Interaction of Various Phenethylamines with the Adrenergic-Adipose Tissue Receptor System, *In Vitro*

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The interaction of several phenethylamines with the rat adipose tissue adrenergic receptor system has been studied utilizing the release of free fatty acids as an index of the interaction. It has been established that structural modifications of the basic phenethylamine molecule produced marked changes in the affinity of the agonists for this system and, to a lesser degree, in the intrinsic activity constants. *N*-Substitution of large alkyl functions increased affinity most markedly. Hydroxylation of the β carbon of the ethylamino side chain also enhanced affinity, while changes in the ring substitution produced changes varying from complete loss of all activity to alterations in the affinity constants. Phenethylamine, tyramine, ephedrine, amphetamine, and metanephrine were found to be inactive in this system. The catechol nucleus appears to confer optimal activity upon the agonist molecule, but structural modifications, particularly upon the side chain nitrogen, markedly affect the ability of the molecule to release free fatty acids from adipose tissue, *in vitro*.

RECENT STUDIES have indicated the increasing importance of the mobilization of free fatty acids (FFA) in (*a*) normal body metabolism as a primary energy source (1, 2) and (*b*) as a factor or contributing factor in certain disease states (3). For these reasons, considerable research emphasis has most recently been placed on studies designed to elucidate the controlling mechanisms involved in the mobilization of FFA from triglycerides in adipose tissue.

The fact that the sympathetic nervous system and in particular the catecholamines play an important role in the mobilization of FFA has been recognized for a number of years (4, 5). Recent work has shown that the catecholamine-induced mobilization of FFA occurs along pathways similar to those elucidated by Sutherland and Rall (6) for the catecholamine-induced glycogenolytic processes of liver, *i.e.*, it appears that the catecholamines stimulate the conversion of ATP to 3′,5′-cyclic AMP, which, in turn, catalyzes the conversion of an inactive lipase to an active lipase (7, 8). The lipolytic enzymes thus activated catalyze the stepwise hydrolysis of triglycerides to yield free fatty acids.

Many studies have been reported concerning the effects of catecholamines on the release of free fatty acids both *in vivo* and *in vitro*. However, few, if any, have defined the structure-activity relationships involved in the interaction of catecholamines with the adipose tissue-adrenergic receptor or have provided a sufficient degree of quantitation to allow such relationships to be made. It is the purpose of this report to present findings with respect to the structure-activity relationships involved in the interaction of substituted phenethylamines with the rat epididymal fat tissue, *in vitro*. This knowledge is deemed important for an understanding of the nature of drug–receptor interaction in this tissue and for the development of highly selective agonists and antagonists of FFA release in the future.

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EXPERIMENTAL

Materials.—The chemicals[1] employed in this study and their source of supply are as follows: tyramine HCl, *d,l*-metanephrine HCl, dopamine HCl, *l*-phenylephrine HCl, *l*-isopropyl arterenol HCl, phenylpropanolamine HCl, and β-phenethylamine (Mann Research Laboratories); metaraminol bitartrate and ephedrine sulfate (Merck, Sharp & Dohme Laboratories); isoxuprine HCl (Mead Johnson Laboratories); nylidrin HCl (U. S. Vitamin Corp.); *l*-epinephrine bitartrate (Winthrop Laboratories); protochylol (Lakeside Laboratories); dextroamphetamine sulfate (K & K Laboratories); *l*-norepinephrine bitartrate and bovine albumin, fraction V (Nutritional Biochemicals Corp.). All concentrations expressed in this paper refer to the free base.

Methods.—Nonfasted, male Sprague–Dawley rats, weighing between 200 and 250 Gm., were maintained in their animal quarters at least 1 week prior to being employed in an experiment. The animals were sacrificed by stunning and decapitation. The anterior one-third of the epididymal fat pads were rapidly removed, placed in freshly prepared Krebs-Ringer bicarbonate buffer (pH 7.4), and minced with a small scissors to yield pieces weighing 5–10 mg. Tissue slices from six rats were pooled for each experiment.

Incubations of fat pads and the determination of the rate of FFA release as function of agonist concentration were conducted by procedures previously described (9).

[1] The authors thank their colleagues in the pharmaceutical industry for generously supplying many of the chemicals used in this study.

