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[29] 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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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-^3H, and the effect of various classes of compounds on the normal physiological depletion of norepinephrine-^3H 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-^3H. The loss of norepinephrine-^3H 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.

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Results

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

TABLE I

RESERPINE, TETRABENAZINE, AND RELATED COMPOUNDS

Compd	Dose, mg/kg	Release of norepinephrine-^{3}H, % of control
Reserpine	1	18
Deserpidine	2.5	26
Syrosingopine	2.5	38
Recinnamine	2.5	37
Serpentine	5	116
Yohimbine	5	51
Yohimbine methiodide	10	121
Raunitidine	2.5	110
Tetrabenazine methanesulfonate	10	45
Dihydrotetrabenazine	10	14

TABLE II

PHENOTHIAZINES AND RELATED COMPOUNDS

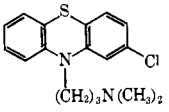
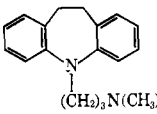
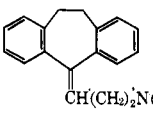
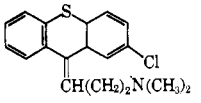
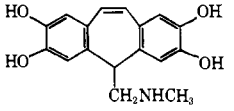
Compd	Dose, mg/kg	Release of norepinephrine-^{3}H, % of control
		
Chlorpromazine	5	107
7-Hydroxychlorpromazine	5	104
Norchlorpromazine	5	106
Chlorpromazine sulfoxide	5	97
Promethazine	5	102
Prochlorperazine	5	98
Promazine	5	104
Trifluoperazine	5	90
Nortrifluoperazine	5	95
		
Imipramine	5	120
Demethylimipramine	5	129
		
Amitryptyline	5	130
		
Chlorprothixene	5	125
Protryptyline	5	121
		
Adnamine HCl	5	115
Noradnamine HCl	5	122

TABLE III

GANGLIONIC BLOCKING AGENTS AND GUANIDINE DERIVATIVES

Compd	Dose, mg/kg	Release of norepinephrine-^{3}H, % of control
Ganglionic Blocking Agents		
Bretylium	10	121
Chlorisondamine	10	129
Choline 2,6-xylyl ether bromide	10	127
Pentolinium bitartrate	10	107
Pempidine	10	140
Mecamylamine	10	111
Guanidine Derivatives		
Guanethidine	10	49
Benzylguanidine sulfate	10	84
O-Bromobenzylguanidine	10	79
Phenethylguanidine sulfate	10	67
3,4-Dimethoxyphenethylguanidine sulfate	10	89
N-Benzyl-N′,N″-dimethylguanidine	10	73
N-O-Chlorobenzyl-N′,N″-dimethylguanidine	10	111

TABLE IV

SYMPATHOLYTIC AGENTS

Compd	Dose, mg/kg	Release of norepinephrine-^{3}H, % of control
Tolazoline HCl	10	102
2-(4-*t*-Butyl-2,6-dimethylphenylmethyl)imidazoline HCl	10	101
Naphazoline HCl	10	110
Tetrahydrozoline HCl	10	107
Phenazoline phosphate	10	88 ←
Phentolamine	10	68 ←
Dibenamine	10	77 ←
Phenoxybenzamine	10	81 ←
Azapetine phosphate	10	92
Promethalol	10	106
Propranalol	10	94
dl-4-(2-Isopropylamino-1-hydroxyethyl)methanesulfonanilide HCl	10	93
Dihydroergotamine	5	98

used in the assays were obtained from commercial sources or as acknowledged.

The effect of drugs on norepinephrine release was assayed as described previously.[2] The effect of reserpine, tetrabenazine, and related compounds is reported in Table I. The results for phenothiazines and structurally related molecules including imipramine, amitryptyline, and chlorprothixene are compiled in Table II. The effect on norepinephrine release of various ganglionic blocking agents and guanethidine analogs is reported in Table III. The effects of various sympatholytic agents on norepinephrine release are found in Table IV. Compounds which inhibit various enzymes involved in the biosynthesis and metabolism of norepinephrine were tested and the results are presented in Table V. Antihistaminics, including pheniramine, phenindamine, tripellenamine, and chlorpheniramine, were inactive at a dose level of 10 mg/kg. The analgesics tested which included phenazocine, morphine, meperidine, *d*-propoxyphene, and normorphine at 5 or 10 mg/kg were inactive. Nalorphine at a dose of 10 mg/kg caused slight release (84% of control). Barbiturates, such as phenobarbital (50 mg/kg) and secobarbital (20 mg/kg) did not differ from control values. Certain cardioactive glycosides, including ouabain, strophanthidin, and digitoxin, caused slight release of norepinephrine at 2.5 mg/kg (86–92% of control). Nicotine at 2.5 mg/kg caused slight release (87% of control); higher doses could not be given because of its toxicity.

