbolic models (such as model I) in accommodating the steepness of dose–response relations elicited by hormones and drugs has been recognised in a variety of systems [6, 9, 11, 17]. This particular difficulty has generally been resolved by using functions such as model II which is based on the Hill equation [9–11]. This model appears to be adequate for the lipolytic system of adipocytes if the hormone concentration range is limited to that at which the hook effect is not evident (Table 6; see also [26]). However, the results imply that in order to avoid spurious assessments of apparent co-operativity when using model II, it is necessary to confine the analysis to data that fall within an empirically selected segment of the dose–response relationship.

The rational quadratic function (model III), which is an alternative to model II as an extension of the hyperbolic function (model I), has been frequently adopted in enzymology where a bell feature is evident. Since it accommodates a wide range of apparent co-operativities in dose–response relationships [18, 19, 37], it is not surprising that it compares favourably with model II as a basis for analysing this feature of the dose–response curve obtained using L-noradrenaline, even though the auto-inhibitory feature is only marginally detectable in the data analysed [26].

A preliminary effort has been made to determine whether models I, II and III can be extended so as to accommodate simultaneously the gross features of the interactions between noradrenaline and various concentrations of β-adrenergic antagonists (propranolol) and partial agonists (dichloroisoproterenol). In the noradrenaline concentration range where the hook effect is not evident, the kinetics of its interaction with propranolol (which is without agonistic effects) is about equally well accommodated by the models II–II and III–III, the extensions of models II and III respectively. Where the action of noradrenaline is modulated by dichloroisoproterenol, which has both stimulatory and inhibitory activities only model III–III yields an acceptable solution. The multiplicity of parameters in model III–III provides considerable scope for mutual compensatory adjustments to occur, particularly where the experimental data are imprecise. Nevertheless, the solutions obtained in optimising the models II–

II and III–III are realistic in that, for the parameters that related specifically to one or the other of the effectors, the values obtained are similar to those yielded when analysing the responses to those effectors individually (Tables 2, 8 and 9).

It has been pointed out that where families of dose–response curves exist, all the information available in the experimental data cannot be extracted unless curves are analysed simultaneously [38]. These results indicate that there is a prospect of using models II–II, and to a greater extent model III–III, not only in the mechanistic analysis of dose–response curves, but also to determine the operational parameters of importance in pharmacology.

Mechanisms of catecholamine-induced dose–response relationships

The superiority of the rational quadratic model consistently observed in the present study is due to the domination of these dose–response curves by distinct stimulatory and auto-inhibitory phases. The biochemical mechanisms that dictate these features remain uncertain. It has been pointed out, for instance, that apparent co-operativity may be affected if the system contains a saturable enzyme system which degrades the hormone [39; cf. 40]. In the present study, an effort was made to detect such degradative activity by adding [^{3}H]noradrenaline to adipocyte preparations under the normal incubation conditions and analysing and recovered radioactivity by paper-chromatography [41]. No appreciable hormone degradation was detected at this concentration of the substrate (1 μM; results not shown), and thus kinetic models designed to deal with such effects [42] were not given detailed consideration.

Similar kinetic effects would be expected if L-noradrenaline were bound to the serum albumin of the incubation medium. Such binding does occur [43–45], although the affinity and capacity of the albumin used is not precisely known. Nevertheless, since the affinity appears to be relatively low it is probable that the binding of noradrenaline is approximately proportional to its concentration in a range that is relavant to the adipocyte dose–response curves, and that such effects were minor.

More complex effects might emerge due to the operation of various feedback loops which are associated with the lipolytic cascade. Many of the substances considered to be involved, such as feedback regulators, FR, non-esterified fatty acids, prostaglandins and adenosine (for reviews, see [4, 46]) become effective when their concentrations in the external medium reaches a critical level. Although precautions were taken to incubate fat cell preparations which were of low 'density' and to ensure that the molar non-esterified fatty acid: albumin ratio in the medium did not exceed 3:1, the influence of these substances on the dose–response curves warrants further investigation.

Although numerous factors are involved in linking hormone–receptor interactions to the lipolytic response, they are dominated by the cyclic AMP-mediated cascade (for review, see [3, 4]). The position with regard to phenylephrine is somewhat ambiguous since it stimulates both α- and β-adrenergic receptors [47]. However, it appears that only the latter has an appreciable effect of cyclic AMP accumulation and lipolysis in the rat (for review, see [4]).