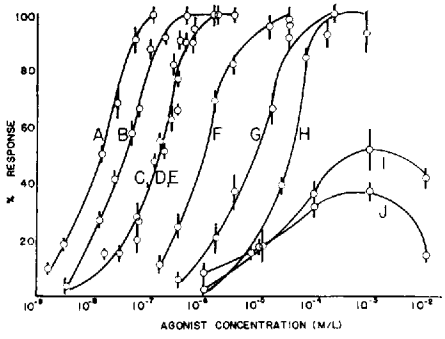


Fig. 1.—Dose-response curves for various phenethylamine agonists on mobilization of FFA from rat epididymal fat tissue, *in vitro*. Key: A, protochylol; B, isopropyl arterenol; C, epinephrine; D, norepinephrine; E, nylidrin; F, isoxuprine; G, metaraminol; H, dopamine; I, phenylephrine; J, phenylpropanolamine. From four to six determinations were made at each agonist concentration for all compounds presented in this paper. The values plotted represent the mean ± the standard error as indicated by the vertical lines.

The rates of FFA release, expressed in terms of μmoles of FFA released/Gm. of adipose tissue/hr., were calculated from the data obtained by serially sampling the incubation vessel at 0, 20, 40, and 60 min. after addition of the agonist to the media. In these studies, a maximal rate of FFA release of 18 μmoles/Gm./hr. was obtained and was found to be independent of the agonist employed. This maximal figure was employed to calculate the per cent response of the system in all studies described in this report.

RESULTS AND DISCUSSION

Dose - Response Relationships.—The dose-response relationships obtained for the active compounds employed in this study are presented in Fig. 1. It is apparent from these data, that with the exception of phenylephrine and phenylpropanolamine, all compounds were capable of producing a maximal release of FFA from adipose tissue slices, *in vitro*. The compounds varied, however, in their relative ability to mobilize FFA. Thus, in considering compounds A through H shown in Fig. 1, the rank order of compounds listed in order of decreasing potency is protochylol > isopropyl arterenol > epinephrine = norepinephrine = nylidrin > isoxuprine > metaraminol > dopamine. Phenylpropanolamine (compound J) and phenylephrine (compound I) possess properties which sharply differentiate them from the others. Thus, these latter two molecules show the property of auto-inhibition which manifests itself in a decrease in FFA release as the concentration of agonist is increased beyond a certain value.

Analysis of these data suggest that the catechol nucleus conveys optimal activity upon the molecule in agreement with the postulates of Belleau (10) regarding the interaction of catecholamines and ATP at the adrenergic receptor. Structural modifications of the catecholamine structure, however, produced pronounced alterations in the relative ability of these moieties to mobilize FFA from the adipose tissue slices. Norepinephrine and dopamine differ only in the presence or absence of the hydroxyl function on the β carbon of the side chain, yet these two compounds differ by a factor of about 160 in their activity in mobilizing FFA. Thus, side chain hydroxylation appears to increase FFA mobilizing activity.

Similarly, *N*-substitution of large alkyl functions enhanced activity as can be seen by comparing the dose-response curves for protochylol and isopropyl arterenol with those of epinephrine and norepinephrine. Indeed, the larger *N*-substitution found in protochylol enhanced its potency to a value approximately twice that of isopropyl arterenol, the compound previously found to be the most potent mobilizer of FFA in this system (9). Methylation

