Dose-response curves for the adipokinetic action of aromatic amines and adrenocorticotropin upon the isolated adipose tissue of the hamster

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SUMMARY Slices of hamster adipose tissue were incubated with various concentrations of an aromatic amine or of adrenocorticotropin (ACTH). The curve relating response (measured in terms of FFA produced) to logarithm of dose was sigmoid for all adipokinetic substances tested. The basic requirement for adipokinetic activity was found to be a phenyl group attached to an ethyl- or propylamine side chain. Structural features incompatible with adipokinetic activity are described. Three groups of sigmoid log dose-response curves were distinguished, characteristic of (*a*) ACTH, and the catechol amines norepinephrine, epinephrine, isoproterenol, ethylnorepinephrine, and 3,4-dihydroxy-α(isopropylamino)-acetophenone; (*b*) dichloroisoproterenol; and (*c*) nine aromatic amines related in structure to phenylethylamine, but lacking the combination of a catechol ring and a hydroxy or keto group on the β-carbon. A modification of the Clark-Stetten model of hormone action is presented. Dose-response data for the action of aromatic amines and of ACTH upon hamster adipose tissue, and for the action of ACTH upon rabbit adipose tissue, are compatible with the equations derived from the model. Consideration of the structural features of aromatic amines, which influence certain parameters of these equations, supports the concept that the various adipokinetic peptides and the catecholamines act at the same site and by the same mechanism to stimulate lipolysis within the fat cell.

At least 10 naturally occurring substances stimulate mammalian adipose tissue to convert stored triglyceride into free fatty acids (FFA): the hypophyseal peptides: adrenocorticotropin (ACTH), thyroid-stimulating hormone, α- and β-melanocyte stimulating hormones, arginine vasopressin, "fraction H" ("peptide II") and "peptide I"; the pancreatic peptide, glucagon; and the amines, epinephrine and norepinephrine, of the sympathetic nervous system (1–5). The adipokinetic potency of each of these substances varies markedly in different mammalian species (3–5). These observations have given rise to the following questions: Do these substances stimulate lipolysis within the fat cell by the same mechanism? What is the structural basis for the adipokinetic activity of the specified amines and peptides? If the various adipokinetic substances operate by the same mechanism, what common structural feature may account for their sharing of this biological property?

Comparison of the dose-response relationships of the several adipokinetic substances may yield information relevant to these problems. Two applications of dose-response data may be visualized: (*a*) Plotting of logarithm of dose vs. magnitude of response for a series of structurally related, biologically active substances may give a series of parallel curves (6). The distances between such curves reveal the relative potencies of the substances, and thereby illustrate the relation of chemical structure to biological activity (6). (*b*) Dose-response data for certain biological actions of hypophyseal peptides (7, 8) and of catechol amines (9, 10) appear to conform to a theoretical model, proposed and discussed by Clark (9), Stetten (7), Ariens (11), and Furchgott (12, 13), which postulates a reversible combination between hormone and cell receptor, the abundance of the hormone-receptor complexes determining the magnitude of the biological response.

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The model contains four variables: (*a*) The number of target cell receptors available to the biologically active substance (7, 9); (*b*) the affinity of the receptor for the substance (7, 9); (*c*) the "intrinsic activity" of the complex between receptor and a particular substance in stimulating the response (11); and (*d*) the coefficient for distribution of the active substance between the extracellular medium and the receptor site (12, 13). Information concerning these variables can be derived by suitable analysis of dose-response data (7). Stetten suggested (7) that this type of analysis might be useful in comparing the actions of different hormones that produce the same biological effect.

An earlier report (14) from this laboratory described a method (adapted from the work of Gordon and Cherkes [15] and of White and Engel [16]) for obtaining reproducible dose-response data for the action of adipokinetic substances upon isolated adipose tissue from the rabbit, guinea pig, or hamster. Adipose tissue from the former two species is highly responsive to most adipokinetic hypophyseal peptides but shows little or no response to the catechol amines (5, 14). In contrast, adipose tissue from the hamster is highly responsive to catechol amines, but responds only to ACTH among the hypophyseal peptides (5, 14). Dose-response data for the action of hypophyseal peptides upon rabbit and guinea pig adipose tissue were presented previously (14). The present paper describes dose-response data for the action of aromatic amines and ACTH upon hamster adipose tissue. The relationship between structure and activity for the amine class of adipokinetic substance is discussed. In addition, Stetten's model (7) (modified according to Ariens [11] and Furchgott [12, 13]) is applied to the dose-response data for adipokinetic peptides and amines reported in the earlier study (14) and in the present study.

MATERIALS AND METHODS

The magnitude of lipolytic response by slices of hamster adipose tissue to various concentrations of adipokinetic substance was determined in the previously described (14) albumin-free assay system. Adult male hamsters, weighing 150–170 g, and fed a diet of Purina Laboratory Chow ad libitum, were used. The epididymal and perirenal adipose tissues were removed from a hamster immediately after death from a blow on the head, and cut by hand into slices weighing 50–100 mg.[1] Forty to sixty such slices were obtained from a single animal. Each slice was promptly placed in 2 ml Krebs-Ringer phosphate buffer solution (pH 7.40) containing a given concentration of adipokinetic substance. After incubation for 2 hr at 37° under air in a Dubnoff shaker, the slices were removed from the medium, blotted, weighed, and analyzed for FFA concentration (14).