The bell-shaped feature of the catecholamine-induced dose–response curves appears to originate at the level of cyclic AMP generation in the lipolytic cascade. Thus, auto-inhibition is detectable in the cyclic AMP response of intact fat cells to both L-noradrenaline and L-adrenaline [48, 49]. Furthermore, the partial agonists hydroxybenzylpindolol, hydroxybenzylpropranolol and dichloro-*t*-butylisoproterenol have been shown to yield pronounced bell-shaped responses from the adenylate cyclase of fat cell membranes [50, 51].

The manner in which the catecholamines elicit their auto-inhibitory effect on the adenylate cyclase system remains uncertain. It is unlikely that it can be dismissed as a general inhibition by high concentrations of a very active agent. For instance, although the inhibition of lipolysis by L-noradrenaline becomes apparent when its concentration exceeds 2 μM, 100-fold higher concentrations elicit a second stimulatory phase, referred to as lipolysis II [52], which is accompanied by increases in the concentration of cyclic AMP [53, 54]. The hook feature may be an important characteristic of the dose–response curve elicited by catecholamines: it disappears or is grossly displaced in the presence of insulin [53, 54] and in the absence of endogenous adenosine (J. E. Souness and J. I. Davies, unpublished observations), and is modified during ageing [55].

Among the effects of some of these agents is an interaction with membranes which is revealed as a local anaesthetic activity. It is frequently detected by isolating the contribution to their overall effect that lacks stereo-specificity. The binding of catecholamines to their functional membrane receptors is highly stereo-specific [56, 57] but the non-stereo-specific effects of these substances include lipolysis II [52].

The stereo-specificity of the bell-shaped dose–response curves elicited by phenylephrine is also complex. The stimulatory effect of the L-isomer (presumably due to β-adrenergic agonism, cf. [47, 58]) is shared by 30-fold higher concentrations of the D-isomer. However, the auto-inhibitory phase may be completely lacking in stereo-specificity (Fig. 2), suggesting that this phase of the response is due to the well established local anaesthetic effect of phenylephrine [58]. Similarly, certain β-adrenergic blocking agents, including propranolol have quite well characterised local anaesthetic activity [59], which is responsible for their inhibition of the lipolytic response to polypeptide hormones and theophylline-stimulated lipolysis, as well as that due to the catecholamines [60, 61].

The mode of action of adenylate cyclase is complex and poorly understood (for review, see [62]). In the fat cell, distinct species of GTP-binding proteins, designated N_s and N_i, appear to mediate respectively the stimulatory and inhibitory effects of hormones and related substances (see [62]). Over a range of concentrations, GTP has a well characterised biphasic effect on enzyme activity, which apears to be due to its interaction with these components [63].

An interesting possibility exists that the balance between the stimulatory and inhibitory phases of the lipolytic action of the catecholamines reflects the extent to which the N_s and N_i proteins participate in the response.

The same underlying mechanisms may both account for the hook feature of dose–response relationships and dictate the degree of apparent cooperativity that they exhibit. This has been established in enzymes that undergo either multiple binding by a single ligand, or random-order binding by multiple ligands [19, 37].

The relationship between the concentration of catecholamine derivatives and their binding to receptors has been investigated in fat cells. Direct binding studies using the catecholamine antagonist dihydroalprenolol to fat cell membrane preparations yielded Hill coefficients of 0.65 and 0.70 [56, 64] and suggestions of negatively co-operative binding.

However, other explanations were later shown to be more likely, such as the existence of multiple receptor species [65]. Our finding that the stimulation of lipolysis by catecholamines lacks any appreciable co-operativity would be conveniently reconciled with this proposal if it were hypothesised that only one of the ligand-binding species was functional with respect to the lipolytic system. Under these circumstances, the analysis of dose–response curves could provide valuable information concerning the interaction between the catecholamines and their physiologically functional receptors (cf. [66]).

As the molecular components of the lipolytic system are characterised, there is an increasing prospect of substituting the models used in this investigation with more mechanistic functions and thus to interpret the modification of dose–response curves by insulin, adenosine, ageing, etc. Some efforts have been made to predict the behaviour of systems comprising a protein kinase and a phospho-protein phosphatase [40, 67]. There have also been studies of the relationship between hormone concentration and both the steady and pre-steady state levels of cyclic AMP in cyclic AMP generating systems consisting of adenylate cyclase, one or more phosphodiesterase and a 'cyclic AMP-leakage' mechanism [16, 20, 68–70]. Although the equations obtained by Davies and Williams [16, 20] included a rational quadratic function which described the effect of multiple binding of hormone–receptor complexes to adenylate cyclase, far more work is required in order to reconcile model III with the known mechanisms of the lipolytic cascade. It would appear that this pursuit. which would for instance allow the parameters of the models used in this study to be resolved in terms of the kinetic constants of the enzymes involved, promises to make a valuable contribution to our understanding of cyclic AMP-mediated systems.

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