PHARMACOLOGICAL REVIEWS
Vol. 18, No. 1, Part I

Printed in U.S.A.

F. INHIBITION OF NOREPINEPHRINE BIOSYNTHESIS AT THE DOPAMINE-β-HYDROXYLATION STAGE

M. GOLDSTEIN[1]

Neurochemistry Laboratories, Department of Psychiatry and Neurology, New York University School of Medicine

The enzyme dopamine-β-hydroxylase is a mixed-function oxidase and catalyzes the terminal step in the biosynthesis of norepinephrine (NE) according to the following equation (18):

$$\text{Dopamine} + \text{Ascorbate} + O_2 \xrightarrow{\text{Fumarate}} \text{NE} + H_2O + \text{Dehydroascorbate}$$

Fumarate and related dicarboxylic acids markedly stimulate the enzymatic β-hydroxylation (18). Recently it was found that fumarate stimulates the enzymatic β-hydroxylation only at low substrate concentrations (3). Fumarate lowers the K_m but does not change the V_{max}. Catalase (17) and peroxidase (16) protect the enzyme from inactivation by peroxides during the incubation period.

The enzyme is nonspecific and accepts a variety of sympathomimetic amines structurally related to dopamine as substrates (4, 8, 17). Several sympathomimetic amines which were found to be substrates of dopamine-β-hydroxylase and their corresponding β-hydroxylated products are listed in table 1. From inhibition studies on conversion of dopamine to NE by competitive substrates and from spectrophotometric changes in the quinone test, the following conclusions were drawn concerning the substrate affinity for the enzyme (8): a) primary phenylethyl and phenylpropylamines are better substrates than their corresponding secondary amines; b) the *p*-hydroxy and 3,4-dihydroxy ring-substituted amines are better substrates than the corresponding unsubstituted amines; and c) the O-methylation in the ring decreases substrate affinity for the hydroxylating enzyme.

The relative nonspecificity of dopamine-β-hydroxylase may be an important factor in the pharmacological activities of the sympathomimetic amines. The β-hydroxylated products of the sympathomimetic amines (*i.e.* octopamine, aramine, *etc.*) may enter NE storage sites and displace the adrenergic neurotransmitter. Of considerable interest is the finding that only the (+) α-methylamines are substrates for dopamine-β-hydroxylase (12). The decarboxylation of L-α-methyl amino acids *in vivo* produces (+) α-methylamines (24) which are excellent substrates for the hydroxylating enzyme. It should be pointed out, however, that not all substrates for dopamine-β-hydroxylase *in vitro* undergo β-hydroxylation *in vivo*. (+) Amphetamine is not converted *in vivo* to its β-hydroxylated product (+) norephedrine, but (+) *p*-hydroxyamphetamine, which is formed *in vivo* from (+) amphetamine, is converted to (+) *p*-hydroxynor-

[1] Research Career Development Awardee of the United States Public Health Service, Grant No. 5-K3-MH-14, 1918-04. Supported by the grant of USPHS No. MH-02717-06 and by a grant of Licensed Beverage Industries, Inc.

TABLE 1

Sympathomimetic amines which are substrates of dopamine-β-hydroxylase and their corresponding β-hydroxylated products

Substrate	Product
Dopamine	Norepinephrine
Tyramine	Norsynephrine
Phenylethylamine	β-Phenylethanolamine
Epinine	Epinephrine
3-Methoxy-dopamine	3-Methoxy-norepinephrine
(+) α-Methyldopamine	Cobefrine
(+) α-Methyl-*m*-tyramine	Aramine
(+) Amphetamine	Norephedrine
(+) *p*-Hydroxyamphetamine	*p*-Hydroxynorephedrine
Mescaline	β-Hydroxymescaline

ephedrine (7). The β-hydroxylation of β-phenylethylamine occurs *in vivo* to a much smaller extent than should be expected from the substrate affinity for the hydroxylating enzyme. Thus, the substrate affinity for the enzyme *in vitro* is not the only factor which is decisive for the β-hydroxylation *in vivo*.

