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The Chemorelease of Norepinephrine from Mouse Hearts. Structure–Activity Relationships. I. Sympathomimetic and Related Amines

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A rapid method for determining the chemorelease of cardiac norepinephrine in the mouse has been developed. Endogenous cardiac norepinephrine is prelabeled with 5 μcuries of norepinephrine-7-^{3}H. The cardiac norepinephrine-^{3}H remaining in the heart after 3 hr averages 340 mμcuries. Lower levels of radioactivity are found in heart when animals are injected 1 hr after the norepinephrine-^{3}H with a compound that causes chemorelease. The releasing potencies of a wide variety of sympathomimetic amines and related compounds have been determined. Structure–activity correlations are discussed.

The general importance of catecholamines and, in particular, norepinephrine in the function of the cardiovascular and central nervous system has prompted investigations of drugs which effect changes in the disposition and metabolism of such amines. Many of these drugs have now become indispensible in the treatment of hypertension and nervous disorders. Thus the tranquilizing and hypotensive effect of reserpine has been linked to its depletion of biogenic amines in central stores[1] and of norepinephrine in sympathetically innervated tissues of the cardiovascular system.[2] Similarly, the hypotensive α-methyl aromatic amino acids, such as α-methyldopa, originally synthesized as inhibitors of aromatic amino acid decarboxylase, have been shown to owe their hypotensive action to a long-lasting depletion of norepinephrine stores by release of the endogenous amine rather than by inhibition of its synthesis.[3] Other hypotensive agents, such as guanethidine,[4] bretylium,[5] and the monoamine oxidase inhibitors,[6] also effect the storage and/or release of norepinephrine.

The sympathomimetic amines are another class of compounds which owe their physiological actions in large part to their effect on norepinephrine stores. On the basis of classical studies, the sympathomimetic amines in various sympathetically innervated tissues have been divided into three groups: 1, amines with direct action; 2, amines with indirect action; 3, mixed function amines.[7] The first class of amines interact directly with the receptor sites, while the amines with indirect action owe their pharmacological effect to release of norepinephrine. This free norepinephrine is the actual initiator of the pharmacological response. Both activities are manifest in the mixed function amines. Many sympathomimetic amines which release norepinephrine are themselves retained in storage sites as "false transmitters."[8]

The release (depletion) of norepinephrine is of diagnostic value for the pharmacology of many drugs. The existing methods used for assaying norepinephrine levels are time consuming and require large numbers of animals in order to obtain significant results.[9] Axelrod and co-workers[10] have described a more convenient method for studying cardiac levels of norepinephrine. They have shown in rats that tracer amounts of radioactive norepinephrine are taken up in the heart, equilibrated with endogenous norepinephrine, and affected in a similar manner by agents that cause or inhibit release. A rapid, simple, economical, and reliable method for the correlation of structure with activity has now been developed in mice. Mice weighing 18 g or less have been found to be best suited for this screening program. The stores of endogenous norepinephrine are prelabeled with an intravenous injection of norepinephrine-^{3}H. The decline in total radioactivity in the heart over a period of 3 hr is reproducible and a convenient measure for the additional norepinephrine released by sympathomimetic amines. New classes of compounds which cause or inhibit the release of norepinephrine[11] have been detected by this method.

(1) B. B. Brodie, J. S. Olin, R. Kuntzman, and P. A. Shore, *Science*, **125**, 1293 (1957).

(2) A. Carlsson, E. Rosengren, A. Bertler, and J. Nilsson in "Psychotropic Drugs," S. Garattini and V. Ghetti, Ed., Elsevier Publishing Co., Amsterdam, 1957, p 363.

(3) A. Carlsson and M. Lindqvist, *Acta Physiol. Scand.*, **54**, 83 (1962).

(4) R. Cass, R. Kuntzman, and B. B. Brodie, *Proc. Soc. Exptl. Biol. Med.*, **103**, 870 (1960).

(5) A. L. A. Boura and A. F. Green, *Brit. J. Pharmacol.*, **14**, 536 (1959).

(6) P. A. Shore, *Pharmacol. Rev.*, **14**, 531 (1962).

(7) U. Trendelenburg, *ibid.*, **15**,225 (1963).

(8) (a) I. J. Kopin, J. E. Fischer, J. M. Musacchio, W. D. Horst, and V. Weise, *J. Pharmacol. Exptl. Therap.*, **147**, 186 (1965); (b) E. Muscholl and L. Maitre, *Experientia*, **29**, 658 (1963).

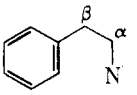
(9) (a) U. S. von Euler, *Pharmacol. Rev.*, **11**, 262 (1959); (b) H. Weil-Malherbe, *ibid.*, **11**, 278 (1959).

(10) (a) G. Hertting, J. Axelrod, and R. W. Patrick, *Biochem. Pharmacol.*, **8**, 246 (1961); (b) J. Axelrod, G. Hertting, and L. Potter, *Nature*, **194**, 297 (1962); (c) L. T. Potter and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **140**, 199 (1963); (d) G. Hertting in "Pharmacology of Cholinergic and Adrenergic Transmission," G. B. Koelle, W. W. Douglas, and A. Carlsson, Ed., Pergamon Press Inc., New York, N. Y., 1965, p 277.

(11) J. Daly, C. R. Creveling, and B. Witkop, *J. Med. Chem.*, **9**, 280 (1966).

