CHANGES IN HUMAN SERUM DOPAMINE-β-HYDROXYLASE ACTIVITY IN VARIOUS PHYSIOLOGICAL AND PATHOLOGICAL STATES

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INTRODUCTION

DOPAMINE-β-hydroxylase (DβH) (EC 1.14.2.1) catalyses the β-hydroxylation of dopamine (DA) to norepinephrine (NE) (FRIEDMAN and KAUFMAN, 1965). DβH is localised in the chromaffin granules of the adrenal medulla (KIRSHNER, 1957) and storage vesicles of sympathetic nerves (POTTER and AXELROD, 1963). Recent evidence indicates that DβH is released concomitantly with catecholamines from sympathetic nerve terminals and the adrenal medulla (SCHNEIDER *et al.*, 1967; DE POTTER *et al.*, 1969; WEINSHILBOUM *et al.*, 1971a). DβH has been found in the serum of man and animals (WEINSHILBOUM and AXELROD, 1971; GOLDSTEIN *et al.*, 1972). In animals activation of the sympathetic nervous system by immobilisation or swim stress, results in increases in circulatory DβH (WEINSHILBOUM *et al.*, 1971; ROFFMAN *et al.*, 1973). Thus experimental evidence suggests that serum DβH might reflect peripheral sympathetic nerve activity. In order to determine whether DβH could also serve as an index of human sympathetic nervous system activity, serum DβH levels were monitored in patients with sympathetic nervous system dysfunction and aberrant catecholamine metabolism.

RESULTS

Developmental aspects of human serum DβH

Serum samples were analysed from 141 normal subjects of varying age groups. Serum DβH activity was measured by a previously described sensitive coupled enzymatic reaction (GOLDSTEIN *et al.*, 1971; WEINSHILBOUM and AXELROD, 1971). Serum DβH levels vary widely in the normal population, but are maintained at a relatively constant level in each individual (FREEDMAN *et al.*, 1971). DβH activity exhibits a marked developmental rise with age (Table 1). Low levels of activity are a striking feature of the first year of life and thereafter enzyme activity progressively increases attaining adult values in the 16–20 year age group. These findings emphasised the necessity of the use of proper age controls in any clinical study.

Neuroblastoma

Neuroblastoma is the most common solid malignant tumor in infants and children Abnormal and variable urinary catecholamine secretion is a common clinical finding. Some patients excrete mainly DA and its major metabolite homovanillic acid (HVA), others secrete both DA, NE and their respective metabolites HVA and vanillylmandelic acid (VMA). Serum DβH activity and urinary catecholamine levels were

TABLE 1. SERUM DβH ACTIVITY: VARIATION WITH AGE

Age (yr)	Serum DβH activity (units*)
0–1	7·5 ± 1·50 (12)
1–5	22·5 ± 5·01 (16)
6–10	50·7 ± 8·25 (18)
11–15	43·2 ± 6·78 (20)
16–20	105·0 ± 13·04 (15)
21–40	92·1 ± 8·46 (38)
21–40	92·1 ± 8·46 (38)
41–60	101·7 ± 8·76 (23)

* Units, nm^{14}C product/hr/ml; results are means ± S.E.M.; number of individuals in parenthesis. Control individuals (141) were male and females from NYU pediatric and adult clinics, and volunteers. None of these patients had neurological or psychiatric disorders. Values of nonclinic individuals were slightly higher than other patients.

monitored in 20 children with active neuroblastoma and 11 patients with inactive or "cured" disease (GOLDSTEIN *et al.*, 1972; FREEDMAN, ROFFMAN, GOLDSTEIN and HELSON, unpublished data). Serum DβH activity was elevated* in 9 of 20 active cases. In these patients urinary catecholamine excretion was characterised by abnormal levels of NE and VMA. No correlation of serum DβH activity and DA or HVA was apparent. Thus, serum DβH levels paralleled the catecholamine secretory processes of the active tumor. However, high serum DβH activity was also observed in 6 of 10 "cured" patients. Long term studies are underway to ascertain whether serum DβH activity may be predictive of tumor reoccurrence in these patients. Thus, the monitoring of serum DβH in conjunction with other diagnostic tests might be of significant usefulness in the diagnosis and prognosis of neuroblastoma.