Oxycellulose-purified ACTH (100 units/mg), and 33 amines and amine derivatives (listed in Table 1), were tested for adipokinetic activity. The ACTH preparation was obtained from Wilson Laboratories (Chicago, Ill.). The amines and related compounds were supplied as follows.–Compounds numbered 1, 2, 9, 11, 29, 30, 32: Mann Research Laboratories, Inc. (N.Y.C.); compounds 3, 4, 14: Winthrop Laboratories (N.Y.C.); compounds 5, 8, 21, 23, 24, 25, 27: Aldrich Chemicals Co. (Milwaukee, Wis.); compound 6: Lilly and Co. (Indianapolis, Ind.); compounds 7, 16, 17, 19, 20, 22: Eastman Organic Chemicals (Rochester, N.Y.); compound 10: Smith, Kline, and French Laboratories (Philadelphia, Pa.); compounds 12, 28, 31: K and K Laboratories, Inc. (N.Y.C.); compounds 13, 15: Merck, Sharp, and Dohme (West Point, Pa.); compound 18: Matheson, Coleman, and Bell (Cincinnati, Ohio); compound 26: Wyeth Laboratories (Philadelphia, Pa.); compound 33: Burroughs Wellcome and Co., Inc. (Tuckahoe, N.Y.).

RESULTS

1. *Dose-Response Data for the Adipokinetic Action of Norepinephrine*

In a representative experiment, the concentration of FFA in adipose tissue incubated in a phosphate medium alone was 1.0 ± 0.2 μEq/g of tissue (mean ± SE). The minimal concentration of norepinephrine in the medium that produced a statistically significant ($p < 0.05$) increase in the concentration of FFA in the tissue slice was 1×10^{-6} M. Progressive increments in concentration of norepinephrine up to 3×10^{-5} M produced progressive increments in tissue concentration of FFA up to a maximum of 15.2 μEq/g. When the increase in FFA concentration of slices exposed to norepinephrine over that in control slices (response) was plotted against the logarithm of the molar concentration of norepinephrine in the medium at the beginning of incubation (log dose), a sigmoid curve was obtained (Fig. 1.) The midportion of this curve (log dose −6 to −5) is linear and has a slope of 9.4. The experiment was repeated with adipose tissue from 4 different animals. Minimal effective dose (MED) was 1×10^{-6} M in each experiment. Maximal response (R_{max}) varied between 12.7 and 16.4 (mean ± SE, 14.8 ± 1.5). Slope of the midportion of the curve varied between 7.3 and 11.0 (mean ± SE, 9.2 ± 0.8).

[1] No difference in magnitude of response to a given concentration of adipokinetic substance was found for slices of epididymal and of perirenal adipose tissue obtained from the same hamster.

2. *Dose-Response Data for the Action of Other Amines and Amine Derivatives*

Dose-response experiments similar to that shown in Fig. 1 were done for the 33 compounds listed in Table 1, where values for MED, R_{max}, and slope of the midportion of the log dose-response curve are given. These values represent the averages derived from two or more separate experiments with each compound. On the basis of similarities in R_{max} and slope, four categories are recognizable.

Group A. Five amines showed similar values for R_{max} (range 13.2–16.8), and also similar values for slope (range 7.9–10.0). These compounds, listed as Group A in Table 1, are isoproterenol, epinephrine, norepinephrine, ethylnorepinephrine, and 3,4-dihydroxy-α-(isopropylamino)-acetophenone. Values for MED in these experiments were, respectively, 1×10^{-7} M, 3×10^{-7} M, 1×10^{-6} M, 1×10^{-6} M, and 3×10^{-6} M. Dose-response experiments with this group of five amines were now repeated; in each experiment two amines were tested upon the adipose tissue of a single hamster. A representative experiment is illustrated in Fig. 2. Parallelism of the midportion of the log dose-response curves for all five amines was demonstrated. Calculation from these curves (6) of the relative potencies of these amines, with a potency of 100 assigned to isoproterenol, gave the following results: epinephrine 18, norepinephrine 10, ethylnorepinephrine 10, 3,4-dihydroxy-α-(isopropylamino)-acetophenone 6.

Group B. Dichloroisoproterenol, the chlorinated derivative of isoproterenol, showed the following characteristics: MED 1×10^{-7} M; R_{max} 9.4; slope of midportion of log dose-response curve, 4.2.

Group C. The nine amines in this group were characterized by MED 3×10^{-4} M, R_{max} 2–4, and slope 1–2.

Group D. The 18 aromatic and aliphatic amines in this group did not produce a statistically significant ($p < 0.05$) increase in tissue FFA concentration at the dosage 3×10^{-4} M.

The different forms of the log dose-response curves for the amines of Groups A, B, and C are illustrated in Fig. 3.

