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Relative lipolytic activities *in vitro* of a series of phenethyl amines

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THE RELATIVE *in vitro* and *in vivo* lipid mobilizing effects of epinephrine, norepinephrine, and, in some instances, isoproterenol have been reported by numerous investigators.[1–17] While comparing the relative lipolytic effects of these and related phenethyl amines, it was noted[18, 19] that their activity in the release of free fatty acids *in vitro* from adipose tissue could be shown to correlate significantly with their cardiac effects, while differing from their vasodepressor and bronchodilator effects. The lipolysis data *in vitro*, on which the earlier discussions[18, 19] were based, along with structure–activity comparisons subsequently obtained, are given in this communication.

EXPERIMENTAL

The method *in vitro* followed that of Černohorsky *et al.*[20] and utilized epididymal fat tissue of overnight fasted Sprague–Dawley strain rats generally weighing about 175 g each. The rats were sedated with pentobarbital prior to sacrifice by decapitation using a guillotine. For the test, 75-mg portions of finely minced fat pads were incubated (at 37°) per ml of Krebs–Ringer phosphate buffer (pH 7·4), which contained, in addition, 5% bovine albumin (Fraction V) and varying concentrations of the phenethyl amines. Samples were taken at 30 and 60 min, the reaction was stopped with trichloroacetic acid, and the extent of lipolysis was measured as glycerol (1·5 to 12 μg per 0·5-ml aliquot), which was determined by the method of van Handel and Zilversmit.[21] The activities of the amines were calculated[22] relative to *l*-norepinephrine on a molar basis from the dose : response lines by using the mean of the 30-min (corrected to 60 min) and 60-min glycerol released. In some instances limited supplies of test material precluded more than two multiple-level tests.

RESULTS AND DISCUSSION

The results summarized in Table 1 show that, with the exception of the *N*-methylated compound,

TABLE 1. RELATIVE LIPOLYTIC EFFECTS *in vitro* OF SOME PHENETHYL AMINES

Phenethyl amines	No. of tests	No. of levels each test	Activity Rel. to *l*-NE = 1·0*
l-Norepinephrine, *l*-arterenol			1·0
l-Isoproterenol, *l*-*N*-isopropylnorepinephrine	7	3	11·7
d-*N*-isopropylnorepinephrine	3	3	0·003
dl-*N*-1,4-dimethylpentylnorepinephrine	4	4	5·97
dl-*N*-*sec*.butylnorepinephrine	3	5	3·06
l-*N*-ethylnorepinephrine	4	3–4	2·75
d-*N*-ethylnorepinephrine	4	2	0·005
dl-*N*-cyclopentylnorepinephrine	2	4	2·51
dl-*N*-1,3-dimethylbutylnorepinephrine	4	4	1·93
l-Nordefrine, *l*-α-methylnorepinephrine	5	3–4	1·67
dl-*N*-*tert*.butylnorepinephrine	7	3	1·04
dl-*N*-cyclopentyl-α-methylnorepinephrine	4	3–5	0·79
Epinephrine	5	3–4	0·72
d-Epinephrine	5	2–3	0·009
dl-*N*-methyl-α-methylnorepinephrine	4	4	0·53
dl-*N*-isopropyl-α-methylnorepinephrine	4	4	0·33
dl-*N*-cyclopentyl-α-ethylnorepinephrine	3	3–4	0·076
Isoetharine, *dl*-*N*-isopropyl-α-ethylnorepinephrine	5	2	0·051
N-Isopropyldopamine	4	4	(0·026)†
l-Octopamine	3	1–4	0·02
dl-*N*-cyclopropyl-α-ethylnorepinephrine	3	4	0·01
N-*n*-butyldopamine	2	2–3	(<0·01)†
dl-*N*-*tert*.butyl-α-ethylnorepinephrine	2	1–4	0·008
Dopamine, oxytyramine	3	1–3	0·002
l-Metaraminol	5	1–3	(<0·001)†
l-Phenylephrine	2	1	<0·001
l-Dopa	2	1–2	inactive

* Molar basis.
† Estimates approximate only; response slopes divergent.

epinephrine, *N*-substitution increased the lipolytic effectiveness of the catecholamines. In nordefrine, α-methyl carbon substitution increased the lipolytic effectiveness of the unsubstituted amine. In three instances α-methyl carbon substitution decreased the lipolytic effectiveness of the catecholamines. α-Ethyl carbon substitution markedly decreased the activity of the resultant amines, in certain compounds, to amines with negligible activity. The amines with fewer than two phenolic hydroxyls or amines lacking an α-carbon hydroxyl also showed decreased activity.