Table V

Inhibitors of Enzymes Concerned with Norepinephrine Biosynthesis and Metabolism

Compd	Dose, mg/kg	Release of norepinephrine-^{3}H, % of control
Tyrosine Hydroxylase Inhibitors		
α-Methyltyrosine	10	85
3,5-Diiodo-*l*-tyrosine	10	89
Aromatic Amino Acid Decarboxylase Inhibitors		
α-Methyldopa	10	103
α-Methyl-*m*-tyrosine	10	46
N-DL-Seryl-N-2,3,4-trihydroxybenzylhydrazine	10	131
1-(3-Hydroxybenzyl)-1-methylhydrazine	10	105
1-(4-Bromo-3-hydroxybenzyl)-1-methylhydrazine	10	90
Dopamine β-Hydroxylase Inhibitors		
p-Hydroxybenzyloxyamine	10	108
m-Hydroxybenzyloxyamine	10	91
4-Bromo-3-hydroxybenzyloxyamine	10	95
Disulfiram	20	107
Diethyldithiocarbamic acid sodium salt	20	110
Monoamine Oxidase Inhibitors		
Benzylhydrazine	10	115
Phenethylhydrazine	10	111
Phenylisopropylhydrazine	10	104
Nialamid	10	109
Iproniazid	10	125
Isocarboxazid	10	107
Hydralazine	10	105
N-Methyl-N-benzylpropynylamine	10	108
trans-Phenylcyclopropylamine (tranylcypromine)	10	91
α-Ethyltyrptamine acetate	10	96
Harmine	10	105
Harmaline	10	116

Cocaine (10 mg/kg) slightly inhibited release of norepinephrine (111% of control), but this inhibition decreased at higher doses. Tranquilizers (10 mg/kg) effected norepinephrine release in the following manner: azacyclonol, 104%; chlordiazepoxide HCl, 90%; droperidol, 89%; haloperidol, 76%; meprobamate, 102%; and oxypertine, 63%. Two antidepressants, pipradol and methylphenidate (10 mg/kg), inhibited normal release of norepinephrine, to the extent of 128 and 112%, respectively.

Discussion

Reserpine has been modified in order to separate its hypotensive effects, *i.e.*, peripheral depletion of norepinephrine, from its sedative effects, *i.e.*, central depletion of amines. These two activities are partially separated in syrosingopine[3] which is a hypotensive drug with reduced sedative activity. Another class of tranquilizers, the benzoquinolizines (tetrabenazine), centrally deplete endogenous amines in rabbits without much effect on peripheral norepinephrine.[4]

Minor structural changes of reserpine markedly influence release of norepinephrine (Table I). Deserpidine which lacks the aromatic methoxyl group and derivatives in which the acid moiety of the ester linkage of ring E has been changed, as in syrosingopine and recinnamine, are less active. Yohimbine, a weak sympatholytic agent, which differs both in substitution and stereochemistry from reserpine still causes release, an observation which has not been previously reported. Ranntidine, a heteroyohimbine, is inactive. Quaternization, as in serpentine, and yohimbine methiodide changes the activity from that of promotor to an inhibitor of release. Tetrabenazine and the corresponding alcohol cause marked release of cardiac norepinephrine-^{3}H in mice (Table I) in contrast to their reported lack of peripheral activity in rabbits. This release of tritiated norepinephrine is accompanied by a depletion of endogenous norepinephrine.[5] Dihydrotetrabenazine is much more active as a releasing agent than the parent ketone, tetrabenazine.

Another class of tranquilizing agents, the phenothiazines, *e.g.*, chlorpromazine, are reported to have no effect on endogenous norepinephrine either in the central nervous or the cardiovascular systems.[6] They do, however, inhibit the uptake of circulating norepinephrine-^{3}H into cardiac storage sites.[7,8] Another group of structurally related drugs which do not effect endogenous norepinephrine[5a] but do inhibit the uptake of norepinephrine-^{3}H into heart are various antidepressants such as imipramine, desipramine, amitryptyline, chlorprothixene, etc. The most effective inhibitor of uptake is the demethyl derivative of imipramine, desipramine.[9] In the central nervous system both classes of compounds (chlorpromazine and imipramine) inhibit the disappearance of tritiated norepinephrine, but only the antidepressant drug, imipramine, inhibits uptake of norepinephrine-^{3}H into *central* storage sites.[10]

In rats neither chlorpromazine nor imipramine caused significant changes in the rate of release of *cardiac* norepinephrine-^{3}H.[11] In mice, as shown in Table II, the antidepressants imipramine, desipramine, amitryptyline, and chlorprothixene inhibited the release of norepinephrine-^{3}H while the tranquilizers, such as chlorpromazine and certain of its metabolites,[12] had no or a slight inhibitory effect. Two compounds derived from norepinephrine by the action of acid, *i.e.*, adnamine and noradnamine,[13] resemble amitryptyline (Table II). Their pharmacological activity is unknown but, like the antidepressants, they inhibit release of norepinephrine-^{3}H in heart. Certain other antidepressants, such as pipradol and methylphenidate are also active. These findings may serve as a guide in the research for antidepressants.