Table I

The Chemorelease of Norepinephrine-^{3}H from Mouse Hearts by Catecholamines and Related Compounds[a]



Compd	Substituent β	Substituent α	Substituent N	Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control
2,3-Dihydroxyphenethylamine HCl	...	...	...	5	43
2,3-Dihydroxy-N,N-dimethylphenethylamine HCl	...	...	$(CH_3)_2$	10	84
2,3-Dihydroxy-N,N-dimethylphenethylamine methochloride	...	...	$(CH_3)_3$	10	65
Dopamine HCl	...	...	...	5	50
3,4-Dihydroxy-N-methylphenethylamine HCl	...	...	CH_3	5	55
				10	31
3,4-Dihydroxy-N,N-dimethylphenethylamine HCl	...	...	$(CH_3)_2$	10	93
3,4-Dihydroxy-N,N-dimethylphenethylamine methochloride	...	...	$(CH_3)_3$	10	100
3,4-Dihydroxy-N-(2-hydroxyethyl)phenethylamine HCl	...	...	CH_2CH_2OH	10	84
3,4-Dihydroxy-N-benzylphenethylamine HCl	...	...	C_7H_7	10	88
3,4-Dibenzoxy-N-methylphenethylamine HCl	...	...	CH_3	10	42
3,4-Diacetoxy-N-methylphenethylamine HCl	...	...	CH_3	10	30
3,4-Di-O-carboethoxy-N-methylphenethylamine HCl	...	...	CH_3	10	31
3,4-Di-O-carboethoxy-N,N-dimethylphenethylamine HCl	...	...	$(CH_3)_2$	10	98
3,4-Di-O-carboethoxy-N,N-dimethylphenethylamine methochloride	...	...	$(CH_3)_3$	10	96
α-Methyldopamine HCl	...	CH_3	...	5	39
3,4-Dihydroxy-N-ureidophenethylamine	...	...	$CONH_2$	10	97
6-Hydroxydopamine HBr	...	...	...	5	28
6-Hydroxynorepinephrine	OH	...	...	5	41
6-Hydroxyepinephrine	OH	...	CH_3	5	43
6-Methoxydopamine HCl	...	...	...	5	80
3,4,5-Trihydroxyphenethylamine HCl	...	...	...	5	22
3-Methoxy-4,5-dihydroxyphenethylamine HCl	...	...	...	5	87
d-Norepinephrine bitartrate	OH	...	...	2.5	57
l-Norepinephrine bitartrate	OH	...	...	2.5	33
l-3,4-Dihydroxynorephedrine	OH	CH_3	...	2.5	20
α-Ethylnorepinephrine HCl	OH	C_2H_5	...	2.5	38
N-Acetylnorepinephrine	OH	...	$COCH_3$	10	88
β-O-Methylnorepinephrine HCl	OCH_3	...	...	5	60
d-Epinephrine bitartrate	OH	...	CH_3	2.5	62
l-Epinephrine bitartrate	OH	...	CH_3	2.5	36
l-N-Ethylnorepinephrine HCl	OH	...	C_2H_5	2.5	44
Propylnorepinephrine sulfate	OH	...	*n*-C_3H_7	5	105
Isoproterenol HCl	OH	...	*i*-C_3H_7	5	98
				10	86
Isotherine	OH	C_2H_5	*i*-C_3H_7	10	93
Butylnorepinephrine HCl	OH	...	*n*-C_4H_9	5	102
Isobutylnorepinephrine HCl	OH	...	*i*-C_4H_9	5	94
Acetbutylnorepinephrine sulfate	OH	...	$C_6H_{11}O$	5	103
Protokylol HCl (caytine)	OH	...	$C_{10}H_{11}O_2$	5	104
l-N-2-(*p*-Hydroxyphenyl-1-hydroxypropyl)norepinephrine	OH	...	$C_9H_{11}O_2$	10	89
N-Methylepinephrine HCl	OH	...	$(CH_3)_2$	10	95
Arterenone HCl	=O	...	...	5	43
Adrenalone HCl	=O	...	CH_3	5	52
N-Isopropylnoradrenalone sulfate	=O	...	*i*-C_3H_7	10	100
Phenisonone HBr	=O	CH_3	*i*-C_3H_7	10	110
N,N-Dibenzylarterenone	=O	...	$(C_7H_7)_2$	10	93
2-(3,4-Dihydroxyphenyl)morpholine HCl	$-OC_2H_4-$	...	$-C_2H_4-$	5	44

[a] Assay as described in the text.

Experimental Section

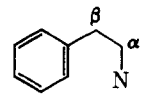
Materials.—The compounds were obtained from commercial sources or as acknowledged. DL-Norepinephrine-7-^{3}H was obtained from the New England Nuclear Corp. (specific activity 5 mcuries/μmole).

Assay of Norepinephrine Release.—A 0.1-ml solution (isotonic NaCl (0.9%) containing 50 mg of heparin/l.) of 1.0 mμmole (5 μcuries) of norepinephrine-^{3}H was injected into the tail vein of 14–18-g male white mice (NIH general purpose). Drugs were administered subcutaneously after 1 hr. The mice were sacrificed after 3 hr by a blow on the head. The hearts (5 mice/assay) were immediately excised, exsanguinated, and homogenized in 10 vol (w/v) of 0.4 *N* $HClO_4$. After centrifugation, 0.2 ml of the supernatant solution was added to 10 ml of Brays phosphor solution and the radioactivity was determined by liquid scintillation counting (counting efficiency 6.7%).

The uptake and gradual release of 5 μcuries of injected norepinephrine from mouse hearts is presented in Figure 1. After 3 hr a 0.2-ml aliquot of the supernatant solution of a heart homogenate contained 1000 ± 60 cpm, which corresponds to 340 ± 20 mμcuries/g of tissue. The activity of amines is expressed as per cent of this control. Lower values were found in the hearts of animals which had received an agent that released norepinephrine. The results obtained for various sympathomimetic amines and similar compounds are presented in Tables I–VI.

