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LIPOLYTIC ACTIVITY OF STRUCTURALLY RELATED AGONISTS IN RAT EPIDIDYMAL FAT TISSUE *IN VITRO**

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Abstract—Utilizing the rat epididymal fat pad, the rates of free-fatty acid (FFA) release were quantitated in the presence of various concentrations of agonists. The calculation of intrinsic activity and affinity constants from standard dose–response relationships and double reciprocal plots were employed to establish the structure–activity relationships. Marked changes in the affinity, and to a lesser degree, in the intrinsic activity constants of phenethylamines for the adrenergic-adipose tissue receptor system were produced by alterations of the (a) side chain nitrogen, (b) α- and β-carbon substituents and (c) *meta*- and *para*-ring positions of the phenyl nucleus. While optimal activity was possessed by all catecholamines, results with certain noncatecholamines indicated that the catechol moiety was not obligatory for FFA mobilization. The presence of a phenolic group in either ring position was sufficient to produce a maximal response when the β-carbon atom was also hydroxylated. In addition to β-hydroxylation, *N*-substitution of a bulky alkyl or aralkyl group greatly enhanced the lipolytic activity of the phenethylamine molecule. Optimal activity was obtained with either primary or secondary amines, whereas tertiary amines were characterized as having a decreased affinity. Furthermore, the presence of a β-keto or α-ethyl substituent on the ethylamino-side chain decreased the ability of the agonist to release FFA from adipose tissue *in vitro*.

NUMEROUS reports have indicated an important role for the sympathetic nervous system and the catecholamines, norepinephrine (NE) and epinephrine (E), in the mobilization of free-fatty acids (FFA). For instance, the injection of E or NE elevates plasma FFA[1–3] and the addition of these catecholamines to an incubation medium containing adipose tissue enhances the release of FFA.[4, 5] Moreover, chemical or electrical stimulation of the adrenergic nervous supply to adipose tissue has resulted in the activation of a lipase system.[6, 7] Other lines of evidence, particularly the location of adrenergic nerve elements near the fat cell[8] and the presence of NE in the epididymal fat pad,[9–10] have led to considerable interest in the underlying mechanism of adrenergic stimulation in fat tissue.

Many studies have reported the potent FFA mobilizing actions *in vivo* and *in vitro* possessed by NE, E and isopropyl arterenol. However, few investigators[11–13] have attempted to quantitate differences between structural analogues of the phenethylamine molecule. The purpose of this investigation was to elucidate the structure–

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activity relationships of importance in the mobilization of FFA from rat epididymal fat tissue *in vitro* by substituted phenethylamines. In earlier reports,[14, 15] the catechol nucleus appeared to confer optimal activity while the presence of a β-hydroxyl group or large alkyl or aralkyl groups on the amino nitrogen enhanced the fat mobilizing activity of the agonist molecule. A sufficient number of analogues (thirty) are included in this paper to evaluate the chemical specificity shown by adipose tissue. This knowledge may be useful in the design of therapeutic agents as agonists or antagonists of FFA mobilization.

EXPERIMENTAL

Materials. The chemicals* employed and their sources are: tyramine HCl, DL-metanephrine HCl, dopamine HCl, l-phenylephrine HCl, *d,l*-isopropyl arterenol HCl, phenylpropanolamine HCl, β-phenylethylamine and DL-3-methoxy-4-hydroxymandelic acid (Mann Research Labs.); 3,4-dihydroxymandelic acid, 2-isopropylaminoethanol, adrenalone HCl, octopamine HCl, *N*-α-methylbenzylmonoethanolamine and *d*-amphetamine sulfate (K & K Labs.); *l*-nordefrin, *l*-ethylnorepinephrine *d*-bitartrate monohydrate and *l*-epinephrine bitartrate (Sterling–Winthrop Research Inst.); metaproterenol sulfate (Geigy Pharm.); nile blue A indicator (Chicago Apparatus); *l*-norepinephrine bitartrate and bovine albumin, fraction V (Nutritional Biochemical Corp.): norphenylephrine HCl and *N*-benzylethanolamine (Aldrich Chemical Co.); homarylamine HCl, phenisonone HBr, metaraminol bitartrate and ephedrine sulfate (Merck Sharp & Dohme Labs.); amidephrine methylsulfate and isoxuprine HCl (Mead Johnson Co.); nylidrin HCl (U.S. Vitamin Corp.) and protochylol HCl and *N*-methylepinephrine HCl (Lakeside Labs.). Unless stated otherwise, the compounds were used as racemates and their concentrations are expressed as the free acid or free base.