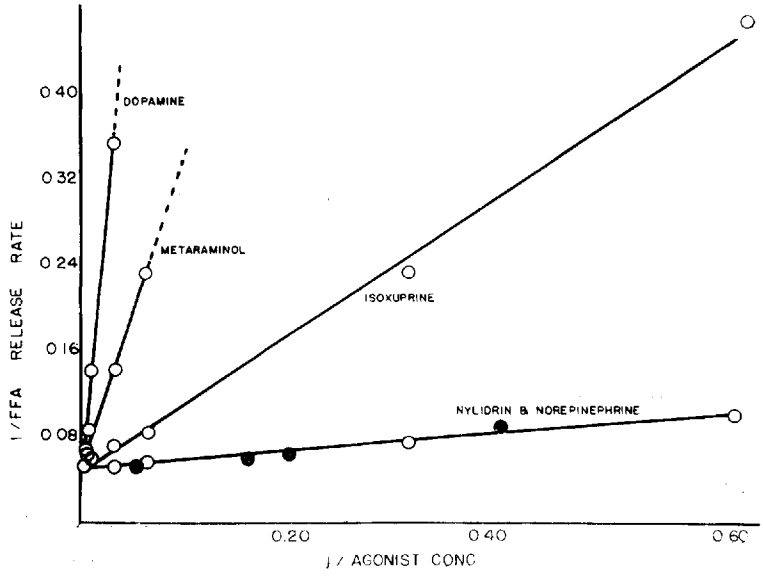


Fig. 2.—Double reciprocal plot illustrating effects of agonists on the rate of FFA release, *in vitro*. Concentrations of agonists were in terms of moles/L. × 10^{-7}.

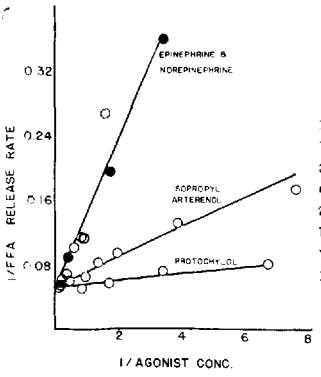


Fig. 3.—Double reciprocal plot illustrating effects of agonists on the rate of FFA release, *in vitro*. Concentrations of agonists were in terms of moles/L. $\times 10^{-7}$.

of the amino nitrogen did not, however, produce a statistically significant alteration in the dose-response relationship (compare epinephrine with norepinephrine).

The influence of *N*-substitution is also evident when one compares the dose-response relationships found for the noncatecholamines tested. Nylidrin, a compound lacking a catechol nucleus but possessing a rather large substituent on the side chain nitrogen, was found to be equipotent with epinephrine and norepinephrine while isoxuprine was found to be approximately one-fifth as potent as epinephrine or norepinephrine. The effect of this type of molecular alteration is also evident when comparing the dose-response relationships of nylidrin and isoxuprine with the other noncatecholamines shown in Fig. 1, namely metaraminol, phenylpropanolamine, and phenylephrine.

In contrast to the above-mentioned alterations in dose-response curves obtained by varying chemical structure, substitution on the α carbon of the side chain did not appear to alter biological activity in any significant manner.

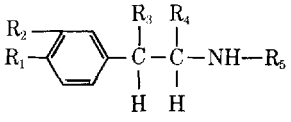
Double Reciprocal Relationships.—The equation described by Ariens (11) relating the relative response of a tissue to an agonist and the concentration of agonist employed has been modified slightly to yield

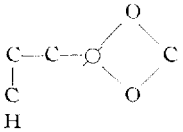
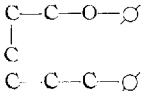
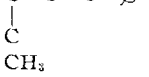
$$R = \frac{(I)(A)}{Ka + A}$$

where R is the response of the tissue measured as the rate of FFA release/Gm. of tissue/hr., I is the intrinsic activity constant or maximal response obtainable at an infinity concentration of the agonist, A is the concentration of agonist employed, and Ka is the apparent dissociation constant of the agonist–receptor tissue complex. Placing the above expression in the double reciprocal form yields

$$1/R = (Ka/I)(1/A) + 1/I$$

Table I.—Intrinsic Activity and Affinity Constants for Substituted Phenethylamines on the Mobilization of FFA from Rat Adipose Tissue



Compd.	Name	R_1	R_2	R_3	R_4	R_5	I^a	pD_2
1	Epinephrine	OH	OH	OH	H	CH_3	1.0	6.8
2	Norepinephrine	OH	OH	OH	H	H	1.0	6.8
3	Isopropyl arterenol	OH	OH	OH	H	$CH(CH_3)_2$	1.0	7.6
4	Protochylol	OH	OH	OH	H		1.0	7.9
5	Dopamine	OH	OH	H	H	H	1.0	4.4
6	Isoxuprine	OH	H	OH	CH_3		1.0	6.1
7	Nylidrin	OH	H	OH	CH_3		1.0	6.8
8	Phenylephrine[b]	H	OH	OH	H	CH_3	0.5	4.3
9	Metaraminol	H	OH	OH	CH_3	H	1.0	5.2
10	Phenylpropanolamine[b]	H	H	OH	CH_3	H	0.37	4.7
11	Phenethylamine	H	H	H	H	H	0.0	...
12	Tyramine	OH	H	H	H	H	0.0	...
13	Ephedrine	H	H	OH	CH_3	CH_3	0.0	...
14	Amphetamine	H	H	H	CH_3	H	0.0	...
15	Metanephrine	OH	OCH_3	OH	H	CH_3	0.0	...