INHIBITORS OF DOPAMINE-β-HYDROXYLASE

1. Compounds structurally related to phenylethylamine

The relative nonspecificity of the enzyme suggested that compounds structurally related to phenylethylamine, which are not substrates, might act as inhibitors. The α-amino acetophenone analogues of epinephrine (E) and NE, adrenalone and arterenone, proved to be potent inhibitors of the enzyme *in vitro* and *in vivo* (13). A Lineweaver-Burk plot indicates that the inhibition by adrenalone or arterenone is of a competitive nature. Substitution of the amino nitrogen increases the inhibitory activity of the α-amino acetophenones.

Several benzylhydrazines and benzyloxyamines are potent inhibitors of the enzyme *in vitro* and *in vivo* (23). Tryptamine, serotonin and histamine produce some inhibition at 10^{-3} M (9). Whether this has physiological significance is yet to be established. Imipramine and desmethylimipramine show some inhibitory activity at 10^{-4} M. Sympathetic blocking agents such as guanethidine, TM-10 and bretylium are inactive (9). Thus, it is unlikely that these drugs owe their action, as has been previously postulated, to the inhibition of dopamine-β-hydroxylase.

2. Chelating agents

The finding that dopamine-β-hydroxylase is inhibited by various chelating agents suggested that a metal is involved in the catalytic activity of the enzyme (9, 10). More recently it was shown that dopamine-β-hydroxylase[2] is a copper enzyme and that the oxidation reduction state of copper changes during the en-

[2] Dopamine-β-hydroxylase was purified by a modification of previously described procedure A (11). The enzyme was eluted from the DEAE column with a gradient of increasing NaCl concentration.

TABLE 2

Inhibition of dopamine-β-hydroxylase with chelating agents

Chelating Agent	Activity
	%
EDTA, 10^{-4} M	60
o-Phenanthroline, 10^{-5} M	30
8-Hydroxyquinoline, 10^{-5} M	40
2,2-Dipyridyl, 10^{-5} M	30
Kethoxal bis (thiosemicarbazone), 10^{-5} M	50
4-Isopropyltropolone, 10^{-5} M	25
Colchiceine, 10^{-5} M	30
Colchicine, 10^{-5} M	100
Sodium diethyldithiocarbamate, 10^{-6} M	25
Disulfiram, 10^{-6} M	<10

zymatic β-hydroxylation (3, 5, 11). Ascorbate reduces the cupric copper of the enzyme to cuprous copper and the latter is reoxidized during the enzymatic β-hydroxylation (3, 5). It has recently been found that cysteine also reduces the cupric copper of the enzyme, and that the reduced enzyme catalyzes the enzymatic β-hydroxylation of tyramine-H^3 without addition of ascorbate (16).

The effects of various chelating agents on dopamine-β-hydroxylase activity are shown in table 2. All the tested chelating agents, and especially those with a high affinity for binding with copper, were potent inhibitors of dopamine-β-hydroxylase. Of considerable interest is the finding that tropolones are inhibitors of dopamine-β-hydroxylase (10). The restoration of activity upon addition of Fe^{++} or Co^{++} ions, and the inhibition by colchiceine, which has the acyloin structure, but not by colchicine, which does not have the acyloin structure, suggest that the enzymatic inhibition by tropolones is due to their metal chelating properties. The simultaneous inhibition of dopamine-β-hydroxylase and catechol methyl transferase by tropolones (1, 14) and the affinity of tropolones for adrenergic β-receptors (1), warrant further biochemical and pharmacological studies with this class of compounds.

The data in table 2 also show that disulfiram is a potent inhibitor of dopamine-β-hydroxylase (6, 11). The finding that disulfiram becomes completely reduced to diethyldithiocarbamate by ascorbate suggests that the inhibition by disulfiram is a consequence of its reduction to the carbamate, which is then effective as a chelating agent. More recently it was also shown that the absorption spectrum in the visible region of a highly purified dopamine-β-hydroxylase[2] (11) in the presence of disulfiram and ascorbate shows a maximum at 450 mμ. The spectrum is identical to that of the copper-diethyldithiocarbamate complex.