TABLE II

THE CHEMORELEASE OF NOREPINEPHRINE-^{3}H FROM MOUSE HEARTS BY O-METHYLATED CATECHOLAMINES[a]



Compd	Ring OH	Ring OCH_3	Substituent β	Substituent α	Substituent N	Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control
3-O-Methyldopamine HCl	4	3	...	...	...	10	104
3-O-Methyl-α-methyldopamine HCl	4	3	...	CH_3	...	10	105
l-Normetanephrine HCl	4	3	OH	...	...	10	104
Metanephrine HCl	4	3	OH	...	CH_3	10	104
3-O-Methylarterenone HCl	4	3	=O	...	...	10	102
3-O-Methyladrenalone HCl	4	3	=O	...	CH_3	10	110
3-O-Methylisoproterenol HCl	4	3	OH	...	i-C_3H_7	10	98
3,5-Dimethoxy-4-hydroxyphenethylamine HCl	4	3,5	...	...	...	10	105
4-O-Methyldopamine HCl	3	4	...	...	...	10	100
Norparanephrine HCl	3	4	OH	...	...	10	83 ←
Paranephrine HCl	3	4	OH	...	CH_3	10	94
4-O-Methylarterenone HCl	3	4	=O	...	...	10	78 ←
4-O-Methyladrenalone HCl	3	4	=O	...	CH_3	10	95
2-Hydroxy-3-methoxyphenethylamine HCl	2	3	...	...	...	10	90
3,4-Dimethoxy-5-hydroxyphenethylamine HCl	5	3,4	...	...	...	10	94
2,4-Dihydroxy-5-methoxyphenethylamine HCl	2,4	5	...	...	...	10	88
2,4-Dihydroxy-5-methoxy-N-methylphenethylamine HCl	2,4	5	...	...	CH_3	10	92
2,5-Dihydroxy-4-methoxyphenethylamine HCl	2,5	4	...	...	...	10	78 ←
3,5-Dihydroxy-4-methoxyphenethylamine HCl	3,5	4	...	...	...	2.5	23 ←
						5	16 ←
						10	12 ←

[a] Assay as described in text.

The radioactivity in the heart at the end of 3 hr was almost entirely due to norepinephrine-^{3}H as was ascertained by alumina column, paper and thin layer chromatography.

The Release of Norepinephrine-^{3}H. A. Catecholamines.—A variety of catecholamines containing modifications both in the aromatic ring and in the side chain were tested for norepinephrine-releasing potency (Table I). Almost all catecholamines increased release of norepinephrine. Structure–activity correlations will be discussed below. Catecholic compounds without the phenethylamine structure did not effect norepinephrine release at 10 mg/kg. These compounds included catechol, hydroxyhydroquinone, pyrogallol, 3,4-dihydroxyphenylacetamide, 3,4-dihydroxypropylacetamide, quercetin, and 3,4-dihydroxyphenylacetic acid. Tropolones including tropolone and β-aminomethyltropolone (10 mg/kg)[12] were inactive in this assay.

B. O-Methylated Catecholamines.—Various O-methylated catecholamines which are formed *in vivo* by enzymatic methylation of catecholamines[13] were tested (Table II). All showed negligible activity with sole exception of 3,5-dihydroxy-4-methoxyphenethylamine, an extremely potent releaser of norepinephrine.[14] Nonbasic methylated catecholamine metabolites such as homovanillic acid, 3-methoxy-4-hydroxyphenylglycolic ("vanillylmandelic") acid, and 3-methoxy-4-hydroxyphenylglycol did not affect norepinephrine release.

C. Phenolic Amines.—A variety of phenolic sympathomimetic amines were tested (Table III). As with the catecholamines, most of the phenolic phenethylamines were active chemoreleasers of norepinephrine. Benzylic or γ-phenylpropylamines such as *p*-hydroxybenzylamine and γ-(*p*-hydroxyphenyl)propylamine (homotyramine) were inactive at 10 mg/kg. Thyroxamine and 5-hydroxykynuramine[15] caused very slight chemorelease of norepinephrine at 10 mg/kg. *p*-Hydroxyphenylacetic acid, *p*-hydroxyphenylglycol, *p*-hydroxymandelic acid, and *p*-hydroxyphenethanol were inactive.

D. Methoxyphenethylamines.—A variety of ring methoxylated phenethylamines were tested as chemoreleasers of norepinephrine. Almost all were inactive (Table IV).

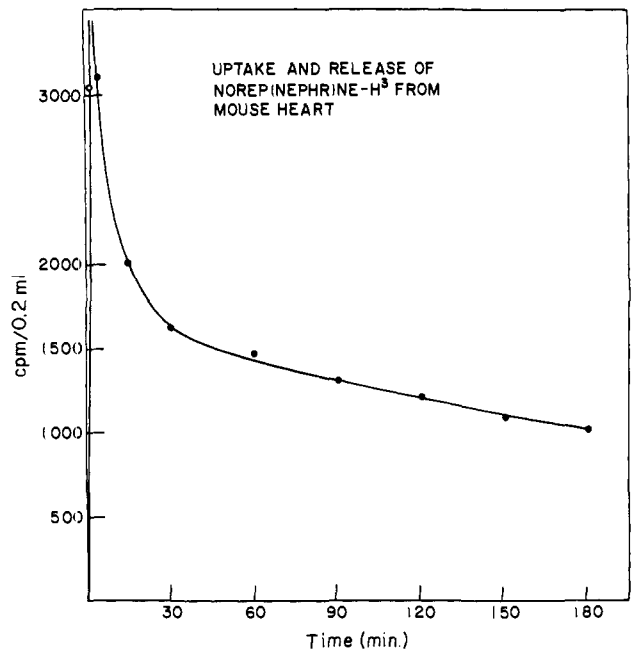


Figure 1.—Depletion of cardiac norepinephrine-^{3}H as a function of time following a single intravenous injection of 5 μcuries of norepinephrine-^{3}H.