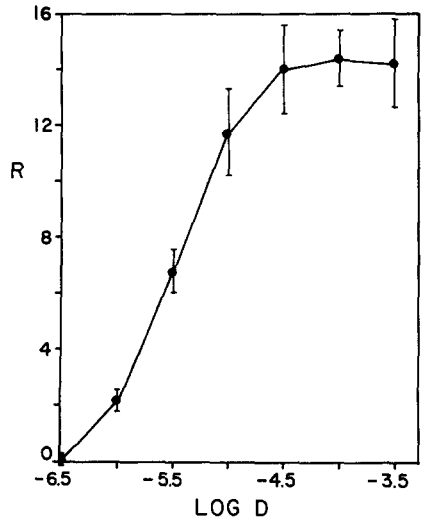


FIG. 1. Relationship between logarithm of the dose of norepinephrine and the response of slices of hamster adipose tissue. Abscissa: logarithm of the molar concentration of norepinephrine in the medium at the beginning of incubation. Ordinate: increase in concentration of FFA in the tissue slices at the end of incubation over that in slices incubated in KRP medium not containing adipokinetic substance. Each point represents the mean of four observations. Standard error of the mean is also shown. All the tissue slices were obtained from a single hamster.

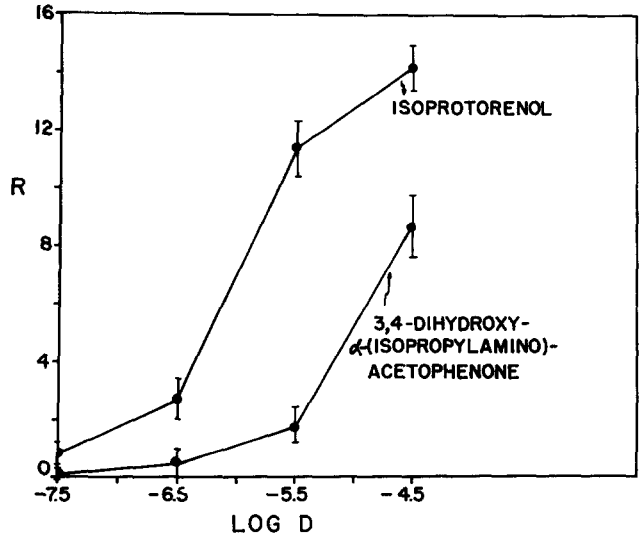


FIG. 2. Log dose-response curves for the action of isoproterenol and 3,4-dihydroxy-α-(isopropylamino)-acetophenone upon the isolated adipose tissue of the same hamster. Ordinates, etc., as in Fig. 1.

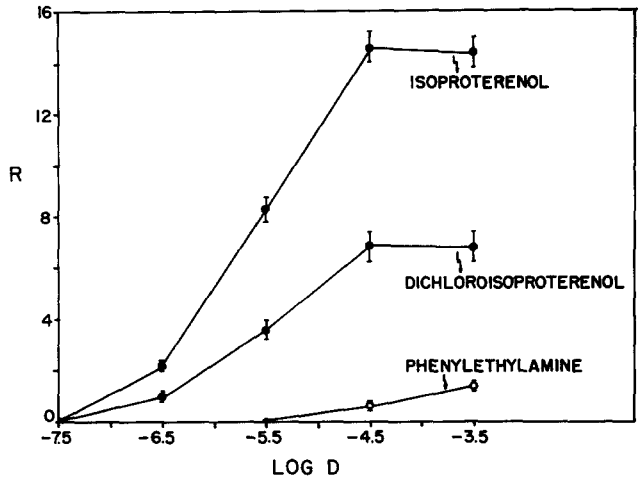


FIG. 3. Comparison of the log dose-response curves for the action of isoproterenol (Group A), dichloroisoproterenol (Group B), and phenylethylamine (Group C) upon the isolated adipose tissue of the same hamster. Ordinates, etc., as in Fig. 1.

In this experiment, dose-response curves were delineated for the action of isoproterenol (Group A), dichloroisoproterenol (Group B), and phenylethylamine (Group C) upon the adipose tissue of a single hamster. R_{max} and slope of the midportion of the curve were highest for isoproterenol, intermediate for dichloroisoproterenol, and lowest for phenylethylamine.

3. *Dose-Response Data for the Adipokinetic Action of ACTH*

MED for oxycellulose-purified ACTH (100 units/mg) was 0.03 μg/ml, or 4.4×10^{-9} M (assuming a potency of 150 units/mg, and molecular weight 4567, for the pure peptide). R_{max} in three experiments averaged 14.0. The log dose vs. response curve was sigmoid (Fig. 4), and the slope of the midportion of the curve averaged 7.4.

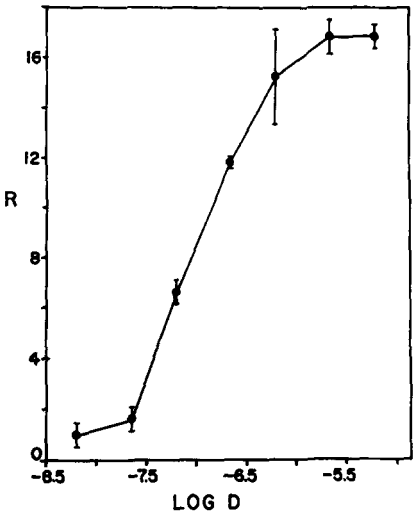


FIG. 4. Log dose-response curve for the action of ACTH upon the isolated adipose tissue of a hamster. Ordinates, etc., as in Fig. 1.