Various hypotensive ganglionic blocking agents such as bretylium, chlorisondamine, hexamethonium, and pempidine inhibit release of cardiac norepinephrine-^{3}H

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in rats.[14] It has been proposed[15] that the "blocking action" of these drugs is due to a cholinergic aspect of adrenergic transmission. Guanethidine and related guanidine derivatives may stimulate this same system and cause release of norepinephrine.[16,17] This view is supported by the observation that bretylium and related compounds block the release of norepinephrine caused by guanethidine.[15] Table III shows that ganglionic blocking agents inhibit the normal physiological release while almost all of the guanidine derivatives increase it. N-O-Chlorobenzyl-N′,N″-dimethylguanidine is, however, an inhibitor of norepinephrine-^{3}H release.[16] Most of the ganglionic blocking agents are quaternary amines. Other quaternary amines such as the quaternary derivatives in the reserpine series (Table I), and the methochloride of *p*-hydroxy-N,N-dimethylphenethanolamine[2] also inhibit release of norepinephrine-^{3}H. By contrast a quaternary amine with releasing activity is the methochloride of 2,3-dihydroxy-N,N-dimethylphenethylamine.[2]

Various sympatholytic drugs (Table IV) show little activity. The 2-substituted imidazolines have both sympathomimetic (tolazoline, otrivin, naphazoline) and sympatholytic activity (phentolamine). Only phenazoline, an antihistaminic, and phentolamine which contain an amine nitrogen in addition to the imidazoline nitrogen caused release. The others tested showed little or no activity (see Results). Antihistamines have been reported to inhibit uptake of norepinephrine,[18] a property they share with many other classes of compounds.[7] The β-chloroethylamines, such as Dibenamine or phenoxybenzamine have been previously reported to release norepinephrine.[14a,19] Other sympatholytics such as azapetine, dihydroergotamine, and the β-blocking agents, prometholol, propanalol, *dl*-4-(2-isopropylamino-1-hydroxyethyl) methanesulfonanilide hydrochloride, and dichlorisoproterenol[14] either have no effect or cause only slight release of norepinephrine-^{3}H. As mentioned above, yohimbine, a sympatholytic agent, causes release of norepinephrine (Table I).

The synthesis of norepinephrine involves the enzymes, tyrosine hydroxylase,[20] aromatic amino acid decarboxylase,[21] and dopamine β-hydroxylase.[22] Metabolism of norepinephrine involves monoamine oxidase and catechol O-methyltransferase.[23] The use of norepinephrine-^{3}H makes available a useful method for assaying short-term effects of inhibitors of these enzymes on the release of norepinephrine. Amino acids, including tyrosine hydroxylase inhibitors, such as α-methyltyrosine and 3,5-diiodo-L-tyrosine,[24] and the long-term norepinephrine depletors, α-methyldopa and α-methyl-*m*-tyrosine,[25] cause only slight release of norepinephrine-^{3}H except for α-methyl-*m*-tyrosine. α-Methyl-*m*-tyrosine, a potent releasing agent, does not release *per se*, but only after decarboxylation to the amine, α-methyl-*m*-tyramine.[26-28] This conversion and subsequent release of norepinephrine-^{3}H by the resulting α-methyl-*m*-tyramine has been developed into a convenient and novel assay of the efficacy of aromatic amino acid decarboxylase inhibitors *in vivo*.[28] Presumably other amino acids release by the same mechanism.

The decarboxylase inhibitor, N-DL-seryl-N-2,3,4-trihydroxybenzylhydrazine[29] inhibits release in contrast to other decarboxylase inhibitors, such as the various hydrazine analogs of amino acids[30] and benzylmethylhydrazines.[31] The benzyloxyamines, isosteric inhibitors of dopamine β-hydroxylase,[32] have little effect on norepinephrine release, while another dopamine β-hydroxylase inhibitor, disulfiram,[33] and its active metabolite, diethyldithiocarbamic acid,[34] are slightly inhibitory.

As shown in Table V, harmaline,[35] as well as many of the monoamine oxidase (MAO) inhibitors of the hydrazine type, inhibits release. Inasmuch as MAO inhibitors find clinical application as antidepressants, they provide another example of inhibition of norepinephrine-^{3}H release by compounds with antidepressant activity.