E. Phenethylamines.—Simple phenethylamines and certain ring-substituted analogs were active as chemoreleasers of norepinephrine (Table V). Amines containing a γ-phenylpropylamine skeleton such as 1-phenyl-3-aminobutane, γ-phenylpropylamine, γ-phenyl-N,N-dimethylpropylamine, and kynuramine were inactive as releasers of norepinephrine as were benzylamine, α-methylbenzylamine, and N,N-diethylbenzylamine. 1-Aminoindan hydrochloride (10 mg/kg) was a moderately active chemoreleaser (82%). Structure–activity correlations are discussed below

F. Tryptamines.—The norepinephrine-releasing activity of tryptamines and methoxy- or hydroxytryptamines are tabulated

(12) We are indebted to Dr. B. Belleau for a sample of this amine.

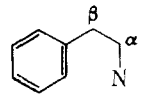
(13) J. W. Daly and B. Witkop, *Angew. Chem., Intern. Ed. Engl.*, **2**, 421 (1963).

(14) C. R. Creveling, J. W. Daly, and B. Witkop, in preparation.

(15) We are indebted to Dr. K. Makino for a sample of this amine.

Table III

The Chemorelease of Norepinephrine-^{3}H from Mouse Hearts by Phenolic Amines and Related Compounds[a]



Compd	Substituents				Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control
	Ring	β	α	N		
p-Tyramine HCl	4-OH	...	...	...	5	48
					10	50[b]
α-Methyl-*p*-tyramine HCl	4-OH	...	CH_3	...	10	38
β-Methyl-*p*-tyramine HCl	4-OH	CH_3	...	...	10	84
N-Methyltryamine HCl	4-OH	...	...	CH_3	10	64
Hordenine sulfate	4-OH	...	...	$(CH_3)_2$	10	103
Norbelladine HCl	4-OH	...	...	$CH_2C_6H_5N_2$	10	110
N-Formyltyramine	4-OH	...	...	CHO	10	92
N-Acetyltyramine	4-OH	...	...	$COCH_3$	10	105
m-Tyramine HCl	3-OH	...	...	...	10	46
α-Methyl-*m*-tyramine	3-OH	...	CH_3	...	5	38
					10	34
o-Tyramine HBr	2-OH	...	...	...	10	95
p-Octopamine HCl	4-OH	OH	...	...	5	53
p-Hydroxy-α-(methylaminomethyl)benzyl alcohol tartrate	4-OH	OH	...	CH_3	5	68
N,N-Dimethyl-*p*-octopamine methiodide	4-OH	OH	...	$(CH_3)_3$	10	126
p-Hydroxynorephedrine HCl	4-OH	OH	CH_3	...	5	40
l-*p*-Hydroxyephedrine HCl	4-OH	OH	CH_3	CH_3	5	49
Nylidrin HCl	4-OH	OH	CH_3	$C_{10}H_{13}$	10	94
Isoxsuprine	4-OH	OH	CH_3	$C_9H_{11}O$	10	73
l-*m*-Octopamine tartrate	3-OH	OH	...	...	5	38
l-Phenylephrine HCl	3-OH	OH	...	CH_3	5	40
l-Metaraminol bitartrate	3-OH	OH	CH_3	...	5	22
1-(3-Hydroxyphenyl)-2-amino-1-butanol HCl	3-OH	OH	C_2H_5	...	5	57
1-(3-Hydroxyphenyl)-2-methylamino-1-propanol HCl	3-OH	OH	CH_3	CH_3	5	34
o-Octopamine[b]	2-OH	OH	...	...	10	83
N-Methyl-*o*-octopamine benzoate	2-OH	OH	...	CH_3	10	108
3,5-Dibromo-4-hydroxyphenethylamine HCl	3,5-DiBr, 4-OH	...	...	...	10	81
2-(3-Hydroxyphenyl)morpholine sulfate	3-OH	$-OC_2H_4-$	...	$-C_2H_4-$	5	62
2-(3-Hydroxyphenyl)-3-methylmorpholine HCl	3-OH	$-OC_2H_4-$	CH_3	$-C_2H_4-$	5	98
2,4-Dihydroxyphenethylamine[c]	2,4-DiOH	...	...	...	10	47
3,5-Dihydroxyphenethylamine HBr	3,5-DiOH	...	...	...	5	50
3,5-Dihydroxyphenethanolamine sulfate	3,5-DiOH	OH	...	...	5	31

[a] Assay as described in text. [b] Four 2.5-mg/kg doses were given 1, 1.5, 2, and 2.5 hr after norepinephrine-^{3}H resulted in a value of 16% control. [c] We would like to thank Dr. Marvin Armstrong for these compounds.

in Table VI. Isotryptamine and 1-aminoethylindole were inactive as releasers of norepinephrine-^{3}H. Structure-activity correlations are discussed below.

Discussion and Results

The effects of drugs on the levels of norepinephrine have been the subject of numerous investigations. The present method of using norepinephrine-^{3}H to label endogenous stores of norepinephrine in mice has allowed the development of a rapid sensitive assay for screening the effects of sympathomimetic amines and other classes of compounds on the continuous physiological release of norepinephrine. Our own work[14] and previous publications[10,16] have shown that this is a valid method for studying levels of endogenous norepinephrine. At these low levels the subcellular distribution of endogenous and tritiated norepinephrine is the same, and chemorelease of endogenous and tritiated norepinephrine is comparable. Similar release has been reported for certain amines in rat heart.[10b,c] These were (+)-epinephrine, dopamine, (±)-phenylephrine, (±)-synephrine, tyramine, (+)-amphetamine, (±)-ephedrine, phenethylamine, (±)-phenethanolamine, and dichlorisoproterenol.