DISCUSSION

1. *Structure-Activity Relationship for Adipokinetic Amines*[2]

(*a*) *Basic Requirement.* The basic requirement for adipokinetic activity is a phenyl group attached to an ethylamine or propylamine side chain (compounds 7 and 8). Replacement of the phenyl group by an aliphatic chain (compounds 16–21), shortening (compound 22) or lengthening (compound 23) of the ethyl or propyl side chain, or replacement of the amine group by a hydroxyl group (compound 24) abolishes activity.

(*b*) *Influence of Oxygen Functions.* Hydroxylation of the phenylethylamine structure on the β-carbon, on the meta position, on the para position, or on two of these three positions, does not alter adipokinetic activity (compare compound 7 with compounds 9–15 of Group C). However, when all three of these positions are hydroxylated, adipokinetic activity is markedly enhanced, as evidenced by a 300 × reduction in MED, a 5 × increase in R_{max}, and a 5 × increase in the slope of the log dose-response curve (compare compounds 9, 11, and 12 of Group C with compound 1 of Group A). Insertion of a keto group instead of an hydroxy group on the β-carbon, in association with hydroxylation of the meta and para positions, causes a lesser (100 ×) reduction in MED, but the same 5 × increase in R_{max} and in slope of the midportion of the log dose-response curve (compare the compounds of Group C with compounds 3 and 5 of Group A).

(*c*) *Influence of Substituents on the Amine Group.* Comparison of compounds 1, 2, 9, and 25 shows that either a primary or secondary amine group is compatible with activity, but that a tertiary amine group abolishes activity (confirming the conclusion of Mueller and Horwitz [17]). Substitution on the amine group appears to influence MED but not R_{max}. This is illustrated by the progressively lower MED for the series norepinephrine, epinephrine, and isoproterenol; by contrast, R_{max} is the same for these three compounds (Table 1).

(*d*) *Other Structural Influences.* Substitution of a single methyl or ethyl group on the α-carbon is compatible with activity (see compounds 4, 13, and 15) (as noted by Mueller and Horwitz [17]), but two methyl groups at this position abolish activity (compound 26). The presence on the α-carbon of a free or esterified carboxyl group (compounds 27–29) or of a hydroxymethyl group (compound 30) also abolishes activity. Presence on the phenyl ring of methoxy groups (compounds 32 and 33) (as observed by others [17, 18]), or of chlorine groups (compound 6), abolishes or reduces activity.

Bogdonoff et al. (18) investigated the relationship between the structure and adipokinetic activity of aromatic amines by measuring the effect of intravenous infusion of various compounds upon the plasma FFA concentration of human subjects. They found compounds 1, 2, and 3 (Table 1) to be active and compounds 11, 14, 15, 26, 32, and 33 to be inactive. Similar observations in man with compounds 1, 2, 3, 11, and 14 were reported by Mueller and Horwitz (17). The present study of the effect of amines upon hamster adipose tissue in vitro confirms the high adipokinetic activity of the first three compounds. The detection of a low order of activity for compounds 11, 14, and 15 in the present experiments upon hamster

[2] The structural positions are designated thus:

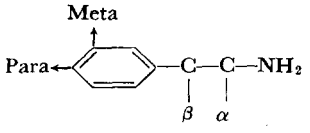


TABLE 1 MAXIMAL RESPONSE, SLOPE OF MIDPORTION OF LOG DOSE-RESPONSE CURVE, AND MINIMAL EFFECTIVE DOSE FOR THE ACTION OF VARIOUS AMINES UPON HAMSTER ADIPOSE TISSUE