EFFECTS OF DOPAMINE-β-HYDROXYLASE INHIBITORS ON THE β-HYDROXYLATION OF EXOGENOUS SYMPATHOMIMETIC AMINES

In disulfiram-treated animals, or in animals treated with other dopamine-β-hydroxylase inhibitors, the conversion of dopamine-H^3 to NE-H^3 is inhibited and

dopamine-H^3 is the major labeled amine which accumulates in tissues (6, 13). Thus, it was demonstrated that when dopamine-β-hydroxylase is ihhibited, dopamine becomes bound in the tissue without being further metabolized. In animals treated with disulfiram or with tropolone the formation of the β-hydroxylated products from other substrates of dopamine-β-hydroxylase (*i.e.*, tyramine-H^3, *m*-tyramine-H^3, α-methyl tyramine-H^3 and α-methyl dopamine-H^3) is markedly inhibited (10, 11, 20, 21).

THE EFFECT OF DISULFIRAM ON ENDOGENOUS CATECHOLAMINE TISSUE LEVELS

The inhibition of dopamine-β-hydroxylase *in vivo* by disulfiram results in a lowering of NE tissue levels (19, 20) and in an increase of dopamine in the brain (16). The NE levels sharply decrease the first 6 hr after administration of disulfiram and they remain at a low level even 24 hr after administration of the inhibitor (fig. 1). The initial lowering of NE levels is more pronounced in the brain than in the heart. This may be due to the more rapid turnover of NE in the brain. On repeated administration of disulfiram every 6 hr, there is a continuous decrease in the tissue's NE content. After the second administration of disulfiram, the rate of the inhibition decreases, and even after repeated administration of the inhibitor the NE tissue levels were not completely depleted. It is conceivable that disulfiram does not completely inhibit dopamine-β-hydroxylase *in vivo*. With less than complete inhibition, the rate of NE depletion will depend on the rate of NE utilization and on the rate of NE resynthesis. It can therefore be assumed that the NE from a faster turnover store will be depleted to a greater extent than from a store with a slower turnover rate.

The following findings support the conclusion that the depletion of NE tissue levels by disulfiram is related to dopamine-β-hydroxylase inhibition. 1) There is a good time correlation between the lowering of endogenous NE tissue levels and

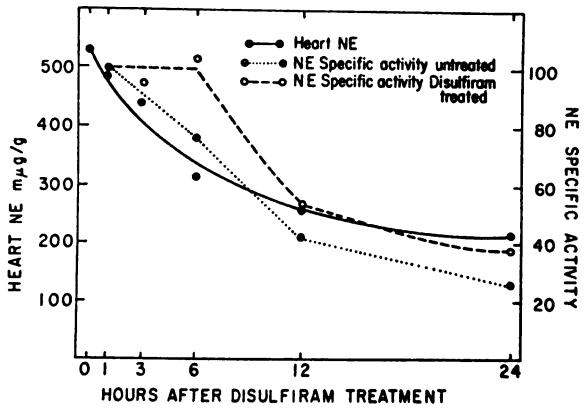

FIG. 1. Specific activities and endogenous levels of NE in the heart of the rat following administration of NE-H^3 in untreated and disulfiram-treated animals. (Disulfiram and NE-H^3 were administered simultaneously.)

the inhibition of the β-hydroxylation of exogenous sympathomimetic amines. 2) Following administration of disulfiram and NE-H^3 there is no change in the specific activity of NE within the first 6 hr after administration of the inhibitor. This indicates that the synthesis of new unlabeled NE has been inhibited: the heart loses endogenous and exogenous NE-H^3 at approximately the same rate, and NE levels progressively decrease. As the effect of disulfiram diminishes and synthesis begins, there is a progressive decrease in the specific activity of NE (fig. 1). 3) The decrease in exogenous and endogenous NE levels in the tissues of disulfiram-treated animals is associated with an increase in exogenous and endogenous dopamine levels (6, 16). 4) Disulfiram does not increase the rate of disappearance of NE-H^3 from the heart and does not prevent the uptake of NE-H^3 into the heart (6, 20).

