simple acetylation of the *N*-terminal extremity of this only slightly active endecapeptide brings about a twenty five times increase of the melanophore stimulating activity (analogue C).

We see two possible explanations for this phenomenon: either the *N*-terminal structure of the chain has to be Ac-Ser- for the appearance of a strong melanophore stimulating activity, or the presence of an acetyl group at the extremity of the peptide chain ensures its resistance against degradation by aminopeptidases, thus increasing the apparent biological activity of the acetylated molecules by simply lenghtening their life.

Zusammenfassung. Drei strukturelle Analoge des α-MSH wurden synthetisiert und auf ihre Melanophoren-stimulierende Wirksamkeit untersucht. Für das Auftreten einer ausgeprägten biologischen Aktivität scheint die Anwesenheit einer *N*-Acetyl-Gruppe an dem Aminoende der Peptidkette wichtiger zu sein als das Vorhandensein der zwei ersten amino-endständigen Aminosäurereste der α-MSH-Kette.

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Studies on Inhibition of 3,4-Dihydrophenylethylamine (Dopamine) β-Oxidase *in vitro*[1]

The availability of a method for the preparation of purified dopamine β-oxidase[2] makes it possible to study the mechanism of the hydroxylation and the substrate specificity of the enzyme. The hydroxylation of the side chain of dopamine may be the rate-limiting step in the biosynthesis of norepinephrine and, consequently, inhibitors of dopamine hydroxylation may be important therapeutic agents in lowering norepinephrine levels *in vivo*.

This is a preliminary report of a study designed to explore the possibility of structural analogues of dopamine as well as structurally unrelated compounds being inhibitors of dopamine β-oxidase.

The test compounds were added simultaneously with 40 μg dopamine-1-C^{14} to a mixture which contained the following components (in μMol): potassium phosphate buffer, pH 6.4, 100; (1-methyl-2-phenyl)-ethyl hydrazine hydrochloride, 1.3; ascorbic acid, 6; fumaric acid, 10; ATP, 12.5. To this mixture 0.2 ml of the enzyme was added[3] and the final volume was adjusted with phosphate buffer pH 6.4 to 1 ml. The reaction mixture was incubated for 1 h at 37°C using air as a gas phase. At the end of the incubation the reaction was stopped by the addition of 3% acetic acid in ethanol, and the solution heated at 55°C for 5 min. The precipitate proteins were removed by centrifugation. The alcohol in the supernate was evaporated under nitrogen and the solution was analyzed for dopamine-1-C^{14} and norepinephrine-1-C^{14}. Dopamine-1-C^{14} and norepinephrine-1-C^{14} were separated as the acetylated derivatives by paper chromatography in the 'C' solvent system of Bush[4]. The relative inhibition rate of dopamine β-oxidase by each compound was determined by the comparison of the amount of norepinephrine-1-C^{14} formed in an incubation mixture which contained only the substrate and the incubation mixture which contained the compound to be tested and the substrate.

The accompanying Table shows the effects of the test compounds on the conversion of dopamine to norepinephrine. Several generalizations may be deduced from the Table. Primary phenylethylamines and phenylpropylamines are active inhibitors, while secondary amines are less active. Phenolic amines are even more potent inhibitors than phenyl amines, but methoxy amines are less active. The nature of the inhibition and the activity of the inhibitors *in vivo* are under investigation.

Added in proof: It was also found that homarylamine and impramine (Tofranil) are inhibitors of dopamine β-oxidase. We have also been able to show that phenylethylamine, 3-methoxydopamine, and *p*-hydroxyemphetamine are substrates of dopamine β-oxidase and are converted by it to the corresponding β-hydroxy compounds.

Zusammenfassung. Die enzymatische Umwandlung von Dopamin in Norepinephrine wird durch primäre Phenylethylamine und Phenylpropylamine sowie durch Pentobarbital gehemmt.

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Department of Psychiatry and Neurology, New York University, College of Medicine, New York, January 16, 1961.

[1] This work was supported by grants from the National Institutes of Health.

[2] E. Y. LEVIN et al., J. biol. Chem. *235*, 2080 (1960).

[3] The enzyme was prepared by the method of E. Y. LEVIN et al.[2] but the purification on calcium phosphate gel was omitted.

[4] M. GOLDSTEIN et al., Proc. Soc. exp. Biol. Med. *103*, 137 (1960).

Inhibition of dopamine-to-norepinephrine conversion *in vitro*

Active compounds	Amount added in μg	% of Inhibition	Inactive compounds	Amount added in μg	% of inhibition
p-hydroxyphenylethylamine	400	70–80	N-acetyl dopamine	400	0
β-phenylethylamine	400	40–50	3,4-dihydroxyphenylethylmethylamine (Epinine)	400	0–5
dl-d-methylphenethylamine (Amphetamine)	800	30–40			
2-phenylcyclopropylamine (SKF-385)	800	30–40	3-methoxy-4-hydroxyphenylethylamine (3-methoxydopamine)	400	0–5
Pentobarbital sodium	800	20–30			
3,4-dihydroxyphenylethylamine (Epinine)	800	10–20	3,4,5-trimethoxyphenylethylamine (Mescaline)	400	0
3-methoxy-4-hydroxyphenylethylamine (3-methoxydopamine)	800	10–20	N,N-diethyl-*d*-lysergamide (LSD-25)	400	0
			Phenylmethylaminopropane (*d*-Desoxyephedrine)	400	0–5