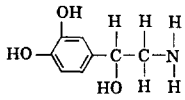
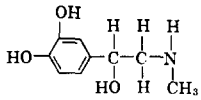
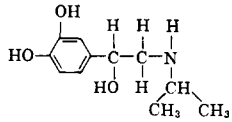
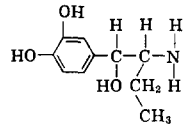
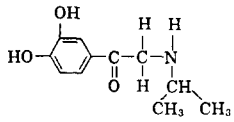
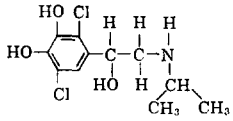
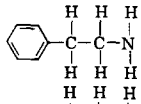
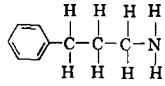
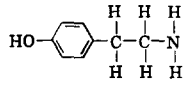
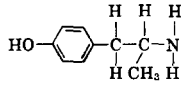
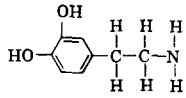
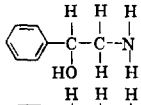
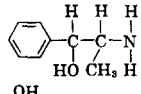
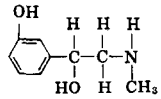
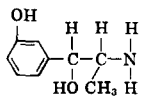
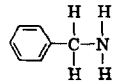
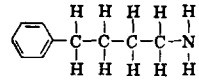
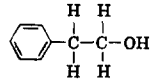
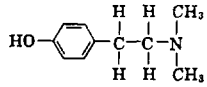
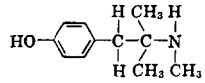
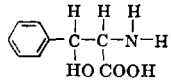
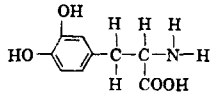
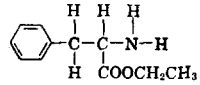
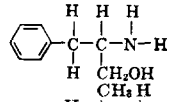
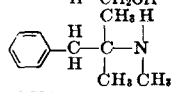
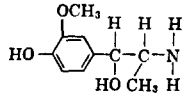
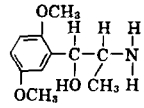
No.	Substance	Structure	Number of Experiments	R_{max}	Slope	MED
				μEq FFA per g tissue		*molar concn.*
1.	Norepinephrine		4	14.8 ± 1.5	9.2 ± 0.8	1×10^{-6}
2.	Epinephrine		6	15.8 ± 1.3	9.5 ± 1.0	3×10^{-7}
3.	Isoproterenol		17	16.8 ± 0.7	10.0 ± 0.7	1×10^{-7}
4.	Ethylnorepinephrine		3	13.2 ± 1.4	8.6 ± 0.6	1×10^{-6}
5.	3,4-Dihydroxy-α-(isopropyl-amino)-acetophenone		4	14.6 ± 1.8	7.9 ± 1.5	3×10^{-6}
		Group B				
6.	Dichloroisoproterenol		4	9.4 ± 0.2	4.2 ± 0.9	1×10^{-7}
		Group C				
7.	Phenylethylamine		4	3.2 ± 0.1	1.8 ± 0.1	3×10^{-4}
8.	Phenylpropylamine		2	3.7	1.8	3×10^{-4}
9.	Tyramine		2	3.6	3.0	3×10^{-4}
10.	Hydroxyamphetamine		3	3.2 ± 0.8	1.6 ± 0.2	3×10^{-4}
11.	Dopamine		2	3.5	2.0	3×10^{-4}
12.	β Phenyl-β-hydroxy-ethyl-amine		2	4.0	3.0	3×10^{-4}
13.	Phenylpropanolamine		2	3.6	2.1	3×10^{-4}
14.	Phenylephrine		2	3.2	2.3	3×10^{-4}
15.	Metaraminol		2	3.9	2.0	3×10^{-4}
		Group D				
16.	Propylamine	$CH_3CH_2CH_2NH_2$				
17.	N-Butylamine	$CH_3CH_2CH_2CH_2NH_2$				

TABLE 1 *Continued*

No.	Substance	Structure
		Group D
18.	N-Pentylamine	$CH_3CH_2CH_2CH_2CH_2NH_2$
19.	N-Hexylamine	$CH_3CH_2CH_2CH_2CH_2CH_2NH_2$
20.	N-Heptylamine	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2NH_2$
21.	N-Octylamine	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2NH_2$
22.	Benzylamine	
23.	4-Phenyl-1-butylamine	
24.	Phenylethyl alcohol	
25.	4-(β-Dimethylaminoethyl) phenol	
26.	Mephentermine	
27.	β-Phenylserine	
28.	3,4-Dihydroxyphenylalanine	
29.	Phenylalanine ethyl ester	
30.	Phenylalaninol	
31.	N-Methyl phenylisobutylamine	
32.	Metanephrine	
33.	Methoxamine	

adipose tissue may reflect the greater sensitivity of the in vitro assay system, or species differences, or both.

2. *Application of Stetten's Model of Hormone–Target Cell Interaction (Modified According to Ariens [11] and Furchgott [12, 13]) to Dose-Response Data for Adipokinetic Amines and Peptides*

Stetten postulated (7):[3]

(*a*) The concentration of hormone at the site of action is equal to the concentration of hormone in the extracellular fluid; (*b*) the hormone forms a complex with a cellular receptor; this process is reversible and achieves

[3] Stetten's model is analogous to that proposed earlier by Clark (9) for the action of acetylcholine and epinephrine upon smooth and striated muscle. Clark's model is analogous to the models devised by Michaelis and Menten (19) and by Langmuir (20, 21) to describe the interaction between catalyst and substrate, and adsorption phenomena. The applications and limitations of this type of model in the interpretation of dose-response data for adrenergic amines have been discussed by Furchgott (12, 13), Ariens (11), and Stephenson (22).

equilibrium according to the law of mass-action; (*c*) the magnitude of the biological response is equivalent to the abundance of hormone-receptor complexes. These postulates led to the equation:

$$\frac{D}{R} = \frac{1}{KQ} + \frac{D}{Q} \qquad \text{(Ia)}$$

where R = magnitude of biological response, D = concentration of hormone in the extracellular fluid, K = association constant[4] of the hormone-receptor complex, Q = number of receptors available to the hormone.