Methods. Nonfasted, white, male Sprague–Dawley and Holtzman rats weighing 220 ± 30 g were employed in this study. Animals were sacrificed by stunning and decapitation and the anterior one-third of the epididymal fat pad was transferred to Krebs–Ringer bicarbonate buffer (pH 7·4). After the pooling of fat pads from at least four rats for each experiment, the tissue was minced to yield pieces weighing about 5–15 mg. For each test, 600 mg of adipose tissue fragments was added to 9·0 ml of a buffer solution containing 4% albumin as an acceptor for the released FFA. The assay of FFA, incubation of fat pads and determination of FFA release as a function of agonist concentration were conducted by procedures previously described.[14]

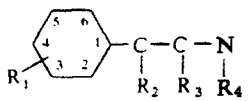
The rates of FFA release, expressed as μmoles of FFA released per gram of adipose tissue per hour of incubation, were calculated from the data obtained by serially sampling the incubation vessel at 0, 20, 40 and 60 min after the addition of the agonist. The FFA released in the absence of any agonist was subtracted from the amount released in the presence of an agonist. A maximal FFA release rate of 22·2 μmoles/g/hr was observed and employed to calculate the per cent response of adipose tissue to obtain the dose–response relationships. Each experiment was repeated three to seven times and the values plotted in the figures represent the mean FFA release rate ± S.E.

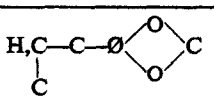
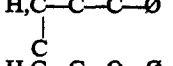
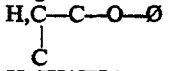
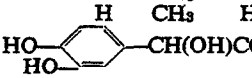
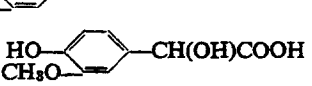
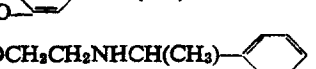
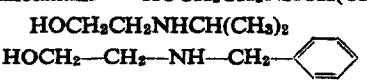
For each of the agonists studied, the affinity and intrinsic activity (efficacy) constants were determined and expressed according to the terminology described by

* The authors wish to thank colleagues in the pharmaceutical industry for supplying many of the chemicals employed.

Ariens and Simonis.[16, 17] The intrinsic activity constants are expressed as a ratio of the maximal response obtained with each agonist to the maximal response obtainable in the system and the affinity constants are expressed as pD_2 values,[18] defined as the negative logarithm of the agonist concentration required to produce a response equal to 50 per cent of its maximal response. In Table 1, the chemical structures of all compounds investigated are presented with their corresponding affinity and intrinsic activity constants.

TABLE 1. INTRINSIC ACTIVITY AND AFFINITY (pD_2) CONSTANTS FOR AGONISTS ON THE MOBILIZATION OF FREE-FATTY ACIDS FROM RAT ADIPOSE TISSUE



