Notes

The Chemorelease of Norepinephrine from Mouse Hearts. III. 3,5-Dihydroxy-4-methoxyphenethylamines and Related Compounds

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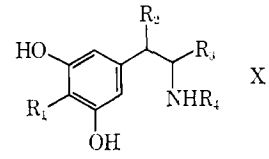
Received November 16, 1967

Compounds which effect the disposition, storage, and metabolism of catecholamines have frequently proved useful as pharmacological tools or therapeutic agents.[1] In order to assess structure–activity correlations in compounds which effect the storage and release of norepinephrine, a rapid and simple method has been developed.[2,3] It involves prelabeling the endogenous cardiac stores of norepinephrine with a tracer amount of norepinephrine-^{3}H. The subsequent loss of norepinephrine-^{3}H from heart can then be influenced by various compounds which may either increase or decrease the normal, physiological rate of release. During studies on structure–activity relationships with some 200 sympathomimetic amines,[2] it was discovered that 3,5-dihydroxy-4-methoxyphenethylamine was an extremely active releasing agent for cardiac norepinephrine. Its unique, norepinephrine-releasing activity among methoxylated phenethylamines, its lack of significant pressor activity, and its apparent storage in cardiac tissue as a false transmitter[4] suggested that it or the related amino acid might prove valuable in the treatment of hypertension.

The results of investigations of a series of related 3,5-dihydroxy-4-substituted phenylamines and amino acids (Table I) indicate that the parent 4-methoxy derivative (**2**) is the most active releasing agent for cardiac norepinephrine-^{3}H in this series of compounds. With both 3,5-dihydroxy-4-methoxyphenethylamine (**2**) and phenethanolamine (**8**), α-methylation causes a slight decrease in activity in contrast to the usual potentiating effect of α-methylation of sympathomimetic amines.[2] Replacement of the 4-methoxy substituent by hydrogen, *n*-propyloxy, or methyl results in diminution of activity (**1**, **4**, **6**). The 4-hydroxy derivative (**5**) which is known to be metabolically converted (catechol O-methyltransferase) to the 4-O-methyl ether[5] retains high activity. The 3,5-dihydroxy-4-methoxyphenethanolamines appear to be slightly less active than the corresponding phenethylamines, again in contrast to the usual potentiating effect of β-hydroxylation.[2] N-Alkylation (**10**, **11**) results in almost complete loss of activity.

Table I

The Chemorelease of Norepinephrine-^{3}H from Mouse Hearts by Compounds Related to 3,5-Dihydroxy-4-methoxyphenethylamine[a]



No.	R_1	R_3	R_4	X	Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control
			Phenethylamines, R_2 = H			
1[b]	H	H	H	HBr	5	50
2[c]	OCH_3	H	H	HCl	2.5	23[g]
3[d]	OCH_3	CH_3	H	HCl	2.5	36
4[e]	OC_3H_7	H	H	HCl	10	64
5	OH	H	H	HCl	5	22
6[f]	CH_3	H	H	HBr	5	54
			Phenethanolamines, R_2 = OH			
7[b]	H	H	H	$0.5H_2SO_4$	5	31
8[b]	OCH_3	H	H	HCl	2.5	26
9[d]	OCH_3	CH_3	H	HCl	2.5	43
10[b]	OCH_3	H	CH_3	HCl	10	73
11[b]	OCH_3	H	$CH(CH_3)_2$	HCl	10	103
			Phenylalanines, R_2 = H			
12[f]	OCH_3	COOH	H	$1.5H_2O$	31	50
13[d]	OH	COOH	H	HCl	30	38

[a] Assay as described[2] with five mice per group. Standard error ±5%. [b] Provided by C. H. Boehringer Sohn. [c] Provided by Sterling-Winthrop Research Institute. [d] Prepared by Regis Chemical Co. under research contract SA-43-ph 3021 for the Psychopharmacology Research Branch, National Institute of Mental Health. [e] Provided by Dr. K. Freter, Pharma Research, Montreal, Canada. [f] Provided by Hoffman-La Roche and Co. [g] Compound **2** at 5 and 10 mg/kg gives, respectively, 16 and 12% of control norepinephrine-^{3}H in heart.

Amino acids such as α-methyldopa and α-methyl-*m*-tyrosine which deplete norepinephrine stores owe their action to prior decarboxylation to the amines.[6] The effects of the amino acids (**12**, **13**) corresponding to 3,5-dihydroxy-4-methoxyphenethylamine and 3,4,5-trihydroxyphenethylamine were, therefore, studied. Both cause profound release of norepinephrine similar to that caused by α-methyl-*m*-tryosine.[7] The release by 3,5-dihydroxy-4-methoxyphenylalanine (50% of control at 31 mg/kg) was partially blocked (76% of control) when the mice were pretreated with 50 mg/kg of the aromatic amino acid decarboxylase inhibitor, N-DL-seryl-2,3,4-trihydroxybenzylhydrazine (*cf.* ref 7), indicating that the amino acid is first decarboxylated to the amine, which then causes the release of norepinephrine-^{3}H. The 3,5-dihydroxy-4-methoxyphenylalanine was tested without signs of toxicity at levels of 1000 mg/kg in mice. Two hours after the administra-

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

(3) J. W. Daly, C. R. Creveling, and B. Witkop, *ibid.*, **9**, 280 (1966).