The data and discussions of Ariens (11) and Furchgott (12, 13), regarding the applications of this type of model to the pharmacology of the autonomic nervous system, suggest that a more general formulation of Stetten's postulates (*a*) and (*c*) is desirable, as follows: (*a*) the concentration of hormone (drug) at the receptor site is equal to the concentration in the extracellular fluid times a distribution coefficient *a;* (*c*) the magnitude of the biological response equals the number of hormone (drug)-receptor complexes times a coefficient *b* ("intrinsic activity"), which may vary for different substances that stimulate the same response.

These modifications in the postulates of the model lead to the following revisions of eq. (Ia):

$$\frac{D}{R} = \frac{1}{(aK)(bQ)} + \frac{D}{(bQ)} \qquad \text{(I)}$$

The relationship between D/R and D has these properties: (*a*) linearity; (*b*) slope = $1/bQ$; (*c*) intercept on vertical axis = $1/(aK)(bQ)$.

Equation I can easily be transformed into three other mathematical representations of the model.[5]

$$\frac{1}{R} = \frac{1}{(bQ)} + \frac{1}{(aK)(bQ)D} \qquad \text{(II)}$$

The relationship between $1/R$ and $1/D$ has these properties: (*a*) linearity; (*b*) slope = $1/(aK)\ (bQ)$; (*c*) intercept on vertical axis = $1/(bQ)$.

$$R = \frac{(aK)(bQ)D}{1 + (aK)D} \qquad \text{(III)}$$

The relationship between R and D has these properties: (*a*) hyperbolic form; (*b*) initial slope = $(aK)(bQ)$; (*c*) as $D \rightarrow \infty$, $R \rightarrow (bQ)$; i.e. $R_{max} = (bQ)$; (*d*) when $R = {}^1/_2R_{max}$, $D = 1/(aK)$.

$$R = \frac{(aK)(bQ)10^{\log D}}{1 + (aK)\ 10^{\log D}} \qquad \text{(IV)}$$

The relationship between R and log D has these properties: (*a*) symmetric sigmoid form; (*b*) $R_{max} = (bQ)$; (*c*) when $R = {}^1/_2R_{max}$, log $D = -\log (aK)$; (*d*) when $R = {}^1/_2R_{max}$, slope = 0.58 (bQ).[6]

The set of relationships exhibits these properties:

(A) intercept I = slope II = 1/initial slope III.

(B) slope I = intercept II = 0.58 × reciprocal of the slope of Curve IV at the point where $R = {}^1/_2R_{max}$.

In order to learn whether the dose-response relationship for the action of isoproterenol upon hamster adipose tissue conforms to the model, the data were plotted in the form of eqs. I, II, III, and IV: that is, D/R as a function of D (eq. I); $1/R$ as a function of $1/D$ (eq. II); R as a function of D (eq. III); and R as a function of log D (eq. IV). Inspection of the set of four curves (Fig. 5) revealed that they exhibit properties (*a*), (A), and (B) above, and hence are consistent with the four equations derived from Stetten's model. Values for aK and bQ may be calculated from each curve by measuring the slope and intercept on the vertical axis (Curves I and II), or by measuring R_{max} and D corresponding to ${}^1/_2R_{max}$ (Curves III and IV). Each curve gives a value for bQ of 15–17, and a value aK of 90×10^4 to 100×10^4.

The dose-response curves for the action of other aromatic amines, and for the action of ACTH, upon hamster adipose tissue, are also consistent with the equations derived from the model. Furthermore, the dose-response data reported previously (14) for the action of ACTH upon the adipose tissue of the rabbit, also show the properties predicted by these equations (Fig. 6). From the dose-response curves, bQ and aK may be calculated for the action of each amine or peptide adipokinetic agent upon hamster or rabbit adipose tissue (Table 2).

The compatibility of the dose-response relationship for the effect of adipokinetic substances upon adipose tissue with that predicted by the Clark-Stetten model suggests that adipose tissue contains "receptors" which form dissociable complexes with adipokinetic substances, and that the magnitude of the accumulation of FFA in the tissue under the conditions of the present experiments is proportional to the abundance of these complexes. The latter conclusion warrants special discussion because the concentration of FFA in adipose tissue is known to be dependent upon at least two metabolic processes. Biochemical studies (24, 25) have shown that following con-

[4] Stetten employed the dissociation constant (reciprocal of the association constant, which is employed in the present paper). Accordingly eq. (Ia) is given, in his paper (7), in the form $D/R = K/Q + D/Q$.

[5] These rearrangements of eq. (Ia) were indicated by Furchgott (12) in his discussion of Clark's model. Equation II is analogous to the Lineweaver-Burk representation (23) of the Michaelis-Menten model for the interaction between enzyme and substrate. Equation III is analogous to the Michaelis-Menten eq. (19) and to Langmuir's adsorption isotherm (20, 21). An alternative expression of eq. III is $D = R/(aK)\ (bQ) - (aK)R$, which is analogous to the equation devised by Clark (9) to represent the action of acetylcholine and epinephrine upon smooth and striated muscle.

[6] For proof of property (*d*), see reference (19).