Compound	Name	R_1	R_2	R_3	R_4	I*	pD_2
1	Protochylol	3—OH,4—OH	OH	H		1·0	7·9
2	Isopropyl arterenol	3—OH,4—OH	OH	H	$H,CH(CH_3)_2$	1·0	7·6
3	Norepinephrine	3—OH,4—OH	OH	H	H,H	1·0	7·1
4	Epinephrine	3—OH,4—OH	OH	H	H,CH_3	1·0	7·1
5	Nordefrin	3—OH,4—OH	OH	CH_3	H,H	1·0	7·1
6	Nylidrin	4—OH	OH	CH_3		1·0	7·1
7	Isoxuprine	4—OH	OH	CH_3		1·0	6·1
8	Metaproterenol	3—OH,5—OH	OH	H	$H,CH(CH_3)_2$	1·0	6·1
9	Ethylnorepinephrine	3—OH,4—OH	OH	CH_2CH_3	H,H	1·0	5·8
10	Metaraminol	3—OH	OH	CH_3	H,H	1·0	5·2
11	Octopamine	4—OH	OH	H	H,H	1·0	4·7
12	Dopamine	3—OH,4—OH	H	H	H,H	1·0	4·4
13	Adrenalone	3—OH,4—OH	=O	H	H,CH_3	1·0	4·2
14	Norphenylephrine	3—OH	OH	H	H,H	1·0	4·2
15	Phenisonone	3—OH,4—OH	=O	CH_3	$H,CH(CH_3)_2$	1·0	3·6
16	*N*-methylepinephrine	3—OH,4—OH	OH	H	CH_3,CH_3	1·0	3·6
17	Phenylpropanolamine		OH	CH_3	H,H	0·37	4·7
18	Phenylephrine	3—OH	OH	H	H,CH_3	0·50	4·3
19	Amidephrine	3—$NHSO_2CH_3$	OH	H	H,CH_3	0·50	
20	Phenethylamine		H	H	H,H	0·0	
21	Metanephrine	3—OCH_3,4—OH	OH	H	H,CH_3	0·0	
22	Tyramine	4—OH	H	H	H,H	0·0	
23	Homarylamine	3,4-dioxymethylene	H	H	H,CH_3	0·0	
24	Ephedrine		OH	CH_3	H,CH_3	0·0	
25	Amphetamine		H	CH_3	H,H	0·0	
26	3,4-Dihydroxymandelic acid					0·0	
27	3-Methoxy-4-hydroxymandelic acid					0·0	
28	*N*-α-methylbenzylmonoethanolamine					0·0	
29	2-Isopropylaminoethanol	$HOCH_2CH_2NHCH(CH_3)_2$				0·0	
30	*N*-benzylethanolamine					0·0	

* Intrinsic activity constant.

RESULTS

The log dose–per cent response relationships obtained for various agonists are presented in Fig. 1–5. A comparison of the dose–response curves reveals that nearly all agonists were capable of producing a similar maximal response and that they differ primarily in their affinities or the concentrations required to mobilize FFA. The influence that structural modifications of the phenethylamine molecule had with respect to FFA mobilizing activities will be discussed with reference to the positions as designated in each figure.

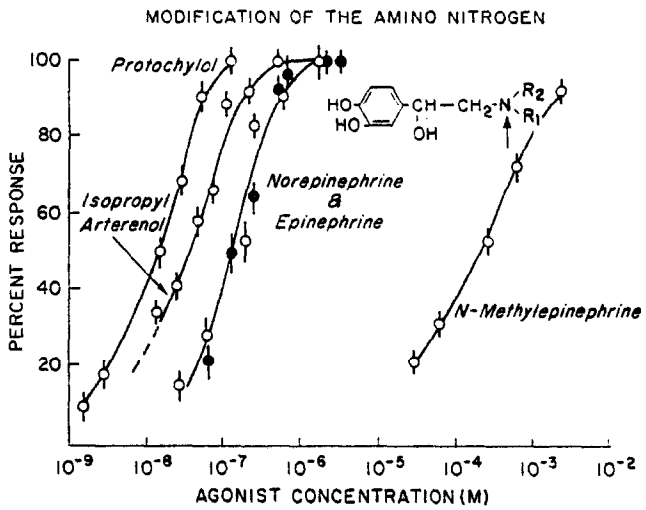


FIG. 1. Dose–response curves for various catecholamines on the mobilization of FFA from rat adipose tissue *in vitro*. Key: norepinephrine (●—●), and epinephrine (○—○).

Modification of the amino nitrogen. In Fig. 1, the dose–response curves of catecholamines which differ in the *N*-substituents are shown. Monosubstitution of large aralkyl or alkyl groups, as in protochylol and isopropyl arterenol, significantly increased the affinity of the agonist molecule when compared to NE. Although methylation of NE as in E did not alter the lipolytic response, formation of the dimethylated analogue, *N*-methylepinephrine, markedly decreased the affinity.