Of the various classes of sympathomimetic amines which release norepinephrine-^{3}H, catecholamines were the most active. Next in activity were phenolic amines and the nonphenolic phenethylamines. The increase in release of heart norepinephrine-^{3}H caused by hydroxytryptamines such as serotonin, N-methylserotonin, 5,6-dihydroxytryptamine, and 6-hydroxytryptamine is of interest inasmuch as these amines occur naturally in higher organisms.[17,18] Another class of biogenic amines, histamine, ω-N-methylhistamine, and 1-methylhistamine, did not release norepinephrine-^{3}H. A catecholamine did not require the usual 3,4-dihydroxyphenethylamine skeleton to serve as a releasing

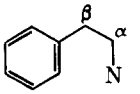
(16) S. M. Hess, *Arch. Intern. Pharmacodyn.*, **138**, 584 (1962).

(17) (a) D. B. Carlisle, *Biochem. J.*, **63**, 32P (1956); (b) K. J. Davey, *Can. J. Zool.*, **38**, 39 (1960).

(18) (a) J. B. Jepson, P. Zaltzman, and S. Udenfriend, *Biochim. Biophys. Acta*, **62**, 91 (1962); (b) G. A. Kerkut and M. A. Price, *Life Sci.*, **2**, 129 (1963).

TABLE IV

THE CHEMORELEASE OF NOREPINEPHRINE-^{3}H FROM MOUSE HEARTS BY METHOXYPHENETHYLAMINES[a]



Compd	Ring: OCH_3	Ring: Other	Substituent: β	Substituent: α	Substituent: N	Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control	
4-Methoxyphenethylamine HCl	4	...	...	...	...	10	102	
4-Methoxy-β-methylphenethylamine HCl	4	...	CH_3	...	...	10	107	
4-Methoxy-3-chloro-N-methylphenethylamine HCl	4	3-Cl	...	...	CH_3	10	93	
3-Methoxy-β-methylphenethylamine HCl	3	...	CH_3	...	...	10	77	←
Methoxyphenamine HCl	2	...	...	CH_3	CH_3	10	66	←
2-Methoxy-4,5-methylenedioxyphenethylamine HCl	2	4,5-CH_2O_2	...	...	...	10	104	
2,3-Dimethoxyphenethylamine HCl	2,3	...	...	...	...	10	87	
2,3-Dimethoxy-N-methylphenethylamine HCl	2,3	...	...	...	CH_3	10	92	
2,3-Dimethoxy-N,N-dimethylphenethylamine HCl	2,3	...	...	...	$(CH_3)_2$	10	100	
2,4-Dimethoxy-N-methylphenethylamine HCl	2,4	...	...	...	CH_3	10	106	
2,4-Dimethoxy-N,N-dimethylphenethylamine methochloride	2,4	...	...	...	$(CH_3)_3$	10	107	
2,5-Dimethoxyphenethylamine HCl	2,5	...	...	...	...	10	98	
2,5-Dimethoxy-N-methylphenethylamine HCl	2,5	...	...	...	CH_3	10	102	
2,5-Dimethoxy-N,N-dimethylphenethylamine methochloride	2,5	...	...	...	$(CH_3)_3$	10	95	
Methoxamine HCl	2,5	...	OH	CH_3	...	10	101	
2,5-Dimethoxy-β-methylphenethylamine HCl	2,5	...	CH_3	...	...	10	97	
2,5-Dimethoxy-β-methyl-N-methylphenethylamine HCl	2,5	...	CH_3	...	CH_3	10	95	
2,5-Dimethoxy-β-methyl-N,N-dimethylphenethylamine HCl	2,5	...	CH_3	...	$(CH_3)_2$	10	100	
2,5-Dimethoxy-β-methyl-N,N-dimethylphenethylamine methochloride	2,5	...	CH_3	...	$(CH_3)_3$	10	103	
3,4-Dimethoxyphenethylamine HCl	3,4	...	...	...	...	10	96	
3,4-Dimethoxy-α-methylphenethylamine HCl	3,4	...	...	CH_3	...	10	109	
3,4-Dimethoxy-N-methylphenethylamine HCl	3,4	...	...	...	CH_3	10	93	
3,4-Dimethoxy-N-ethylphenethylamine HI	3,4	...	...	...	C_2H_5	10	98	
3,4-Dimethoxy-N,N-dimethylphenethylamine	3,4	...	...	...	$(CH_3)_2$	10	99	
3,4-Dimethoxy-N-methyl-N-benzylphenethylamine HCl	3,4	...	...	...	CH_3, C_7H_7	10	101	
3,4-Dimethoxy-N,N-dimethylphenethylamine methochloride	3,4	...	...	...	$(CH_3)_3$	10	104	
3,4-Dimethoxy-N-(2-hydroxyethyl)phenethylamine HCl	3,4	...	...	...	CH_2CH_2OH	10	101	
3,4-Dimethoxyphenethanolamine	3,4	...	OH	...	...	10	94	
Mescaline HCl	3,4,5	...	...	...	...	10	99	
β-Hydroxymescaline	3,4,5	...	OH	...	...	10	101	
Mescalone	3,4,5	...	=O	...	...	10	92	
2,4,5-Trimethoxyphenethylamine HCl	2,4,5	...	...	...	...	10	103	
2,3,4,6-Tetramethoxyphenethylamine HCl	2,3,4,6	...	...	...	...	10	94	
2,3,5,6-Tetramethoxyphenethylamine HCl	2,3,5,6	...	...	...	...	10	90	

[a] Assay as described in text.

agent, since 2,3-dihydroxy compounds were active. Compounds with additional ring hydroxyl groups, such as 3,4,5-trihydroxyphenethylamine and 6-hydroxydopamine,[19] were still very active. Methoxy substitution, such as in 6-methoxydopamine and 3-methoxy-4,5-dihydroxyphenethylamine, decreased activity. 3,4-Diacetoxy-N-methylphenethylamine was active because of the easy *in vivo* hydrolysis of the phenolic esters. These diesters were as active in causing release as the parent catechols.