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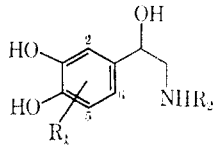
TABLE II

THE CHEMORELEASE OF NOREPINEPHRINE-^{3}H FROM MOUSE HEARTS BY RING METHYL AND METHOXY DERIVATIVES OF NOREPINEPHRINE[a]



No.	R_1	R_2	Dose, mg/kg	Norepinephrine-^{3}H in heart, % of control
14[b]	...	H	2.5	33
15[b]	...	CH_3	2.5	36
16	...	$CH(CH_3)_2$	10	86
17[c]	2-CH_3	H	10	55
18[c]	2-CH_3	CH_3	10	88
19[c]	2-CH_3	$CH(CH_3)_2$	10	76
20[c]	2-OCH_3	H	10	87
21[c]	2-OCH_3	CH_3	10	86
22[c]	2-OCH_3	$CH(CH_3)_2$	10	81
23[c]	5-CH_3	H	10	32
24[c]	5-CH_3	CH_3	10	74
25[c]	5-CH_3	$CH(CH_3)_2$	10	89
26	5-OCH_3	H	10	94
27[c]	6-CH_3	H	10	30
28[c]	6-CH_3	CH_3	10	82
29[c]	6-CH_3	$CH(CH_3)_2$	10	100
30	6-OCH_3	H	5	80
31[c]	6-OCH_3	$CH(CH_3)_2$	10	100

[a] Assay as described[2] with five mice per group. Standard error ±5%. Hydrochloride salts unless otherwise indicated. [b] Bitartrate salt. [c] Provided by C. H. Boehringer Sohn.

tion of this amino acid (50 mg/kg sc), the level of norepinephrine in brain[8] had decreased to 60 ± 15% of normal. This same dose depleted cardiac norepinephrine-^{3}H to a level corresponding to 34 ± 5% of control. 3,4,5-Trihydroxyphenylalanine (**13**) has now been reported to deplete endogenous norepinephrine in both heart and spleen[9] and brain.[10]

Other ring methyl and methoxy analogs of norepinephrine have been tested for releasing activity (Table II, which includes (nor)epinephrine and isoproterenol (**14**–**16**) for comparison). None of the analogs show the very high activity of 3,5-dihydroxy-4-methoxyphenyl derivatives. All of these methoxy derivatives have low activity in agreement with earlier relations between ring methoxylation and lack of norepinephrine-releasing activity.[2,4] The 3,5-dihydroxy-4-methoxy derivatives provide the sole exception to this generalization. Norepinephrine derivatives with methyl substituents in the ring *retained* appreciable activity (**17**, **23**, **27**) which was found to be reduced by N-alkylation.

Studies are now in progress on the pharmacological effects of 3,5-dihydroxy-4-methoxyphenylalanine and 3,4,5-trihydroxyphenylalanine in both the cardiovascular and the central nervous systems.

Acknowledgments.—The authors would like to express their gratitude to the various pharmaceutical firms which generously provided various amines and amino acids. The skilled assistance of Mrs. Louise Atwell is gratefully acknowledged.

(8) Determined on two groups of seven mice by the method of J. R. Crout in "Catechol Amines in Urine, Standard Methods of Clinical Chemistry," Vol. II, D. Seligson, Ed., Academic Press Inc., New York, N. Y., 1961, p 62.

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The Chemorelease of Norepinephrine from Mouse Hearts. IV. Structure–Activity Relationships. Reserpines and Yohimbines

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Received November 16, 1967

In the last 10 years the effect of structural modifications on the sedative and antihypertensive properties of reserpine have been intensively studied (for an excellent review see ref 2). Since reserpine apparently elicits its effects through depletion of biogenic amines, in particular norepinephrine, one method of probing structure–activity correlations in this class of compounds is to determine whether they cause depletion of norepinephrine. The use of an assay based on prelabeled cardiac stores of norepinephrine-^{3}H permitted the scrutiny of a large variety of compounds for their ability to deplete this important neurotransmitter. In a preliminary investigation on various drugs,[3b] it was noted that another *Rauwolfia* alkaloid, yohimbine, also caused depletion of cardiac norepinephrine-^{3}H. The norepinephrine-^{3}H-releasing activity of reserpine, yohimbine, and a variety of congeners have now been studied (Tables I and II). The method employed possesses the great advantages of requiring only 1–2 mg of sample and of providing definitive results within a short time.

Variations in the ester or other oxygen substituents at position 18 of reserpine (**1**–**7**) had little effect on the degree of norepinephrine-^{3}H release in correspondence with pharmacological data.[4] Methyl reserpate (**8**) which contains a free 18-hydroxyl group retained some activity in this assay in contrast to its reported lack of reserpine-like action.[4] 3-Epi-α-yohimbine (**37**), in which the 18-oxygen group is replaced by hydrogen, did not cause release of norepinephrine-^{3}H, nor did it possess marked sedative activity (Table II). Of the congeners tested which are epimers of reserpine at position 18 (**18**–**20**), all retained some, if not all, of their norepinephrine-^{3}H-releasing activity. It has been reported that methyl 18-epireserpate (**18**) has sedative activity,[2] while 18-epireserpine is inactive.[5]

Certain variations in the aromatic substituents caused diminution of norepinephrine-^{3}H-releasing activity (**10**, **11**, and **23**), while other alterations such as the removal of the 11-methoxy substituent of reserpine

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