Familial dysautonomia

Familial dysautonomia (F.D.) is a rare syndrome prevalent in Ashkenazie Jews and clinically characterised by autonomic nervous system dysfunction. Abnormally low serum DβH activity has previously been reported in F.D. (WEINSHILBOUM and AXELROD, 1971; FREEDMAN *et al.*, 1972). Further studies from our laboratory have extended these findings. It is evident (Fig. 1) that serum DβH activity varies over a wide range of activity in these patients. A significant number of patients had low enzyme activity whereas others exhibited high enzyme activity. Reduced serum DβH activity could reflect reduced peripheral sympathetic activity. Recent histologic studies of sural nerve biopsies have suggested morphologic neuronal degeneration in F.D. (AGUAYO *et al.*, 1971). Elevated activity could reflect compensatory release mechanisms in the peripheral sympathetic systems. Therefore, in patients with F.D. serum DβH activity must be cautiously interpreted. It has not yet been established what processes serum DβH activity might reflect in the etiology and/or development of symptomatology of F.D.

* Activity greater than two standard deviations above control mean value.

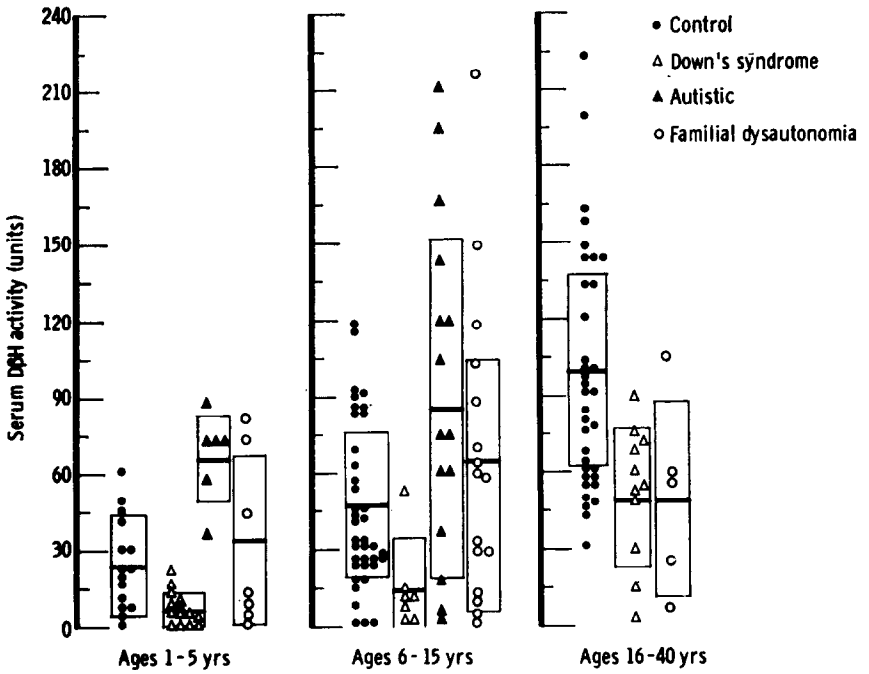

FIG. 1.—Human serum dopamine-β-hydroxylase (DβH) activity. DβH activity units = nmoles ^{14}C-product/hr/ml serum

Down's syndrome

Down's syndrome (D.S.) is characterised by trisomy-21 and mental and growth retardation. Since low blood serotonin levels have been reported (BAZELON *et al.*, 1967; ROSNER *et al.*, 1965) it has been suggested that biogenic amine metabolism is abnormal in D.S. In order to assess the involvement of the peripheral nervous system serum DβH activity was monitored in 32 children with D.S. We and others have observed reduced serum DβH activity in D.S. (WETTERBERG *et al.*, 1972a; COLEMAN, 1973). The results (Fig. 1) indicate that enzyme levels are reduced in all age groups studied (Fig. 1). Serum DBH activity was analysed in non-mongoloid mentally retarded (autistic) children. In this sample of autistic children serum DβH activity was slightly higher than in controls. Thus, in comparison to autistic patients, D.S. serum enzyme activity reduction is more pronounced. Studies are now in progress with radioimmunoassay techniques to determine the levels of circulating DβH protein in these patients.

Parkinson's disease and Huntington's chorea

Parkinson's disease (P.D.) and Huntington's chorea (H.C.) are disorders of the extrapyramidal system. Since central monoamine pathways seem to play a significant role in these diseases, it was interesting to determine whether serum DβH activity could serve as a useful index of clinical status of these diseases.

