Dopamine-β-Hydroxylase: Evidence for Increased Activity in Sympathetic Neurons during Psychotic States

Abstract. *Tritiated dopamine was infused into psychiatric patients during acute psychotic episodes and in remission. An index of the activity of dopamine-β-hydroxylase of salivary gland sympathetic neurons was determined by measuring the distribution of tritiated metabolites in salivary fluid. Increased synthesis of norepinephrine occurred in acute schizophrenia and in the manic state of manic-depressive psychosis but not in the depressed phase.*

Numerous studies of the metabolites in urine and cerebrospinal fluid in manic-depressive and schizophrenic disorders reveal the presence of irregularities in norepinephrine (NE) metabolism (*1*). A recognized inherently confounding factor in this approach is that the metabolites in these fluids are derived from several sources and that differences in the metabolism of catecholamines (CA) between them may cancel out meaningful changes in the common end-product repository (*2, 3*). We sought to circumvent this problem by investigating CA metabolism in the sympathetic neurons of the salivary glands. The latter, in animals and man, are richly innervated by sympathetic nerve terminals in close association with the acinar cells (*4*). These terminals readily take up and concentrate significant quantities of exogenously administered CA tracers (*2, 5*). In a previous study we found measurable amounts of tritiated metabolites in the saliva originating from local neuronal sites after infusions of tritiated CA in man (*6*).

Patients and normal subjects were infused with 1 mc of tritiated dopamine (DA) (specific activity, 5.0 curie/mmole; New England Nuclear Corp., Boston, Massachusetts), and saliva samples were collected over an ice bath under mild suction for 15 minutes every hour for several hours. The percentage contribution of each of the metabolites to the total activity in the particular saliva specimen was calculated, after correction for the loss of one tritium atom from the NE metabolites as compared to the DA metabolites (Table 1). The absence of DA and NE in the saliva is most likely due to the fact that about 70 percent of salivary gland monoamine oxidase (MAO) is found in the acinar cells which the amines traverse upon entering the salivary fluid stream (*7*).

In all patients examined the percentage contribution of the metabolites became relatively constant by the second hour following infusion. This fact prompts the suggestion that in a very short period—less than 1 hour after infusion—a percentage of the DA that has entered the neuron is converted to NE and a small but significant proportion may be bound and protected in granules that have little dopamine-β-hydroxylase (DBH) activity (*8*). An index of dopamine-β-hydroxylase activity (DBHI) was defined as the percentage of radioactivity contributed by the metabolites of NE. This index was calculated for the period in which the distribution of metabolites remained constant or when only a slight change in metabolite distribution was noted. The reported DBHI is the average for three consecutive saliva specimens collected from 2 to 6 hours after infusion (Fig. 1).

We report herein some of our findings on the CA metabolites in salivary fluid following the infusion of tritiated DA in two normal control subjects and four psychiatric patients. Patient E.K., with a 30-year history of manic-depressive illness, was studied over a period of 1 year through a complete cycle of the disease: hypomania, remission, and depression. Patient R.A.'s illness started about the age of 16 and was initially characterized by lability of mood, irrational behavior, loss of motivation for school, and long periods of social withdrawal. The 2 years prior to this study consisted of excited episodes during which his behavior became impulsive and inappropriate; he acted out infantile fantasies and was delusional. These periods, lasting 3 to 4 months, were followed by almost total withdrawal, when he remained in bed for weeks. He complained of severe inner agitation, had feelings of unreality, was depressed, and communicated in monosyllables. He was studied during this phase and again in a period of remission 3 months later. Patient J.G.'s first acute illness occurred upon graduation from college, when he began experiencing severe anxiety, confusion, and auditory and visual hallucinations of God speaking to him. He was hospitalized twice over the next 6 months and treated with phenothiazines and electroshock. The first study was done 4 months after his second hospitalization, at which time he showed hyperactivity, paranoid ideas, grandiose thinking, loose associations, conceptual distortions, and inappropriate affect. He was retested 6 months later during a remission in which only a minimal thought disorder was present. Patient I.L. had a manic-depressive disorder for about 20 years characterized by depressions and marked hypomania. He had been treated with antidepressant drugs and electroshock. In the last 3 years he went through a complete cycle every year. Initial studies were done during a severe depression, after which he received a course of eight electroshock