The previously available inhibitors of dopamine-β-hydroxylase, such as benzyloxyamines, do not inhibit endogenous NE synthesis in the intact animal (23). It has therefore been suggested that the catalysis by tyrosine hydroxylase, and not by dopamine-β-hydroxylase, is the rate-limiting step in NE biosynthesis (25). The finding that disulfiram decreases the levels of endogenous NE in tissues does not necessarily imply that dopamine-β-hydroxylase is the rate-limiting step in NE biosynthesis under physiologic conditions. However, it does show that a sufficiently effective inhibitor of dopamine-β-hydroxylase can regulate NE biosynthesis *in vivo*.

DISULFIRAM AS A TOOL IN PHARMACOLOGICAL AND CLINICAL STUDIES

The inhibition of tyrosine hydroxylase may produce different pharmacological effects from those produced by the inhibition of dopamine-β-hydroxylase. The inhibition of tyrosine hydroxylase results in a lowering of both catecholamines, namely, dopamine and NE, while the inhibition of dopamine-β-hydroxylase results in a lowering of NE tissue levels and in an increase of dopamine tissue levels. Disulfiram may therefore prove to be a useful tool in investigating the role of dopamine in the brain. After it was found that dopamine and tyramine become bound in the tissues of animals treated with dopamine-β-hydroxylase inhibitors (6, 10, 11), the subcellular distribution of these amines was investigated (21). The presence of a catechol group results in a greater retention in the microsomal fraction than the presence of a β-hydroxyl group. Sympathomimetic amines which lack both the catechol and the β-hydroxyl group do not stay in the microsomal fraction (21). Recent studies have shown that when dopamine-β-hydroxylase is inhibited with disulfiram, tachyphylaxis to tyramine develops more rapidly and escape from tachyphylaxis does not occur (2).

In DOCA-hypertensive rats, disulfiram causes a marked and sustained hypotensive effect (26). The antihypertensive effect caused by disulfiram seems to be related to the inhibition of dopamine-β-hydroxylase.

In animals treated with disulfiram the release of NE-H^3 from the heart by tyramine is diminished as compared with animals not treated with disulfiram. These preliminary results indicate that the release of NE from the tissues by the

sympathomimetic amines is mediated to some extent *via* their β-hydroxylated products (16, 22).

Studies on the therapeutic potential of disulfiram as an agent which interferes with formation of the adrenergic neurohumors in the treatment of hypertension, angina, neuroblastoma and pheochromocytoma are warranted. It is noteworthy in this connection, that tyrosine hydroxylase and dopamine-β-hydroxylase activities were found in neuroblastoma and pheochromocytoma tumors (15). Thus, it was demonstrated that the enzymes which catalyze catecholamine production are present in these tumors, and their activity must be responsible for the overproduction of the catecholamines in these diseases.

