Proceedings of the National Academy of Sciences
Vol. 66, No. 2, pp. 453–458, June 1970

Neurally Mediated Increase in Dopamine-β-Hydroxylase Activity

Perry B. Molinoff,* Steven Brimijoin, Richard Weinshilboum, and Julius Axelrod

NATIONAL INSTITUTE OF MENTAL HEALTH, BETHESDA, MARYLAND

Communicated by Bernard B. Brodie, March 27, 1970

Abstract. The development of a sensitive and specific enzymatic assay for dopamine-β-hydroxylase has enabled us to measure the activity of this enzyme in several tissues where it has not previously been measured. The administration of reserpine leads to an increase in dopamine-β-hydroxylase activity in the rat adrenal, heart, salivary gland, and in sympathetic ganglia. The increase in the heart is preceded by a small but significant fall. We have confirmed the increase in tyrosine hydroxylase which follows the administration of reserpine and have found that the activity of phenylethanolamine-*N*-methyltransferase also increases after administration of this drug. The activities of two enzymes not involved in the synthesis of catecholamines, monoamine oxidase and lactate dehydrogenase, are not affected by reserpine treatment. The rise of dopamine-β-hydroxylase activity in the sympathetic ganglia is blocked by surgical decentralization.

Dopamine-β-hydroxylase (DBH) catalyzes the oxidation of dopamine,[1] the last step in the reaction sequence leading to the formation of the neurotransmitter noradrenaline. The enzyme is highly localized within noradrenaline storage vesicles of sympathetic nerves,[2] and it has recently been found to be distributed between the membrane and soluble contents of the adrenal chromaffin granule.[3] Stimulation of the adrenal gland with acetylcholine causes release of the entire soluble contents of the chromaffin granule,[4] including DBH.[5] The enzyme has also been found in the perfusate of the isolated calf,[6] cat,[7] and dog spleen[8] after splenic nerve stimulation.

Purified DBH, obtained from bovine adrenal medulla, has been studied in detail,[1] but relatively few investigations have been made in which the activity of the enzyme has been measured in crude tissue homogenates after pharmacologic or physiologic manipulations. DBH is difficult to assay in such homogenates due to the presence of inhibitory compounds.[9]

Changes in the activities of several enzymes involved in catecholamine synthesis have recently been demonstrated after the administration of reserpine, a drug that interferes with the binding of noradrenaline in the synaptic vesicle.[10] DBH activity, measured in the adrenals of rabbits, falls after the administration of reserpine and then rises after 1–2 days to values above normal.[11] The activity of tyrosine hydroxylase in rat sympathetic ganglia, heart, and adrenal glands also

increases after the administration of reserpine.[12] This increase appears to be an induction, since it is prevented by inhibitors of protein synthesis.[13] By means of surgical denervation and decentralization it has been shown that the reserpine-induced increase in tyrosine hydroxylase activity in the adrenal gland and in the superior cervical ganglia is mediated by changes in neural activity.[14]

We have recently developed a sensitive and specific enzymatic assay for DBH which has made it possible to measure the activity of this enzyme in crude homogenates of tissues where it has not previously been studied. This assay has been used to study the changes in DBH activity, induced by reserpine, in rat heart, salivary gland, and sympathetic ganglia.

Methods. Assays: The *DBH* assay involves the conversion of phenylethylamine to phenylethanolamine, which is then enzymatically *N*-methylated by phenylethanolamine-*N*-methyltransferase (PNMT) to *N*-methyl-phenylethanolamine. PNMT, an enzyme localized to the adrenal medulla[15] and to brain,[16] specifically *N*-methylates β-hydroxylated amines. Tissues are homogenized in 0.005 *M* Tris buffer, pH 7.4, containing 0.1% Triton X-100. Aliquots of these homogenates are then incubated at pH 6 with 2.4 μmoles ascorbic acid, 0.4 μmole phenylethylamine, 25 μmoles sodium fumarate, 10 μmoles Tris, 50 μg pargyline, and catalase and copper sulfate sufficient to obtain optimal activity. After a 20-min incubation, the pH of the reaction is abruptly changed from 6 to 8.6, and the phenylethanolamine formed is *N*-methylated with purified bovine adrenal PNMT and 1 mμmole of ^{14}C-*S*-adenosylmethionine as a methyl donor. The radioactive *N*-methyl-phenylethanolamine formed is extracted into toluene containing 3% isoamyl alcohol. Complete details of this assay will be published elsewhere.