[a] Intrinsic activity constant. [b] Compound shows properties of a dualist or partial agonist.

Plotting the data as $1/R$ *versus* $1/A$ yields a straight line with a slope numerically equal to Ka/I and an intercept of $1/I$. Analysis of the data in this manner provides an efficient means of calculating the intrinsic activity constant (I) and the affinity constant (the reciprocal of the apparent dissociation constant for the drug–receptor tissue complex, Ka) from the intercept and the slope of the line, respectively. The data obtained in this study and plotted in this manner are shown in Figs. 2 and 3. The data for norepinephrine have been included in both graphs for comparative purposes.

In Fig. 2, it is apparent from the steep slopes of the dopamine and metaraminol lines that these compounds are relatively weak agonists in this system as compared to isoxuprine, nylidrin, and norepinephrine. It is also apparent that all curves intercept the y-axis at a common point, indicating equal intrinsic activities.

In Fig. 3, a different scale for the x-axis was employed to permit the plotting of the data for the more active agonists in the double reciprocal manner. It is apparent in this graph that (*a*) the lines extrapolate to an intercept identical with that obtained in Fig. 2 indicating the same intrinsic activities for all compounds, and (*b*) in this system, the highest affinities were shown by protochylol and isopropyl arterenol, both compounds being clearly more potent than epinephrine and norepinephrine.

Structure–Activity Relationships.—The structure-activity relationships obtained in this study have been summarized in Table I. In this table, the intrinsic activity constants are expressed as ratios of the maximal response obtained with an agonist to the maximal response obtainable in the system (11), and the affinity constant is expressed as the pD_2 of Miller, Becker, and Tainter (12), defined as the negative logarithm of the agonist concentration required to produce a response equal to 50% of the maximal response obtainable in the system. These constants can be derived from either the dose-response relationships shown in Fig. 1 or from the intercept and slope value of the lines shown in Figs. 2 and 3. Inactive compounds have also been included in the table of data.

The data summarized in Table I indicate the effects structural modification of the basic phenethylamine structure had on the ability of the compounds to stimulate the mobilization of FFA from adipose tissue, *in vitro*. The parent compound, phenethylamine, was found to be completely inactive in this system, thus possessing zero intrinsic activity. Para-hydroxylation (tyramine), or α carbon methylation (amphetamine), did not increase activity to a measurable level, while β-carbon hydroxylation along with α-carbon methylation had variable results. (Compare the inactive ephedrine with the slightly active phenylpropanolamine.) Placing a hydroxyl function in the 3 position of the ring significantly increased FFA mobilizing activity (metaraminol). Ring hydroxylation in the *para* position coupled with N-substitution markedly enhanced FFA mobilizing activity (isoxuprine and nylidrin). The catechol nucleus appeared to confer optimal activity upon the molecule, however, as seen by the relatively high affinity constants possessed by these type compounds (epinephrine and norepinephrine). The affinity of the catecholamine can be enhanced, however, by N-substitution of large or bulky alkyl functions (isopropyl arterenol and protochylol) and diminished by removal of the β-hydroxyl group of the side chain (dopamine). Methylation of 3-OH group of epinephrine completely abolished FFA mobilizing activity (metanephrine).

CONCLUSION

It is concluded from these studies that (*a*) while the catechol nucleus appeared to confer optimal activity upon the agonist molecule, significant mobilization of FFA from rat epididymal fat tissue was achieved by monohydroxylated ring structures, (*b*) the hydroxyl function on the β carbon atom of the side chain played an important role in determining the affinity of the compound for the adipose tissue receptor system, (*c*) N-substitution of large alkyl functions greatly enhanced fat mobilizing activity, and (*d*) compounds whose action is mediated by release of endogenous catecholamines were not active in this system. Furthermore, from the information obtained in this study, it appears that both α- and β-type adrenergic stimulants are capable of stimulating the mobilization of FFA but that the β-type adrenergic stimulants are much more active in this regard than are the α stimulants.

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