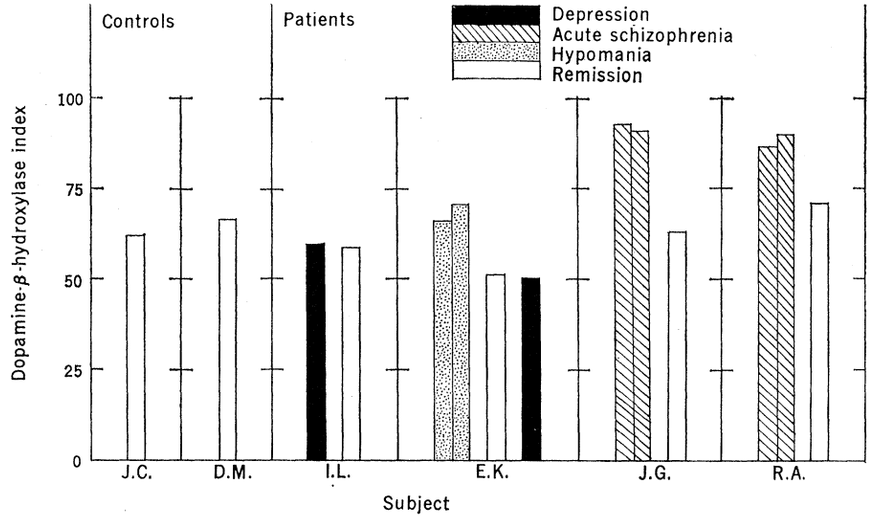

Fig. 1. Dopamine-β-hydroxylase activity index following infusion of tritiated DA in two control subjects and in four patients during various clinical conditions. This index represents the percentage of radioactivity contributed by NE metabolites.

treatments and made a good recovery. He was retested 8 days after his last treatment. At the time of each test all patients (and controls) had not taken any medication for at least 1 month.

The metabolite distribution shows a striking change between remission and acute states in schizophrenia and hypomania, but not in the depressions (Table 1). An index of the synthesis of NE from DA in the sympathetic nerve terminals which contain the DBH is presented in Fig. 1 for all subjects and conditions. Evidence for an increased synthesis of NE in acute psychotic states is seen in the schizophrenic patients J.G. and R.A. and in the manic-depressive patient E.K. during hypomania. No change in the synthesis index was found between remission and depression in both manic-depressive patients I.L. (agitated) and E.K. (retarded). However, a significant difference was found between these states upon gustatory stimulation with citric acid (*9*).

The synthesis of neuronal DBH as well as tyrosine hydroxylase (TH) is under the control of a transsynaptic process and the adrenocorticotropic hormone (ACTH) of the pituitary. Both enzymes are increased by protracted and repeated immobilization stress. The elevated enzyme activity is due to accelerated synthesis, as shown by the ability of cycloheximide to prevent this increase. Psychosocial stimulation also results in a significant increase in TH, but whether a similar effect occurs in DBH has not been reported (*10*). It is conceivable that TH may also be increased in the patients with elevated DBH activity. The stress produced by the illness may itself have an inducing effect on DBH activity and this increased enzyme activity may reflect increased enzyme synthesis. One can also speculate that the increase in DBH occurs in the course of stressful life events preceding the onset of the illness and is maintained throughout the course of the psychotic state. Whether the increased activity is due to a decrease in inhibition or an increased amount of enzyme cannot be determined from these studies.

A reduced ability to oxidize CA due to diminished MAO activity has been demonstrated in platelets from schizophrenic and manic-depressive subjects (*11*). An increased synthesis of central nervous system neurotransmitters, under stress, combined with low MAO activity provides an attractive hypothesis for the mechanisms underlying the functional psychotic states of schizophrenia and mania. Current views of schizophrenia conceptualize it as a genetic predisposition combined with reactions to stress (*12*). Whether the genetic predisposition is related to low MAO activity or increased capacity to form CA-synthesizing enzymes, or both, remains to be determined.

Table 1. Distribution of tritiated salivary metabolites after infusion of 1 mc of tritiated DA in patients and normal controls. Saliva specimens, stored at −15°C, were allowed to thaw slowly at 4°C and then centrifuged to remove solids. Portions of the clear saliva were taken for measurement of total radioactivity. Tritiated water was removed by drying in vacuo at 30°C, and after reconstitution in 5 ml of distilled water portions were again taken for measurement of radioactivity. Each specimen was adjusted to *p*H 5.2, and incubated at 37°C with 0.4 ml of Glusulase (containing 42,000 units of glucuronidase and 30,000 units of sulfatase) for 20 hours. Carrier substances were added in the amount of 1.0 mg of each of the following: homovanillic acid (HVA), homovanillyl alcohol (HVOH), 3-methoxytyramine (MT), 3-methoxy-4-hydroxymandelic acid (VMA), 3-methoxy-4-hydroxyphenylglycol (MHPG), and normetanephrine (NM); X = unknown. After a specimen was concentrated in vacuo to near dryness, 5.0 ml of 0.5*M* borate buffer at *p*H 8.4 (saturated with NaCl) was added. The HVOH and MHPG were extracted by shaking twice with 25 ml of ethyl acetate (previously saturated with the buffer). The extracts were combined and portions were taken for measurement of radioactivity. The HVA and VMA were extracted and measured in the same way, following acidification to *p*H 2.0 with concentrated HCl. The relative amounts of the two alcohols present in the first ethyl acetate extraction were determined by chromatography in butanol, acetic acid, and water (8 : 2 : 2) on Whatman No. 1 paper. Both MHPG and HVOH can be separated adequately (R_F values of 0.67 and 0.80, respectively); the carrier spots are easily seen under ultraviolet light, and the radioactivity is eluted and measured. The two acids present in the second extraction (at *p*H 2.0) were also separated, and their relative amounts were determined by chromatography and elution in the same system (R_F values: VMA, 0.66; HVA, 0.79). The remaining aqueous phase was then adjusted to *p*H 9.3 and extracted with two volumes of water-saturated isoamyl alcohol, portions of which were taken for determination of radioactivity. The remainder of this extract was concentrated in vacuo and chromatographed as above. Sufficient separation can be achieved to allow elution and determination of relative activity of NM (R_F, 0.40) and MT (R_F, 0.51). The radioactivity measured in these three extractions typically accounted for 80 to 85 percent of the activity originally present in the saliva after the removal of the tritiated water. More than 95 percent of the radioactivity in each of the chromatograms coincided with the standards. Only 5 percent of the tritiated metabolites in the saliva were present as conjugates.