Tyrosine hydroxylase was assayed by the method of Levitt *et al.*[17] with modifications as described by Mueller *et al.*[18] *Monoaminoxidase* was assayed by measuring the conversion of ^{14}C-tryptamine to indoleacetic acid as previously described.[19] *Lactate dehydrogenase* was assayed spectrophotometrically by following the disappearance of NADH.[22] *PNMT* was assayed as previously described[15] with the substitution of phenylethanolamine for normetanephrine as a methyl acceptor. The reactions were run in Tris buffer at pH 8.6.[21] ^{14}C-*S*-adenosylmethionine served as a methyl donor.

Protein determinations were by the method of Lowry *et al.* using bovine serum albumin as standard.[22]

Materials. Animals: Sprague-Dawley rats of either sex weighing 100–150 gm were used.

Drugs: Reserpine (Serpasil) was obtained from Ciba Pharmaceutical and pargyline (Eutonyl) from Abbott Laboratories. Catalase was obtained from C. F. Boehringer (Mannheim) and Triton X-100 from the Packard Instrument Company. ^{14}C-*S*-adenosylmethionine (spec. act. 42 mCi/mm) was purchased from New England Nuclear Corporation.

Results. Effect of reserpine on the DBH activity in the stellate ganglia and in the heart: When reserpine was subcutaneously administered to rats every other day for 6 days, the DBH activity rose at least twofold in the heart and stellate ganglia (Fig. 1). The activity began to rise in the cell bodies within a few hours after the administration of the drug, though the rise rarely was statistically significant before 16 hr. In contrast, in the heart, which contains the nerve terminals of the cell bodies found in the stellate ganglion, a small but consistent fall in DBH activity occurred within a few hours after reserpine administration. In experiments in which heart DBH was measured 4 hr after reserpine, activity was 2–27% lower than in controls. The mean fall was 14.8% with a standard error of 5.3%.

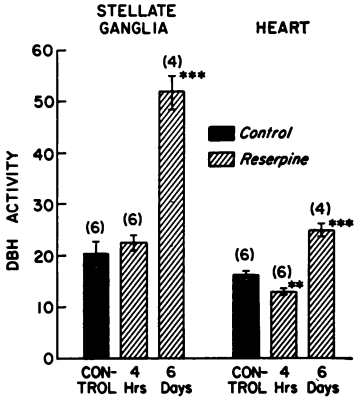

FIG. 1.—Effect of reserpine on DBH activity in rat stellate ganglia and heart. Reserpine (2.5 mg/kg) was administered subcutaneously on alternate days for 6 days. Groups of animals were killed 4 hr and 6 days after the first injection. DBH activity for stellate ganglia is expressed as cpm/μg protein and for heart as cpm/mg weight. The number of animals in each group is in parentheses above each bar. Results are expressed as mean ± SEM.
** $p < 0.01$ compared with control.
*** $p < 0.001$ compared with control.

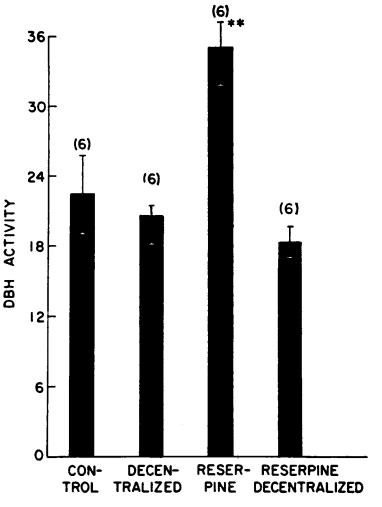

FIG. 2.—Blockade by decentralization of the effect of reserpine on superior cervical ganglia. Animals were decentralized on either the right or the left side under ether anesthesia. Reserpine (2.5 mg/kg) was administered subcutaneously on alternate days beginning 6 days after surgery. The animals were sacrificed on day 14 and DBH activity was measured in each superior cervical ganglion. Results are expressed as for stellate ganglia in Fig. 1.
**$p < 0.01$ compared with innervated ganglion from untreated treated animals.

Effect of decentralization on DBH activity: To examine the possibility that nerve impulses were modulating the increases in DBH activity, superior cervical ganglia were decentralized unilaterally. Under ether anesthesia, a 0.5-cm section of the sympathetic nerve chain was removed low in the neck. This procedure prevents impulses, originating in the central nervous system, from reaching either the superior cervical ganglion or its nerve terminals in the salivary gland. After allowing the animals to recover for 6 days, they were given reserpine on alternate days for 8 days. This resulted in an increase in DBH activity in the innervated superior cervical ganglion but not in the decentralized ganglion (Fig. 2).