Modification of the α-carbon atom. In Fig. 2, methylation at this position did not influence the activity as shown by the dose–response curves of NE and nordefrin. The presence of an ethyl substituent as in ethylnorepinephrine, however, significantly, *N*-methylepinephrine, decreased the lipolytic response. Ethylnorepinephrine was found to be about twenty times less active than the equipotent agonists, nordefrin and NE.

Modification of the β-carbon atom. In Fig. 3, the results of the paired compounds NE-dopamine, E-adrenalone and octopamine-tyramine illustrate the importance of the β-hydroxyl group in lipolysis. Removal of the hydroxyl group from NE as in dopamine produced a 160-fold decrease in affinity while its removal from octopamine completely abolished lipolytic activity as indicated with tyramine. In addition, adrenalone, possessing a keto group instead of the β-hydroxyl as found in E, was approximately four hundred times less active in the mobilization of FFA.

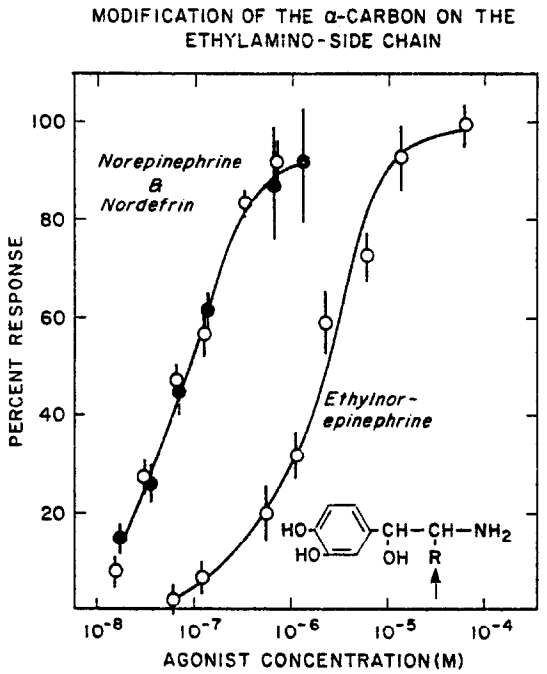


FIG. 2. Dose–response curves for nordefrin, norepinephrine and ethylnorepinephrine on the mobilization of FFA from rat adipose tissue *in vitro*. Key: norepinephrine (●—●), and nordefrin (○—○).

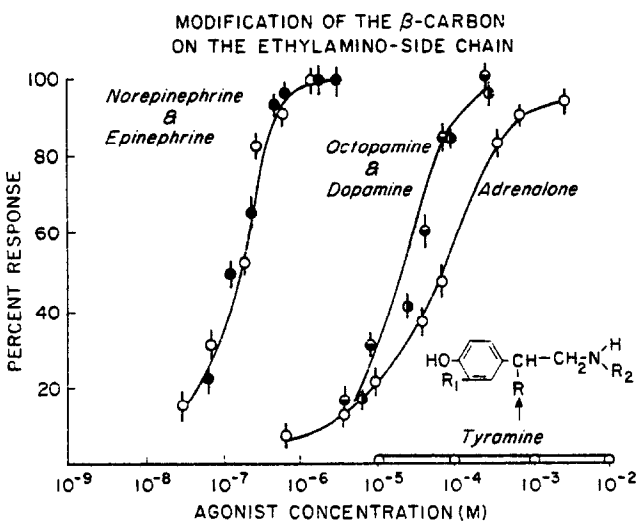


FIG. 3. Dose–response curves for various agonists on the mobilization of FFA from rat adipose tissue *in vitro*. Key: norepinephrine (●—●), octopamine (◒—◒), and dopamine (◑—◑).

Modification of the meta-ring position. The results for the paired compounds NE-octopamine, metaraminol-phenylpropanolamine and E-metanephrine are presented in Fig. 4. Removal of *meta*-hydroxyl from NE significantly decreased the affinity as observed by comparison with the results of octopamine. The loss of this phenolic group from metaraminol decreased the affinity and maximal response of the agonist molecule as indicated with phenylpropanolamine. Also, it was found that methylation of the *meta*-ring hydroxyl of E as in metanephrine completely abolished the lipolytic activity.