These results are qualitatively similar to those noted earlier by others, but extended in some respects. We note, as have others,[4, 6, 8, 9, 12–15] that *l*-norepinephrine and *l*-epinephrine exhibit the same order of lipolytic activity, as seen from the representative data of Table 2, though some[7, 10, 17] have reported *l*-norepinephrine to be twice as active lipolytically as *l*-epinephrine. In agreement with Lech

TABLE 2. RELATIVE LIPOLYTIC EFFECTIVENESS *in vitro* OF γ-NOREPINEPHRINE AND EPINEPHRINE ON THE BASIS OF 5 TESTS

Amine	Concentration (M)	No.	Glycerol released† (μmole/g tissue/hr)	Relative activity‡
l-Norepinephrine	$8{\cdot}1 \times 10^{-8}$	2	0·15	
	$2{\cdot}7 \times 10^{-7}$	5	0·94 ± 0·52	
	9×10^{-7}	5	2·46 ± 0·54	
	3×10^{-6}	4	3·175 ± 0·36	
	10^{-5}	3	3·97 ± 0·23	1·0
Epinephrine	$8{\cdot}1 \times 10^{-8}$	2	0·2	
	$2{\cdot}7 \times 10^{-7}$	5	1·0 ± 0·58	
	9×10^{-7}	5	1·98 ± 0·57	
	3×10^{-6}	5	2·72 ± 0·62	
	10^{-5}	3	3·3 ± 0·25	0·72

* Generally four levels of amine were used in each test. The same four levels of amine were not necessarily used in all tests, however.

† Mean of glycerol released after 30 and 60 min; mean ± S.D.

‡ Relative dose : response activity calculated on the basis of the individual values.

and Calvert,[17] we noted that isoproterenol is considerably more active than *l*-norepinephrine, i.e., 11·7-fold compared with their 20-fold. Barrett[14] reported isoproterenol to be 3 times as effective as *l*-norepinephrine *in vivo*.

In summary, these data serve as one basis for correlating lipolytic activities with other biochemical and pharmacological activities of the phenethyl amines, especially as regards differentiation of the β-1 lipolytic–cardiac adrenergic receptor from the β-2 vasodepressor–bronchodilator one.[18, 19]

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The binding of colchicine by sarcoma 180 cells

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THE METAPHASE arrest of dividing cell populations that is produced by colchicine was first described more than 30 yr ago,[1] but the biochemical mechanism of this action remains unknown. Anu mber of biochemical changes produced by colchicine have been described.[2–6] They include inhibition of the synthesis of DNA and of RNA and protein, a reduction in the level of ATP in regenerating liver, and increased formation of cytosine nucleotides. None of these effects, however, has been related directly to mitotic arrest, which involves some structural deformation.[7] Another biochemical approach to this problem is to study the intracellular disposition of colchicine itself. Taylor[8] has described the kinetics of the uptake and loss of colchicine-^{3}H in cultures of human KB cells, and suggested that the drug may be bound to intracellular components. Further evidence of such binding in grasshopper embryos has been obtained by Wilson and Friedkin.[9] We have studied the uptake of colchicine-^{3}H by sarcoma 180 (S180) cells and its intracellular distribution in order to determine if some type of binding does indeed occur in mammalian cells.

S180 in the ascites form was carried in Swiss mice (CD-1, 25–30 g). The ascitic fluid was withdrawn, contaminating erythrocytes were lysed by exposure to hypotonic saline (0·2%), and the cells finally resuspended in Eagle's medium. Colchicine was custom tritiated by Schwarz BioResearch, Inc. Extensive purification was carried out on this material, first by chromatography on columns of silicic acid that were eluted with mixtures of chloroform and methanol, and then by thin-layer chromatography on 0·5 mm layers of silica gel HF_{254} (Merck). The plates were eluted with three solvents: methanol, chloroform–isopropanol (3 : 1, v/v), and *n*-butanol–95% ethanol–concentrated ammonium hydroxide–water (4 : 1 : 2 9 1, v/v). The colchicine-^{3}H obtained has a sp. act. of 112·9 mc/ m-mole.

Tumor cells (2·1 to 4·4 × 10^8 cells) in 16 ml of Eagle's medium were incubated at 37° with 0·04 μmole of colchicine-^{3}H for up to 120 min. The cells were separated from the medium by centrifuging through sucrose solution (0·25 m) in Shevky-Stafford and McNaught centrifuge tubes.[10] Drug entered the cells rapidly, reaching maximum intracellular levels in about 20 min. After extraction of the cell with cold perchloric acid (0·5 M), a fraction of the radioactivity remained in the residue, but could be released in acid-soluble form by heating with sodium hydroxide (0·2 M) for 30 min at 90°. The amount of this bound fraction increased during the 120-min incubation to about 7 per cent of the total intracellular radioactivity. Similar findings have been reported with another antimitotic alkaloid, vinblastine.[11] Although alkaline hydrolysis decomposes RNA, the bound radioactivity that is released by such treatment does not appear to be associated with this nucleic acid. When the residues obtained by washing cells exposed to colchicine-^{3}H with cold acid were neutralized and incubated with ribonuclease or deoxyribonuclease, so as to produde almost complete hydrolysis of RNA or DNA, the