Effect of reserpine on monoaminoxidase, lactate dehydrogenase, and PNMT: In order to determine whether the effect of reserpine was specific for the enzymes involved in the synthesis of catecholamines, monoaminoxidase and lactate dehydrogenase activities were measured in rat hearts, adrenals, and stellate ganglia. PNMT activity was also measured in rat adrenals. All assays were performed on the same homogenates prepared from chronically reserpinized animals (Table 1). There was no significant change in the activity of monoaminoxidase or lactate dehydrogenase in any of the tissues examined, while a

TABLE 1. *Effect of reserpine on enzyme activities.*

Tissue and treatment	Enzyme		
	DBH	Tyrosine hydroxylase	PNMT
Stellate ganglia			
Control	53.5 ± 5.5	0.61 ± 0.06	
Reserpine	70.4 ± 5.5*	1.27 ± 0.11†	
	cpm/μg protein/20′	mμmoles H_2^3O/mg protein/hr	
Adrenal gland			
Control	81 ± 2	5.89 ± 0.69	6.2 ± 0.3
Reserpine	171 ± 6†	14.93 ± 1.38†	8.6 ± 0.3†
	cpm/pr/20′ × 10^3	mμmoles H_2^3O/pr/hr	mμmoles *N*-methylphenylethanolamine/pr/hr
Heart			
Control	78.3 ± 3.8		
Reserpine	117.4 ± 5.2†		
	cpm/mg wt/20′		

	Enzyme	
	Lactate dehydrogenase	Monoaminoxidase
Stellate ganglia		
Control	0.25 ± 0.02	0.030 ± 0.003
Reserpine	0.27 ± 0.02	0.038 ± 0.005
	mμmoles DPNH/min/μg protein	mμmoles indoleacetic acid/mg protein/hr
Adrenal gland		
Control	1771 ± 180	0.12 ± 0.01
Reserpine	1457 ± 115	0.12 ± 0.01
	mμmoles DPNH/pr/min	mμmoles indoleacetic acid/pr/hr
Heart		
Control	338 ± 97	2.95 ± 0.51
Reserpine	293 ± 6	3.30 ± 0.25
	mμmoles DPNH/min/mg wt	mμmoles indoleacetic acid/gm wt/hr

Two groups of six male rats were used. Reserpine (2 mg/kg) was administered subcutaneously to one group, on alternate days, and all rats were killed on day 7. μg protein = soluble protein; pr = pair of adrenals; mg wt = mg wet weight; gm wt = gm wet weight; mg = protein-soluble protein.
* $p < 0.05$ compared with controls.
† $p < 0.001$ compared with controls.

highly significant increase was observed in the PNMT activity. DBH and tyrosine hydroxylase activities were also significantly increased in each of the tissues examined.

Discussion. The effects of reserpine on adrenal DBH activity[11] and on tyrosine hydroxylase activity in several sympathetically innervated organs[12] led us to study the effects of this drug on DBH activity in sympathetic ganglia and in sympathetic nerve terminals in the heart and salivary glands. Since reserpine has been shown to increase incorporation of ^{14}C-leucine into adrenal protein,[13] the possibility that reserpine was nonspecifically stimulating neuronal protein synthesis was examined. The failure of reserpine to affect monoaminoxidase and lactate dehydrogenase activities argues against a general trophic effect, and suggests that the drug-induced increases in enzyme activity are restricted to the catecholamine biosynthetic pathway. Another enzyme involved in catecholamine biosynthesis, but confined to the adrenal medulla[15] and to brain, is PNMT.[16, 23] The activity of this enzyme is also increased after

reserpine. (The effect of reserpine on PNMT was first observed by Ira Black and Roland Ciaranello, working in our laboratory.) In the case of tyrosine hydroxylase and on the basis of preliminary experiments with DBH, the increase appears to be caused by enzyme induction. These data raise the possibility that all the enzymes involved in catecholamine synthesis are regulated in coordinate fashion. Measurements of dihydroxyphenylalanine decarboxylase levels are currently in progress in our laboratory in order to further examine this possibility.

The increase in cardiac DBH activity after reserpine treatment is preceded by a significant initial decrease which occurs within 4 hr after the drug is administered. This rapid decrease is probably related to the unique relationship of DBH to the sympathetic vesicle. The effect of reserpine could be due to a leakage of the soluble contents of the vesicle, resulting in decreased DBH activity, or alternatively, reserpine could lead to increased nerve firing with quantal discharge of the contents of the sympathetic vesicle with a similar end result.