In general, secondary amines were less active than primary amines. Tertiary amines were inactive. So were secondary amines substituted by alkyl groups larger than ethyl. Quaternary amines were inactive as releasing agents with the exception of the methochloride of 2,3-dihydroxy-N,N-dimethylphenethylamine. N-Acylated catecholamines, simple catechols, and homoprotocatechuic acid were inactive. The slight release shown with N-acetylnorepinephrine was probably due to hydrolysis *in vivo*. α-Methylation of catecholamines tended to increase their activity.

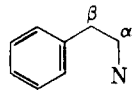
The D-(−) antipode of norepinephrine was a better releasing agent than the unnatural L-(+)-norepinephrine. The β-hydroxy group was, however, not essential for activity, since dopamine, β-O-methylnorepinephrine, and arterenone were active chemoreleasers. Isoproterenol, a vasodilator which has been reported to occur naturally,[20] was relatively inactive. Tritiated

(19) C. C. Porter, J. A. Totare, and C. A. Stone, *J. Pharmacol. Exptl. Therap.*, **140**, 308 (1963).

(20) D. J. Roberts, *Brit. J. Pharmacol.*, **24**, 735 (1965), and preceding papers.

Table V

The Chemorelease of Norepinephrine-^{3}H from Mouse Hearts by Simple and Substituted Phenethylamines[a]



Compd	Ring	Substituent β	Substituent α	Substituent N	Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control
Phenethylamine	...	...	...	...	10	65 ←
β-Phenethanolamine HCl	...	OH	...	...	10	91
N,β-Dimethylphenethylamine HCl (vonedrine)	...	CH_3	...	CH_3	10	104
2,2-Diphenylethylamine	...	C_6H_5	...	...	10	119
d-Amphetamine sulfate	...	...	CH_3	...	10	58 ←
l-Amphetamine sulfate	...	CH_3	...	...	10	86
Norephedrine HCl	...	OH	CH_3	...	10	68 ←
d-Desoxyephedrine HCl	...	...	CH	CH_3	10	62 ←
Ephedrine HCl	...	OH	CH_3	CH_3	10	91
Pseudoephedrine	...	OH	CH_3	CH_3	10	84
N-Ethylamphetamine HCl	...	...	CH_3	C_2H_5	10	64 ←
N-Ethylephedrine HCl (nethamine)	...	OH	CH_3	CH_3, C_2H_5	10	106
N-Methylphenethylamine HCl	...	...	...	CH_3	10	80 ←
N,N-Dimethylphenethylamine HCl	...	...	...	$(CH_3)_2$	10	102
N,N-Dimethyl-β-phenethanolamine HCl	...	OH	...	$(CH_3)_2$	10	92
N-Phenethylpiperidine HCl	...	...	...	$-C_5C_{10}-$	10	97
Prenylamine	...	...	CH_3	$(CH_2)_2CH(C_6H_5)_2$	10	44 ←
α-Methylamphetamine HCl	...	...	$(CH_3)_2$	...	10	95
Mephentermine	...	...	$(CH_3)_2$	CH_3	10	100
4-Chlorophenethylamine	4-Cl	...	...	...	10	101
3-Chlorophenethylamine	3-Cl	...	...	...	10	88
2-Chlorophenethylamine	2-Cl	...	...	...	10	89
4-Chloro-α-methylphenethylamine HCl	4-Cl	...	CH_3	...	10	71 ←
4-Chloro-α-methyl-N-methylphenethylamine HCl	4-Cl	...	CH_3	CH_3	10	74 ←
4-Chloro-α,α-dimethylphenethylamine HCl	4-Cl	...	$(CH_3)_2$	...	10	87
Dichlorisoproterenol HCl	3,4-Cl_2	OH	...	i-C_3H_7	10	104
4-Aminophenethylamine 2HCl	4-NH_2	...	...	...	10	94
2-Aminophenethanolamine 2HBr	2-NH_2	OH	...	...	10	97
2-Acetamido-α-aminoacetophenone	2-NHAc	=O	...	...	10	92
Norkynuramine 2HBr	2-NH_2	=O	...	...	10	87
α-Methyl-*p*-nitrophenethylamine HCl	4-NO_2	...	CH_3	...	10	98
α-Methyl-3,4-methylenedioxyphenethylamine	3,4-CH_2O_2	...	CH_3	...	10	76 ←
4-Methylphenethylamine	4-CH_3	...	...	...	10	94
2-Methylphenethylamine	2-CH_3	...	...	...	10	103

[a] Assay as described in the text.

isoproterenol is not concentrated in the heart,[21] in contrast to tritiated norepinephrine and epinephrine.[22]

Methylation of catecholamines *in vivo* by catechol O-methyltransferase nearly abolishes all physiological activity.[23] In accordance with these observations 3-O-methyldopamine, metanephrine, and normetanephrine were not releasing agents. The products of enzymatic methylation of 6-hydroxydopamine and 2,3-dihydroxyphenethylamines were also inactive. 3,5-Dihydroxy-4-methoxyphenethylamine, however, was a potent, short-acting depletor of norepinephrine.[14] This compound is formed by the action of catechol O-methyltransferase on 3,4,5-trihydroxyphenethylamine[24] (trisnormescaline). In this case the releasing activity of the parent catecholamine was potentiated by enzymatic methylation. The O-methylated metabolites of catecholamines, such as homovanillic, 4-hydroxy-3-methoxymandelic acid, 4-hydroxy-3-methoxyphenylglycol, and 4-hydroxy-3-methoxyphenylethanol were inactive.

Structural modifications affected the activity of phenolic amines in a similar way. Tertiary amines were inactive. Secondary amines were less active than the corresponding primary amines. α-Methylation and β-hydroxylation enhanced activity. *m*-Hydroxyphenethylamines were more active than the *p*-hydroxy isomers. The *o*-hydroxyphenethylamines were almost inactive. The most potent phenolic amine, Aramine, is an α-methyl-*m*-hydroxyphenethanolamine.