Subject	Date	Condition	Distribution (%)						
			HVA	HVOH	MT	X	VMA	MHPG	NM
		Controls							
J.C.	1 December 1972		22	8	7	1	15	45	2
D.M.	5 October 1972		14	9	10	0	9	53	4
		Manic-depressive							
I.L.	27 December 1972	Depressed	33	2	5	1	14	43	2
	16 January 1973	Remission*	34	3	5	0	11	45	2
E.K.	18 February 1972	Hypomania	15	6	11	2	16	44	6
	23 February 1972	Hypomania	14	4	10	2	16	48	6
	26 September 1972	Remission	34	5	9	1	9	40	2
	25 November 1972	Depressed	31	7	12	0	10	36	4
		Schizo-affective							
J.G.	12 April 1972	Acute	4	2	1	0	26	55	12
	28 April 1972	Acute	4	4	0	1	26	51	14
	27 October 1972	Remission	20	6	10	1	13	48	2
R.A.	21 April 1972	Acute	5	4	1	3	18	54	15
	28 April 1972	Acute	4	4	1	1	28	46	16
	28 July 1972	Remission	16	6	6	1	8	60	3

* After electroshock treatment.

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References and Notes

1. M. B. Bowers, G. R. Heninger, F. A. Gerbode, *Int. J. Neuropharmacol.* **8**, 255 (1969); S. Wilk, *Nature* **235**, 440 (1972); W. B. Bunney, J. M. Davis, H. Weil-Malherbe, H. Smith, *Arch. Gen. Psychiat.* **16**, 448 (1967); S. Rosenblatt, J. D. Chanley, W. P. Leighton, *J. Psychiat. Res.* **6**, 321 (1969).
2. I. J. Kopin, *Biochem. Pharmacol.* **14**, 753 (1965).
3. H. Taubin, *Clin. Res.* **19**, 724 (1971); W. R. Burack and P. R. Braskoczy, *J. Pharmacol. Exp. Ther.* **144**, 44 (1964); R. M. Post, F. K. Goodwin, E. Gordon, D. M. Watkin, *Science* **179**, 897 (1973).
4. K. A. Norberg and L. Olson, *Z. Zellforsch. Mikrosk. Anat.* **68**, 183 (1965); M. Fujuvara, *Biochem. Pharmacol.* **15**, 2113 (1966); J. R. Garrett, *Arch. Oral Biol.* **12**, 1417 (1967).
5. B. Fredholm and G. Sedvall, *Life Sci.* **5**, 2023 (1966); J. Hertting and J. Axelrod, *Nature* **189**, 66 (1972).
6. S. Rosenblatt, J. D. Chanley, W. P. Leighton, *J. Neurochem.* **17**, 1105 (1970).
7. O. Almgren, *Acta Physiol. Scand.* **67**, 21 (1966).
8. J. Haggendal and A. Dahlstrom, *J. Pharm. Pharmacol.* **24**, 565 (1972); W. P. DePotter, *Phil. Trans. Roy. Soc. London Ser. B* **261**, 313 (1971).
9. S. Rosenblatt, W. P. Leighton, J. D. Chanley, in preparation.
10. J. Axelrod, *Pharmacol. Rev.* **24**, 233 (1972).
11. R. J. Wyatt, D. L. Murphy, R. Belmaker, S. Cohen, C. H. Donnelly, W. Pollin, *Science* **179**, 916 (1973); D. Murphy and R. Weiss, *Amer. J. Psychiat.* **128**, 35 (1972).
12. W. Pollin, *Arch. Gen. Psychiat.* **27**, 29 (1972); I. I. Gottesman and J. Shields, *Brit. J. Psychiat.* **122**, 15 (1973).
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