Decentralization blocks the reserpine-induced elevation in DBH activity in the superior cervical ganglion, suggesting that this increase is mediated by a transsynaptic process. The increase in DBH activity may be a consequence of increased nerve firing or may reflect an alteration in nerve impulse pattern. It is not clear whether this altered activity is the result of peripheral depletion of catecholamines or is a result of the central effects of reserpine.

It has been reported that nerve firing over short periods of time leads to increased synthesis of noradrenaline from both tyrosine[24] and dihydroxyphenylalanine.[25] The mechanism of the increased conversion of tyrosine to noradrenaline is thought to involve decreased feedback inhibition of tyrosine hydroxylase by noradrenaline. The evidence presented in this report and elsewhere[11, 14] indicates that, during longer periods of time, control may also be exercised over the amount of at least three of the four enzymes involved in the biosynthesis of catecholamines.

We acknowledge with thanks the excellent technical assistance of Mrs. Helen Hunt and Mrs. Dorothy Rutherford.

* Requests for reprints may be addressed to Dr. P. B. Molinoff, Laboratory of Clinical Science, National Institute of Mental Health, Building 10, Room 2D55, Bethesda, Maryland 20014.

[1] Kaufman, S., and S. Friedman, *Pharmacol. Rev.*, **17**, 71 (1965).
[2] Potter, L. T., and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **142**, 299 (1963).
[3] Viveros, O. H., L. Arqueros, R. J. Connett, and N. Kirshner, *Mol. Pharm.*, **5**, 60 (1969).
[4] Schneider, F. H., A. D. Smith, and H. Winkler, *Brit. J. Pharmacol.*, **31**, 94 (1967).
[5] Viveros, O. H., L. Arqueros, and N. Kirshner, *Life Sci.*, **7**, 609 (1968).
[6] de Potter, W. P., A. F. de Schaepdryver, E. J. Maerman, and A. D. Smith, *J. Physiol. (London)*, **204**, 102 (1969).
[7] Gewirtz, G., and I. J. Kopin, *Nature*, in press.
[8] de Potter, W. P., E. J. Maerman, A. F. de Schaepdryver, and A. D. Smith, *Proceedings of the 4th International Congress on Pharmacology Abstracts* (Basal: Schwabe and Co., 1969), p. 146.
[9] Chubb, I. W., B. N. Preston, and L. Austin, *Biochem. J.*, **111**, 245 (1969).
[10] Stjarne, L., *Acta Physiol. Scand. Suppl.*, **62**, 228 (1964).
[11] Viveros, O. H., L. Arqueros, R. J. Connet, and N. Kirshner, *Mol. Pharm.*, **5**, 69 (1969).
[12] Mueller, R. A., H. Thoenen, and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **169**, 74 (1969).
[13] Mueller, R. A., H. Thoenen, and J. Axelrod, *Mol. Pharm.*, **5**, 463 (1969).
[14] Thoenen, H., R. A. Mueller, and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **169**, 249 (1969).

[15] Axelrod, J., *J. Biol. Chem.*, **237**, 1657 (1962).
[16] Ciaranello, R. A., R. E. Barchas, G. S. Byers, D. W. Stemmle, and J. D. Barchas, *Nature*, **221**, 368 (1969).
[17] Levitt, M., J. W. Gibb, S. W. Daly, M. Lipton, and S. Udenfriend, *Biochem. Pharmacol.*, **26**, 1313 (1967).
[18] Mueller, R. A., H. Thoenen, and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **169**, 74 (1969).
[19] Wurtman, R. J., and J. Axelrod, *Biochem. Pharmacol.*, **12**, 1439 (1963).
[20] Kubowitz, F., and P. Oh, *Biochem. Z.*, **314**, 94 (1943).
[21] Molinoff, P., L. Landsberg, and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **170**, 253 (1969).
[22] Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
[23] Pohorecky, L. A., M. Zigmond, H. Karten, and R. J. Wurtman, *J. Pharmacol. Exptl. Therap.*, **165**, 190 (1969).
[24] Weiner, N., and M. Rabadjija, *J. Pharmacol. Exptl. Therap.*, **160**, 61 (1968).
[25] Austin, L., B. O. Livett, and I. W. Chubb, *Life Sci.*, **6**, 97 (1967).