Variations in the length of the side chain, *e.g.*, *p*-hydroxybenzylamine and homotyramine, led to loss of activity. Tyramine and octopamine metabolites or derivatives, such as *p*-hydroxyphenylacetic acid, *p*-hydroxyphenylglycol, *p*-hydroxyphenylethanol, and N-acetyl- or N-formyltyramine were inactive.

Tyramine and other amines show an interesting saturation phenomenon (Table III). A dose of 5 or 10 mg/kg of tyramine released the same amount of norepinephrine-^{3}H. This type of saturation phenomenon has been taken for evidence that tyramine can release only

(21) G. Hertting, *Biochem. Pharmacol.*, **13**, 1119 (1964).

(22) J. Axelrod, H. Weil-Malherbe, and R. Tomchick, *J. Pharmacol. Exptl. Therap.*, **127**, 251 (1959).

(23) (a) J. Champagne and A. D'Iorio, *Science*, **132**, 419 (1960); (b) Z. M. Bacq and J. Rensen, *Bull. Acad. Roy. Med. Belg.*, [6] **25**, 755 (1960).

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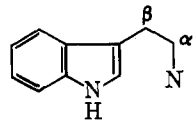
TABLE VI

THE CHEMORELEASE OF NOREPINEPHRINE-^{3}H FROM MOUSE HEARTS BY TRYPTAMINE DERIVATIVES[a]



Compd	Substituent: Ring	β	α	N	Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control
Tryptamine HCl	...	...	...	...	10	91
α-Methyltryptamine acetate	...	...	CH_3	...	10	94
N-Methyltryptamine	...	...	...	CH_3	10	102
1-Methyltryptamine	1-CH_3	...	...	...	10	93
N,N-Dimethyltryptamine	...	...	...	$(CH_3)_2$	5	100
2,3-Dihydrotryptamine	2,3-Dihydro	...	...	...	10	88
Benanserin	1-Benzyl-2-Methyl-5-Methoxy	...	...	...	5	98
5-Methoxytryptamine oxalate	5-OCH_3	...	...	...	5	97
5-Methoxy-N-methyltryptamine oxalate	5-OCH_3	...	...	CH_3	10	99
5-Methoxy-N,N-dimethyltryptamine oxalate	5-OCH_3	...	...	$(CH_3)_2$	10	96
Melatonin	5-OCH_3	...	...	$COCH_3$	10	93
2-Hydroxytryptamine	2-OH	...	...	...	5	91
4-Hydroxytryptamine oxalate	4-OH	...	...	...	10	91
4-Hydroxy-N-methyltryptamine oxalate	4-OH	...	...	CH_3	10	95
Psilocin	4-OH	...	...	$(CH_3)_2$	5	103
4-Hydroxy-N,N-dimethyltryptamine	4-OH	...	...	$(C_2H_5)_2$	10	96
Serotonin oxalate	5-OH	...	...	...	10	56 ←
N-Methylserotonin oxalate	5-OH	...	...	CH_3	10	58 ←
Bufotenine	5-OH	...	...	$(CH_3)_2$	10	90
Bufotenidine iodide	5-OH	...	...	$(CH_3)_3$	10	92
N-Acetylserotonin	5-OH	...	...	$COCH_3$	5	95
6-Hydroxytryptamine creatinine sulfate	6-OH	...	...	...	10	56 ←
6-Hydroxy-N,N-dimethyltryptamine	6-OH	...	...	$(CH_3)_2$	10	94
7-Hydroxytryptamine	7-OH	...	...	...	10	79 ←
5,6-Dihydroxytryptamine	5,6-DiOH	...	...	...	10	57 ←
6-Hydroxy-5-methoxytryptamine formate	6-OH, 5-MeO	...	...	...	10	102
5-Hydroxy-6-methoxytryptamine formate	5-OH, 6-MeO	...	...	...	10	97
2,5-Dihydroxytryptamine	2,5-DiOH	...	...	...	10	98

[a] Assay as described in the text.

a certain compartment or "pool" of the endogenous norepinephrine.[25] If, however, the 10-mg/kg dose is divided into 4 injections of 2.5 mg/kg over a period of 90 min, almost all of the cardiac norepinephrine-^{3}H is released. A similar observation for endogenous norepinephrine has been recently reported by Neff, *et al.*[26]

Simple phenethylamines, which owe virtually all of their pharmacological effect to release of norepinephrine, gave the following results. Tertiary amines were inactive. Secondary amines were usually somewhat less active than primary amines. Segontin, a secondary amine with a bulky alkyl substituent [R = CH_2CH_2-$CH(C_6H_5)_2$][27] was very active. As in the catechol and phenolic amine series α-methylation in nonphenolic amines slightly enhanced activity. Dialkylation of the α position leads to inactive compounds. β-Hydroxylation decreases activity in contrast to higher activity observed on β-hydroxylation in catechol and phenolic amines. Ring substitution with amino, chloro, methoxy, methyl, or nitro groups almost invariably resulted in inactive compounds. Certain 4-chloro-α-methylphenethylamines, and certain alkoxy amines, such as 3-methoxy-β-methylphenethylamine, methoxyphenamine, and 3,4-methylenedioxy-α-methylphenethylamine, were still active as releasing agents.