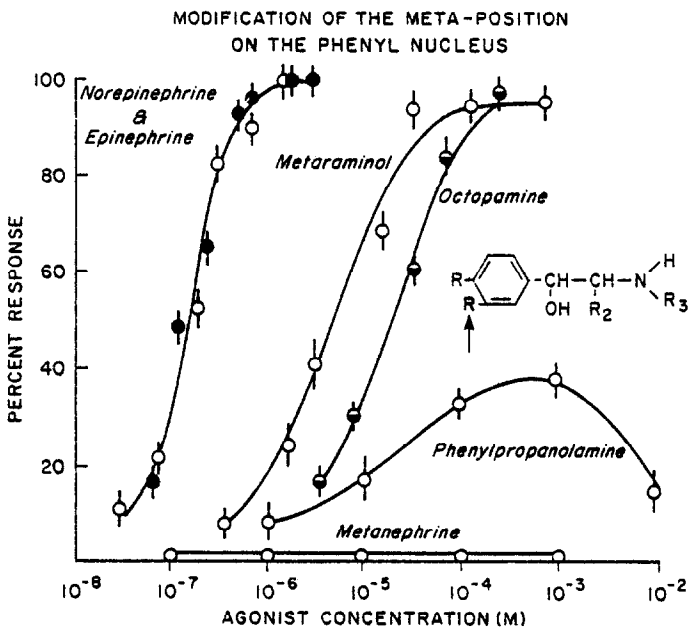


FIG. 4. Dose–response curves for various agonists on the mobilization of FFA from rat adipose tissue *in vitro*. Key: norepinephrine (●—●), and octopamine (◒—◒).

Modification of the para-ring position. In Fig. 5, the catecholamines, NE, E and nordefrin, were found to be indistinguishable in the mobilization of FFA; however, their corresponding noncatecholamines illustrate marked changes in the dose–response relationships. Metaraminol and norphenylephrine possess less affinity for adipose tissue than their *para*-hydroxylated analogues, nordefrin and NE. Phenylephrine, in addition to a reduction in affinity when compared to E, was unable to produce a maximal response.

Intrinsic activity and affinity constants. The data of several agonists were plotted in the double reciprocal manner to facilitate the calculation of the affinity and intrinsic activity constants from the slope and intercept values of the lines, respectively, and to verify the results obtained with the dose–response relationships. The chemical structures, intrinsic activity and affinity constants for the thirty phenethylamine analogues are summarized in Table 1. With the exception of phenylephrine, phenylpropanolamine and amidephrine, all agonists were able to stimulate a maximal FFA

release and thus possess an intrinsic activity constant of unity (1·0). Several inactive compounds are included and represented by intrinsic activity constants of zero (0·0). Structural modifications produced marked changes in the affinity constants (pD_2 values), ranging from 3·6 for the weaker agonists to 7·9 and 7·6 for the most active compounds, protochylol and isopropyl arterenol.

A significant decrease in pD_2 values produced by structural modification of E, NE, nordefrin or isopropyl arterenol was noted: (a) by the presence of a tertiary amine group (*N*-methylepinephrine); (b) by the removal of either ring-hydroxyl group (metaraminol, octopamine and norphenylephrine); (c) by the removal of the β-hydroxyl