REFERENCES

1. Belleau, B. and Burba, J.: Occupancy of adrenergic receptors and inhibition of catechol-O-methyl transferase by tropolones. J. med. pharm. Chem. **6**: 755–759, 1963.
2. Bhagat, B., Gordon, E. K. and Kopin, J. J.: Norepinephrine synthesis and the pressor response to tyramine in the spinal cat. J. Pharmacol. **147**: 319–323, 1965.
3. Blumberg, W. E., Goldstein, M., Lauber, E. and Peisach, J.: Magnetic resonance studies on the mechanism of the enzymic β-hydroxylation of 3,4-dihydroxyphenylethylamine. Biochim. biophys. Acta **99**: 188–190, 1965.
4. Creveling, C. R., Daly, J. W., Witkop, B. and Udenfriend, S.: Substrates and inhibitors of dopamine-β-hydroxylase. Biochim. biophys. Acta **64**: 125–134, 1962.
5. Friedman, S. and Kaufman, S.: 3,4-Dihydroxyphenylethylamine β-hydroxylase: A copper protein. J. biol. Chem. **240**: 552–554, 1965.
6. Goldstein, M., Anagnoste, B., Lauber, E. and McKereghan, M. R.: Inhibition of dopamine-β-hydroxylase by disulfiram. Life Sci. **3**: 763–767, 1964.
7. Goldstein, M. and Anagnoste, B.: The *in vivo* conversion of d-amphetamine to d-p-hydroxynorephedrine. Biochim. biophys. Acta, in press.
8. Goldstein, M. and Contrera, J. F.: The substrate specificity of phenylamine-β-hydroxylase. J. biol. Chem. **237**: 1898–1902, 1961.
9. Goldstein, M. and Contrera, J. F.: The activation and inhibition of phenylamine-β-hydroxylase. Experientia **18**: 334–336, 1962.
10. Goldstein, M., Lauber, E. and McKereghan, M. R.: The inhibition of dopamine-β-hydroxylase by tropolone and other chelating agents. Biochem. Pharmacol. **13**: 1103–1106, 1964.
11. Goldstein, M., Lauber, E. and McKereghan, M. R.: Studies on the purification and characterization of 3,4-dihydroxyphenylethylamine-β-hydroxylase. J. biol. Chem. **240**: 2066–2072, 1965.
12. Goldstein, M., McKereghan, M. R. and Lauber, E.: The stereospecificity of the enzymatic amphetamine β-hydroxylation. Biochim. biophys. Acta **89**: 191–193, 1964.
13. Goldstein, M., Musacchio, J. M., Kenin, M. C., Contrera, J. F. and Rice, M. D.: The inhibition *in vivo* of norepinephrine synthesis by adrenalone. Biochem. Pharmacol. **11**: 809–811, 1962.
14. Goldstein, M. and Musacchio, J. M.: Effects of monoamine oxidase inhibitions on biogenic amine metabolism. Ann. N. Y. Acad. Sci. **107**: 840–846, 1963.
15. Goldstein, M., Prochoroff, N. and Sirlin, S.: A radioassay for dopamine-β-hydroxylase activity. Experientia, in press.
16. Goldstein, M., *et al.*: To be published.
17. Levin, E. Y. and Kaufman, S.: Studies on the enzyme catalyzing the conversion of 3,4-dihydroxyphenylethylamine to norepinephrine. J. biol. Chem. **236**: 2043–2049, 1961.
18. Levin, E. Y., Levenberg, B. and Kaufman, S.: The enzymatic conversion of 3,4-dihydroxyphenylethylamine to norepinephrine. J. biol. Chem. **235**: 2080–2086, 1960.
19. Musacchio, J., Horst, W. D., Kopin, I. J., Anagnoste, B. and Goldstein, M.: Inhibition of dopamine-β-hydroxylase by disulfiram *in vivo*. J. Pharmacol., to be published.
20. Musacchio, J., Kopin, I. J. and Snyder, S.: Effects of disulfiram on tissue norepinephrine content and subcellular distribution of dopamine, tyramine and their β-hydroxylated metabolites. Life Sci. **3**: 769–775, 1964.
21. Musacchio, J., Kopin, I. J. and Weiss, V. K.: Subcellular distribution of some sympathomimetic amines. J. Pharmacol. **148**: 22–27, 1965.
22. Musacchio, J., *et al.*: To be published.
23. Nikodijevic, B., Creveling, C. R. and Udenfriend, S.: Inhibition of dopamine-β-oxidase *in vivo* by benzyloxyamine and benzylhydrazine analogs. J. Pharmacol. **140**: 224–228, 1963.
24. van Rossum, J. M.: Mechanism of the central stimulant action of α-methyl-meta-tyrosine. Psychopharmacologia **4**: 271–280, 1963.
25. Spector, S., Sjoerdsma, A. and Udenfriend, S.: Blockade of endogenous norephedrine synthesis by α-methyltyrosine, an inhibitor of tyrosine hydroxylase. J. Pharmacol. **147**: 86–95, 1964.
26. Wohl, A. J., Nemeth, M. and Korduba. C.: Dopamine-β-hydroxylase inhibition and antihypertensive effect of disulfiram. Fed. Proc. **24**: No. 2, 1441, 1965.