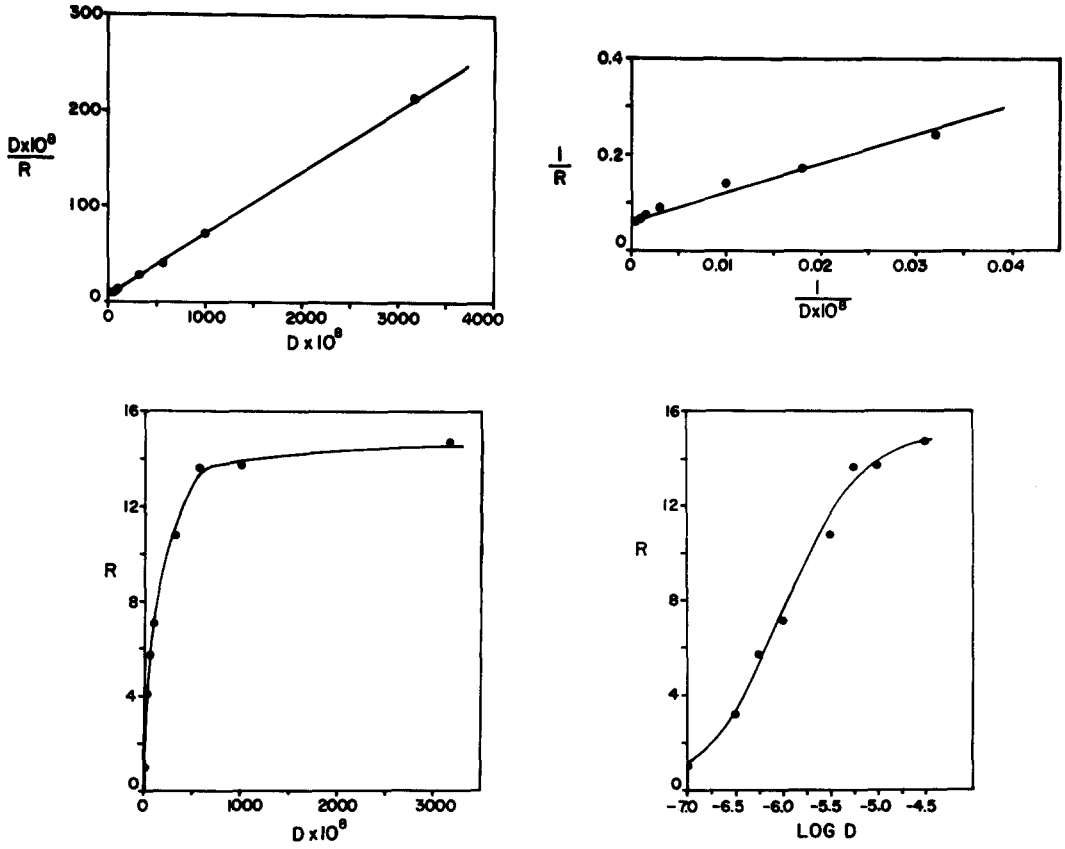


FIG. 5. Four plots of a dose-response experiment on the action of isoproterenol upon the adipose tissue of a hamster. Each point represents the mean of seven observations. D, dose; R, response. The upper left graph corresponds to Eq. I derived from the model, the upper right to Equation II, the lower left to Eq. III, and the lower right to Eq. IV. The type of plot in the lower right graph is the same as that in Figs. 1–4.

tact of an adipokinetic substance with the fat cell, an intracellular lipase is activated and catalyzes the hydrolysis of triglyceride. The newly formed FFA are subject to re-esterification with α-glycerophosphate and reconversion to triglyceride. The rate of reesterification tends to increase when the rate of lipolysis increases (25). When adipose tissue is exposed to an adipokinetic substance in albumin-free medium, the intracellular concentration of FFA rises and then attains a "plateau" value, at which time the rate of reesterification is believed to have increased sufficiently to equal the rate of lipolysis (14). This "plateau" concentration of FFA, which served as the response of the tissue in the present experiments, is thus a function of the rate of lipolysis and of the rate of reesterification; the latter rate, furthermore, is dependent upon the former. Nevertheless these relationships are not incompatible with the possibility of a simple proportional relationship between abundance of receptor–hormone complexes and the "plateau" FFA concentration, as the following hypothesis shows: (*a*) Assume that the concentration of lipase that hydrolyzes intracellular triglyceride is determined by, and is proportional to, the abundance of receptor–drug complexes. Then rate of lipolysis = number of these complexes $\times$ k_1. (*b*) Assume that the rate of reesterification of FFA is proportional to the concentration of FFA; rate of reesterification = intracellular concentration of FFA $\times$ k_2. (*c*) When intracellular FFA concentration attains "plateau" value, assume rate of reesterification = rate of lipolysis. Then "plateau" FFA concentration $\times$ k_2 = number of receptor–drug complexes $\times$ k_1; or, "plateau" FFA value = number of receptor–drug complexes $\times$ a constant.