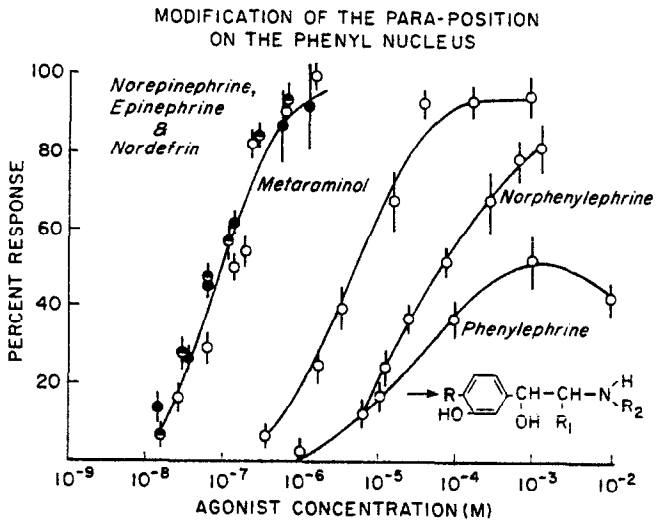


FIG. 5. Dose–response curves for various agonists on the mobilization of FFA from rat adipose tissue *in vitro*. Key: norepinephrine (●—●), and nordefrin (○—○).

group (dopamine) or the presence of a β-keto group (phenisonone and adrenalone); (d) by the presence of an α-ethyl substituent (ethylnorepinephrine); (e) by an alteration in the placement of the ring-hydroxyl groups (metaproterenol); and by the removal of the phenyl nucleus (*N*-α-methylbenzylmonoethanolamine, *N*-benzylethanolamine and 2-isopropylaminoethanol).

It can also be noted that methylation of the *meta*-ring hydroxyl group (metanephrine) and/or removal of the amino nitrogen (3,4-dihydroxymandelic acid and 3-methoxy-4-hydroxymandelic acid) of E abolished lipolytic activity. Phenethylamine analogues lacking a free phenolic group were either inactive (phenethylamine, homarylamine, amphetamine and ephedrine) or possessed little activity (amidephrine and phenylpropanolamine).

DISCUSSION

Several investigators[19–23] have shown that catecholamine-induced lipolysis occurs along pathways similar to the glycogenolytic processes of the liver. That is, the catecholamines initiate the release of FFA by interaction with adenyl cyclase, a cyclizing

enzyme which catalyzes the formation of adenosine 3',5'-monophosphate (3',5'-AMP) from ATP. The cyclic nucleotide is believed responsible for the conversion of an inactive lipase to an active lipase which in turn catalyzes the stepwise hydrolysis of triglycerides to yield FFA. Thus, the rate-limiting step is most likely related to the maximal concentration of 3',5'-AMP in adipose tissue after stimulation.

The dose–response and double-reciprocal relationships reported herein conform to the theory of interaction involving drug and receptor in a reversible complex.[16, 24] The affinity and intrinsic activity constants were employed as parameters of the interaction to establish the relationship between structural modification of the phenethylamine molecule and the ability of the agonist to mobilize FFA *in vitro*.

In the present study, all catecholamines were found capable of producing a maximal release of FFA as indicated by the intrinsic activity constants (Table 1). The optimal activity exhibited by certain monohydroxylated-ring analogues implied that the catechol nucleus was *not* obligatory for effective FFA mobilization. Results pointing out (a) the importance of the β-hydroxyl and ring-hydroxyl groups in the binding of the phenethylamine for adipose tissue receptors and (b) the observed compatibility of primary and secondary amines with lipolysis correlates with earlier reports in humans[11] and in hamster fat tissue.[13] Other small structural changes on the ethylamino-side chain of the catecholamine markedly altered the ability to mobilize FFA. Significant decreases in relative affinity were associated with the presence of a tertiary amine, α-ethyl or β-keto group in the catecholamine molecule, whereas methyl substitution on the amino nitrogen or α-carbon atom did not alter the biological activity.

The characterization of catecholamine-induced lipolysis according to Ahlquist's[25, 26] classification of *alpha* or *beta* adrenergic receptors has met with conflicting views. Nevertheless, it should be pointed out that the chemical specificity shown by adipose tissue has revealed a tendency to respond more favorably to the *beta*-agonists. As reported,[12, 15, 27] the enhancement of affinity obtained by the addition of bulky *N*-monosubstituents to various phenethylamines is characteristic of a *beta*-type adrenergic receptor. Thus, of the *alpha*-agonists studied, phenylpropanolamine, phenylephrine and amidephrine exhibited weak activity whereas only NE was an active compound. In addition to isopropyl arterenol, the *beta*-agonists, nylidrin, isoxuprine, metaproterenol and protochylol, were potent lipolytic agents.

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