Longitudinal studies of 28 patients with H.C. and 49 patients with P.D. have been carried out (LIEBERMAN *et al.*, 1972; LIEBERMAN *et al.*, 1973). P.D. patients on L-dopa therapy ($N = 34$) exhibited a wide range of serum enzyme activity with a

mean value comparable to that of controls ($\bar{X} = 122{\cdot}7 \pm 11{\cdot}70$ vs $102{\cdot}6 \pm 6{\cdot}15$). Untreated P.D. patients ($N = 15$) tended to have low enzyme activity ($\bar{X} = 55{\cdot}2 \pm 9{\cdot}12$). In patients studied prior to and during L-dopa therapy a marked rise in serum DβH activity was observed. In H.C. patients ($N = 28$), a preponderance of high serum DβH values was observed ($\bar{X} = 128{\cdot}7 \pm 13{\cdot}84$). A preliminary study of several H.C. families has not yet established a familial correlation between serum enzyme activity and H.C.

Coma

In order to further attempt to assess the usefulness of monitoring serum DβH in disorders of the central nervous system, a study of 24 comatose patients was undertaken (LIEBERMAN *et al.*, 1972). A wide range of serum enzyme activity was observed. Mean values were significantly lower than controls (control $102 \pm 6{\cdot}15$ vs coma $64{\cdot}8 \pm 10{\cdot}25$). However, in 4 patients who survived, serum DβH activity did not significantly rise. Thus, a correlation of serum DβH activity and functional state of central nervous system activity was not clearly established.

Hypertension

The involvement of the sympathetic nervous system in control of blood pressure has long been suggested. We, in collaboration with Dr. Serrano (Institute of Cardiology, Mexico) have instituted a clinical study of 50 patients with hypertension. A wide range of serum DβH activity was observed. No clear relationship existed between serum DβH activity and blood pressure. In some patients with prolonged history of hypertension, serum DβH levels tend to lower levels of control population.

Psychiatric disorders

Aberrant catecholamine metabolism has been suggested in psychiatric disorders. (BUNNEY and DAVIS, 1965; SCHILDKRAUT, 1965). Serum DβH activity was studied in 56 patients with various psychiatric disorders: manic depressives, schizophrenics and character disorders (SHOPSIN *et al.*, 1972). Serum DβH levels in these patients did not differ from controls.

DISCUSSION

The past 3 years have been a period of active research in many laboratories to evaluate the usefulness of monitoring serum DβH as an index of human sympathetic nervous system dysfunction. At this time a critical evaluation of the results seem warranted. In general, these studies seem to indicate a relative lack of correlation between serum DβH activity and sympathetic nervous system function. However, certain factors should be considered before this issue is finalised. Although serum DβH activity is relatively stable over long periods of time in normal individuals, this stability might not be characteristic of certain pathological conditions. Therefore, longitudinal studies are necessary to more fully evaluate the relationship of serum DβH values to the clinical course of the disease. Since control values vary widely, it would be desirable to use the individual as his own control. This is especially important during prolonged drug treatment. If groups of patients are to be compared to normals, proper age controls are required. These factors are crucial if the monitoring of serum DβH is to be of clinical significance. On the other hand, it is possible

that in man the circulatory DβH values may not reliably reflect sympathetic activity. In contrast to data obtained from animal studies, stress induced changes in human serum DβH activity are small in magnitude and do not occur in all cases (WOOTEN and CARDON, 1972; FREEDMAN and GOLDSTEIN, unpublished data). Also, changes in circulatory levels of NE are not reflected by parallel changes in serum DβH (MUELLER *et al.*, 1972).

The circulating levels of DβH probably reflect not only the rate of release but also the rate of degradation of the enzyme (GOLDSTEIN *et al.*, 1972). DβH, like other plasma proteins may be degraded and removed from the circulation by the reticuloendothelial system. In some preliminary studies we have observed abnormal DβH levels in disorders not associated with dysfunction of the sympathetic nervous system (GOLDSTEIN *et al.*, 1973). It must be considered that these abnormally high values in various neoplastic conditions (leukemia, hepatoma, lymphosarcoma) might reflect a defect in the rate of enzyme degradation. An intensive study of the factors involved in control of human serum DβH activity levels is required. Why do normal individuals exhibit such a wide range of values? Are DβH and NE proportionally released into the human circulation? Do genetic factors play an important role in the individual levels of circulatory DβH?

Also, since serum DβH activity probably reflects enzyme released from peripheral sources, the monitoring of DβH activity in cerebrospinal fluid (CSF) might provide a more direct index of central sympathetic nervous activity. We have recently developed an assay for DβH in the CSF (EBSTEIN, FREEDMAN and GOLDSTEIN, unpublished data) and are presently studying DβH in the CSF of normal and neurological patients.

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