Sympathomimetic amines without an aromatic ring were only slightly active, *e.g.*, 2-methylamino-6-methyl-6-heptanol, isometheptene, 2-methylaminoheptane, cyclopentamine, 1-cyclohexyl-2-aminopropane, propylhexadrine, methylhexaneamine, and tuamine. The sympathomimetic imidazolines, *e.g.*, tolazoline, did not cause release.[11] Hydrazines or benzyloxyamines, isosteric with phenethylamines, were inactive.[11]

Tryptamine, N-methyl- and N,N-dimethyltryptamine, and their 5-methoxy analogs were virtually inactive. Of the hydroxytryptamines the naturally occurring serotonin, N-methylserotonin, and 6-hydroxytryptamine were the most active. 5,6-Dihydroxytryptamine, reported as a neurohormone in crustaceae,[17a] was quite active. As with the catecholamines, enzymatic O-methylation to 5-methoxy-6-hydroxytryptamine and 6-hydroxy-5-methoxytryptamine[28] virtually abolished this activity. The vasoconstrictor activity of serotonin has been interpreted in terms of release of norepinephrine.[29] Our observations support this assumption.

(25) J. Axelrod, E. Gordon, G. Hertting, I. J. Kopin, and L. T. Potter, *Brit. J. Pharmacol.*, **19**, 56 (1962).

(26) N. H. Neff, T. N. Toser, W. Hammer, and B. B. Brodie, *Life Sci.*, **4**, 1869 (1965).

(27) H. H. Schöne and E. Lindner, *Arzneimittel-Forsch.*, **10**, 583 (1960).

(28) J. Axelrod, J. K. Inscoe, and J. Daly, *J. Pharmacol. Exptl. Therap.*, **149**, 16 (1965).

(29) I. R. Innes, *Brit. J. Pharmacol.*, **19**, 427 (1962).

In these structure–activity correlations many factors must be considered, such as enzymatic transformations en route, transport phenomena, and finally the molecular structure of the binding site. The first two factors influence the amount of amine which reaches the binding site. For example, since tyramine is rapidly oxidized by the ubiquitous monoamine oxidase, only a fraction of the administered dose survives to effect release of norepinephrine. The α-methylated derivative which is not a substrate of monoamine oxidase is more active as a chemoreleasing agent. In other analogous pairs of amines, the α-methyl derivative is more active. A monoamine oxidase inhibitor such as pheniprazine potentiates the releasing activity of tyramine but not of α-methyltyramine.[30]

Catecholamines are metabolized and inactivated both by monoamine oxidase and by catechol O-methyltransferase. Catecholamines are thus much more active chemoreleasers than our data indicate.

Enzymatic β-hydroxylation decreases the activity of phenethylamines and increases the activity of phenolic amines. The activity of an amine which is a substrate for dopamine β-hydroxylase is then the sum of its own activity and that of its β-hydroxylated metabolite.[31]

Regarding the question of active transport, extensive studies on the migration of norepinephrine into sympathetically innervated tissues[32] have shown that amines differ markedly in their affinity for the transport mechanism. The molecular specificity of this system results in concentration of certain amines. For example, D-(−)-norepinephrine has a greater affinity for the uptake mechanism than the L-(+) antipode[32b] and exhibits a commensurably higher releasing activity.

The molecular mechanism by which sympathomimetic amines effect release is very poorly understood. An amine with a high affinity for the storage site may simply displace the norepinephrine stoichiometrically, as for example metaraminol.[33] Other amines, such as 6-hydroxydopamine[19] and prenylamine,[34] release more than stoichiometric amounts of norepinephrine. In addition many amines have a long-lasting effect on tissue levels of norepinephrine[14] which is difficult to correlate with the amount of sympathomimetic amine remaining in the sympathetic nerve.[35]

In view of these various parameters the structure–activity relations presented here can serve only as a guide to further investigations into the precise mechanism of release and binding of biogenic amines.

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(30) C. R. Creveling and J. Daly, unpublished observations.

(31) C. R. Creveling and J. B. van der Schoot in "Advances in Drug Research," Vol. II, A. B. Simmons and N. J. Harper, Ed., Academic Press Inc., London, 1965.

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(34) H. Grobecker, D. Plam, and H. J. Schuman, *Arch. Exptl. Pathol. Pharmakol.*, **251**, 158 (1965).

(35) S. Udenfriend and P. Zaltzman-Nirenberg, *J. Pharm. Exptl. Therap.*, **138**, 194 (1963).

The Chemorelease of Norepinephrine in Mouse Hearts. Structure–Activity Relationships. II. Drugs Affecting the Sympathetic and Central Nervous Systems

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Endogenous cardiac norepinephrine in mice has been prelabeled with a 5-μcurie injection of norepinephrine-^{3}H, and the effect of various classes of compounds on the normal physiological depletion of norepinephrine-^{3}H has been studied. The effect of a variety of tranquilizers, antidepressants, ganglionic blocking agents, hypotensive agents, sympatholytics, and compounds that inhibit key enzymes in the biogenesis and metabolism of norepinephrine have been ascertained. The releasing and release-inhibiting activities of these drugs are discussed.

The importance of the release of peripheral and central stores of norepinephrine in the pharmacological action of many drugs finds ample expression in the action of reserpine, tetrabenazine, α-methyldopa, guanethidine, and other drugs.[1] A rapid, sensitive method for assessing this aspect of drug action has been described by Daly, *et al.*[2] It consists of prelabeling the endogenous norepinephrine in the hearts of mice by an injection of a tracer amount of norepinephrine-^{3}H. The loss of norepinephrine-^{3}H from cardiac tissue is influenced by drugs which may either facilitate or inhibit the normal physiological release. The method has been successfully applied to the analysis of structure–activity correlations in the sympathomimetic amines.[2] This paper will describe results obtained with other classes of drugs which change the normal disposition and metabolism of norepinephrine.

(1) P. A. Shore, *Pharmacol. Rev.*, **14**, 531 (1962).

(2) J. W. Daly, C. R. Creveling, and B. Witkop, *J. Med. Chem.*, **9**, 273 (1966).

Results

DL-Norepinephrine-7-^{3}H (5 mcuries/μmole) was obtained from New England Nuclear Corp. Compounds