The parameters of the model are the product bQ (number of receptors available to the drug $\times$ intrinsic activity of the receptor–drug complex), and the product aK (distribution coefficient of the drug $\times$ association constant of the receptor–drug complex). The physiological correlate of bQ is the maximal response the drug is capable of producing; the physiological correlate of aK is the reciprocal of the concentration of drug in the extracellular fluid required to produce a half-maximal response. Alterations in bQ modify the height and slope of the log dose-response curve; changes in aK cause lateral dis-

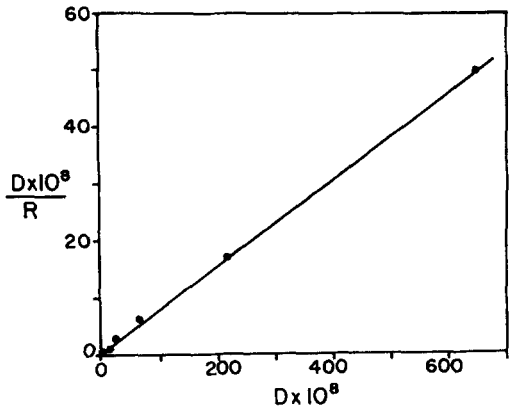


FIG. 6. Dose-response relationship for the action of ACTH upon rabbit adipose tissue. The plot corresponds to Eq. I and to the upper left graph of Fig. 5. The data for this experiment were reported previously (14).

placement of the curve along the horizontal axis. Inspection of the values for bQ and aK for the various amines (Table 2) reveals that certain structural features of the molecule selectively influence bQ, while other features primarily influence aK. The following examples may be cited: (*a*) Amines possessing two hydroxyl groups on the ring and a hydroxyl group on the β-carbon Group A, (compounds 1–5) have values for bQ in the range 13–17. The term bQ is not influenced by the following alterations in the side chain: substitution of a keto group for the hydroxyl group on the β-carbon (compare compounds 3 and 5); introduction of an ethyl group on the α-carbon (compounds 1 and 4); substitution on the amine group (compounds 1–3). In contrast, halogenation of the ring reduces bQ by 50% (compare compounds 3 and 6), and removal of one or both of the hydroxyl groups from the ring reduces bQ by 80% (compare compounds 2 and 14; 1 and 12). (*b*) The five amines of Group A, which have similar values for bQ, differ in their values for aK. These differences are related to alterations in the side chain. The value for aK increases progressively with substitution of a methyl or isopropyl radical on the amino group; it is reduced 17 times by substitution of a keto group for the hydroxyl group on the β-carbon. These alterations in the side-chain do not influence bQ. Thus certain alterations in the ring selectively influence bQ (and its physiological correlate, the magnitude of the maximal response); certain alterations in the side-chain selectively influence aK (and its physiological correlate, the magnitude of dose required to produce half-maximal response). An explanation for these correlations between amine structure and the parameters of adipokinetic activity must await information on the structure and location of the receptors in adipose tissue and on the forces that govern the formation of the receptor-amine complex.

TABLE 2 VALUES FOR bQ AND aK FOR THE ADIPOKINETIC ACTION OF VARIOUS AMINES AND ACTH

Source of Adipose Tissue	Substance (Compound No.)	bQ	aK
Hamster	Norepinephrine (1)	15	10×10^4
"	Epinephrine (2)	16	20×10^4
"	Isoproterenol (3)	17	100×10^4
"	Ethylnorepinephrine (4)	13	10×10^4
"	3,4-Dihydroxy-α-(isopropyl-amino)-acetophenone (5)	15	6×10^4
"	Dichloroisoproterenol (6)	9	100×10^4
"	Phenylethylamine (7)	3	1×10^4
"	Hydroxyamphetamine (10)	3	1×10^4
"	Dopamine (11)	4	1×10^4
"	ACTH	15	$1{,}000 \times 10^4$
Rabbit	ACTH	10	900×10^4

The values of bQ for the action of ACTH and for the action of the catecholamines (Group A) upon hamster adipose tissue are similar (Table 2). This suggests that these two different classes of adipokinetic substance interact with a similar number of receptors in hamster adipose tissue and that the resulting receptor–drug complexes have similar intrinsic activity in stimulating lipolysis. A previous study (14) demonstrated parallelism of the log dose-response curves for the action of ACTH and six other hypophyseal peptides upon rabbit adipose tissue. Since the slope of the midportion of the log dose-response curve is, according to the Clark-Stetten model, determined by bQ (eq. IV, property [d]), this earlier observation may now be interpreted to signify that the family of hypophyseal adipokinetic peptides interact with a similar number of receptors in rabbit adipose tissue, and that the resulting receptor–peptide complexes have similar intrinsic activity. The concept of a common mechanism of action for catecholamine and various peptide hormones has been advanced by Sutherland and co-workers (26). The present findings support this concept.

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Note Added in Proof Two additional aromatic amines have recently been tested upon hamster adipose tissue: Cobefrin and α-methylepinephrine, which are derived from norepinephrine and epinephrine respectively by substitution of a methyl group on the α-carbon. The dose–response curves of cobefrin and α-methylepinephrine were found to be identical with those of norepinephrine and epinephrine respectively. Thus introduction of a methyl group on the α-carbon, like that of an ethyl group on this position (compare compounds No. 4 and No. 1), does not alter adipokinetic activity.

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