Certain of the MAO inhibitors, depending on structure, may interfere with release and at the same time liberate norepinephrine due to an isosteric relationship to phenethylamines. These two opposing effects might result in partial cancellation. Phenylisopropylhydrazine (pheniprazine), for example, is reported to have sympathomimetic activity[36] which may be due to chemorelease of norepinephrine. Other workers have reported inhibition of release by this compound.[37] The MAO inhibitor *trans*-phenylcyclopropylamine[38] seems to have a chemoreleasing component to its activity. Catechol O-methyltransferase inhibitors, *e.g.*, tropolone and 3,4-dihydroxyphenylpropylacetamide, have no effect on norepinephrine-^{3}H release.[2]

The detailed mechanism by which differing classes of compounds liberate norepinephrine is not understood. It may be recalled that norepinephrine released

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by reserpine is metabolized by MAO, while that released by sympathomimetic amines directly into the circulation is methylated by catechol O-methyltransferase.[39] The mode of action of guanethidine differs from both that of sympathomimetic amines and reserpine.[37] The liberation of norepinephrine-^{3}H by these three classes of compounds may be influenced by a variety of inhibitors. Cocaine blocks the release of norepinephrine by sympathomimetic amines.[5,40] Ganglionic blocking agents,[14] MAO inhibitors, and phenothiazines[41] block release caused by reserpine. Ganglionic blocking agents[14] inhibit release caused by guanethidine.

The various relationships between drugs, biosynthesis, and the disposition and metabolism of norepinephrine may be further probed by the rapid assay utilized here. The method provides a simple, convenient method for studying structure–activity relationships with respect to chemorelease of norepinephrine. The method makes possible the study of interactions between drugs which release norepinephrine and drugs which inhibit the normal or drug-induced release of norepinephrine.

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The Depletion of Norepinephrine-^{3}H from Heart by α-Methyl-*m*-tyrosine. A Novel and Convenient Method for Assaying the Inhibition of Aromatic Amino Acid Decarboxylase *in Vivo*

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Cardiac norepinephrine of mice was prelabeled with 5 μcuries of norepinephrine-^{3}H. After 1 hr 10 mg/kg of α-methyl-*m*-tyrosine was administered subcutaneously. After 3 hr the activity of cardiac norepinephrine-^{3}H was 50% of that of control animals. This release of norepinephrine which requires enzymatic decarboxylation of α-methyl-*m*-tyrosine to the active releasing agent α-methyl-*m*-tyramine is blocked by inhibitors of aromatic amino acid decarboxylase. The resulting decrease in α-methyl-*m*-tyrosine-releasing activity is a direct measure of inhibition of the enzyme *in vivo*, and provides a convenient method for determining the effectiveness of decarboxylase inhibitors in intact animals.

The chemorelease of cardiac norepinephrine in mice is conveniently assayed by prelabeling the endogenous norepinephrine with an intravenous injection of 5 μcuries of norepinephrine-7-^{3}H; after 1 hr the drug to be tested is administered and the activity of cardiac norepinephrine-^{3}H after 3 hr is compared with that in control animals.[1,2]

In screening various enzyme inhibitors for their effect on norepinephrine release,[2] we observed that of the α-methyl aromatic amino acids which cause a marked depletion of norepinephrine over a period of days, *only* α-methyl-*m*-tyrosine caused a release of norepinephrine during the assay period of 2 hr.

The depletion of norepinephrine in heart, brain, and other tissues by α-methyl aromatic amino acids, such as α-methyl-*m*-tyrosine and α-methyldopa, is caused by the amine formed *in vivo* by the enzymatic decarboxylation of the amino acid.[3,4]

An earlier report that the amino acid, α-methyl-*m*-tyrosine, had a releasing activity of its own was based on experiments in animals in which aromatic amino acid decarboxylase was only partially inhibited by a hydrazine analog of dopa, *i.e.*, α-(3,4-dihydroxybenzyl)-α-hydrazinopropionic acid.[5]

Because α-methyl aromatic amino acids are poor substrates for aromatic amino acid decarboxylase[6] and because of the unique releasing activity of α-methyl-*m*-tyrosine, the release caused by this amino acid was reinvestigated. The effect of various amino acids on the liberation of norepinephrine-^{3}H from heart is shown in Table I.

α-Methyl-*m*-tyrosine was the only amino acid which caused significant release. The methyl ester of α-methyl-*m*-tyrosine was as active as the amino acid, suggestive of rapid ester hydrolysis *in vivo*. The release by α-methyl-*m*-tyrosine and by its decarboxylation product, α-methyl-*m*-tyramine, as a function of time is shown in Figure 1. Norepinephrine was released by the

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