DOPAMINE-β-HYDROXYLASE ACTIVITY IN SERUM

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INTRODUCTION

CLINICAL research into the role of the sympathetic nervous system in human disease has been hampered by the lack of a convenient and sensitive measure of the level of function of the sympathetic nervous system in man. Blood is among the most easily sampled of human tissues. In the course of evolution processes have evolved at the synaptic terminal which limit access of neuro-transmitter substances to the peripheral circulation. This fact increases the technical problems involved in the determination of levels of neurotransmitters in blood and complicates the interpretation of these values even when it is possible to determine them accurately. The recent discovery that the release of catecholamines from the adrenal medulla and from sympathetic nerves is accompanied by the release of proteins found within catecholamine-containing vesicles (BANKS and HELLE, 1965; GEFFEN *et al.*, 1969; DE POTTER *et al.*, 1969) has raised the possibility that the determination of circulating levels of these releasable vesicular proteins might serve as a measure of the level of function of the sympathetic nervous system, the adrenal medulla, or both.

The development of a sensitive enzymatic radiochemical assay procedure for the determination of the activity of the enzyme dopamine-β-hydroxylase (E.C. 1.14.2.1, DBH) (MOLINOFF *et al.*, 1971; GOLDSTEIN *et al.*, 1971), one of the releasable vesicular proteins found in sympathetic nerve terminals and the adrenal medulla (GEFFEN *et al.*, 1969; DE POTTER *et al.*, 1969; VIVEROS *et al.*, 1968), led to the observation that DBH activity is present in the blood of man and other animals (WEINSHILBOUM and AXELROD, 1970; WEINSHILBOUM and AXELROD, 1971a). In order to interpret measurements of this circulating enzyme activity in a clinical setting, however, it was necessary to determine (a) the biochemical identity of the circulating enzyme activity with that found in tissues, (b) the source of the circulating enzyme activity, and (c) the factors which are important in the regulation of the level of this enzyme activity in the blood.

BIOCHEMICAL DATA

The DBH activity found in blood is biochemically similar to DBH activity in the adrenal medulla and sympathetic nerves with regard to co-factor requirements, requirement for oxygen, Michaelis–Menten constant for substrate, and response to the addition of cupric ion to inhibit endogenous inhibitors of the enzyme (WEINSHILBOUM and AXELROD, 1971a). Starch block electrophoresis of serum DBH activity and the enzyme activity found in sympathetic nerves and the adrenal medulla has demonstrated that there are differences in the electrophoretic mobilities of the DBH activity among various species, but that within a given species the enzymatic activity found in the blood has the same electrophoretic mobility as that found in other tissues (ROSS *et al.*, 1972). Immunochemical studies have shown that there is immunochemical cross reactivity within a given species of serum DBH with antibody formed

in response to the purified adrenal enzyme from that same species (RUSH and GEFFEN, 1971; GOLDSTEIN *et al.*, 1972). All of these data are compatible with the hypothesis that the circulating DBH activity is the same as that found in other tissue in the same species.

SOURCE OF CIRCULATING ENZYME

Circulating DBH activity in the rat is unchanged after adrenalectomy and adrenal demedullation (WEINSHILBOUM *et al.*, 1971a). Partial chemical sympathectomy performed by the use of intravenous 6-hydroxydopamine results in a significant decrease in serum DBH activity in this animal (WEINSHILBOUM and AXELROD, 1971b). When rats are subjected to forced immobilization, a procedure which is known to increase urinary catecholamine excretion, serum DBH activity increases acutely, and the magnitude of the increase is the same in animals with or without adrenal glands (WEINSHILBOUM *et al.*, 1971a). These data suggest that at least a portion of the serum DBH activity originates from sympathetic nerve terminals, and that the adrenal gland is not necessary for the maintenance of serum DBH activity in the rat.

The release of DBH and norepinephrine are directly proportional when the nerves to the isolated perfused spleen or the isolated vas deferens are stimulated (SMITH *et al.*, 1970; WEINSHILBOUM *et al.*, 1971b). This observation is significant with regard to attempts to use circulating DBH activity as a measure of the level of function of the sympathetic nervous system.

FACTORS WHICH AFFECT HUMAN SERUM DBH ACTIVITY

Age

Serum DBH activity in man is age dependent (WEINSHILBOUM and AXELROD, 1971c; FREEDMAN *et al.*, 1972). The activity increases with increasing age for the first 4–5 years of life, and there is little change thereafter at least into the mid-thirties or forties. The greatest increase in DBH activity in blood occurs during the first 2–3 years of life. It has been suggested that the age related increase of serum DBH activity in man may represent the functional or anatomical development of the sympathetic nervous system (WEINSHILBOUM and AXELROD, 1971c; FREEDMAN *et al.*, 1972). Until the factors that are involved in the clearance of DBH activity from blood are better understood, however, it can not be assumed that this change in circulating levels of the enzyme represents either a change in the rate of release or the quantity of enzyme released from sympathetic nerve terminals.

Environmental factors

In man, as in the rat, it has been demonstrated that stress such as the cold pressor test and exercise can lead to transient elevations in serum DBH activity (WOOTEN and CARDON, 1973). The magnitude of the elevations of serum DBH activity in normal humans in response to such stress is variable, and is small when compared to the total range of enzymatic activity in a control population. Changes in circulating DBH activity of greater than 100 per cent have been demonstrated in quadraplegic patients during hypertensive crises (NAFTCHI *et al.*, 1973).

Genetic factors

A wide range of values of enzymatic serum DBH activity in control subjects has been reported in all studies performed thus far. Because changes in the enzyme

activity in the blood of control subjects in response to relatively minor stress are small when compared to the total range of enzyme activity in the entire population, the possibilty arises that genetic factors may play an important role in the determination of baseline values in serum DBH activity in control populations. In a recent study we have determined serum DBH activity in blood samples obtained from 433 children aged 6–12 and from 227 adult control subjects (WEINSHILBOUM *et al.*, submitted for publication). Samples from children were obtained in the morning at school after an overnight fast, and samples from adult subjects were obtained from blood donors receiving no medications.

DBH activity was measured by a sensitive radiochemical enzymatic assay as described elsewhere (MOLINOFF *et al.*, 1971; WEINSHILBOUM and AXELROD, 1971c) except that acetate buffer, 1·0 M, pH 4·9, was used instead of Tris HCl buffer, 1·0 M, pH 6 in the DBH reaction. This change resulted in a final incubation pH of 5·2, the optimal pH for the determination of DBH activity in human serum in this assay system. One unit of DBH activity represented the formation of 1 nmole of β-phenyl-β-ethanolamine from β-phenylethylamine per ml of serum per hr.

The percentage frequency distribution of serum DBH values in 433 control children (233 boys, 200 girls) is shown in Fig. 1. 4·6 per cent of the children (9 boys, 11 girls) had a very low serum DBH activity (less than 50 units). This group of children included 3 out of the 4 children tested in one family and 2 sets of 2 siblings each. Serum DBH values in this population are skewed to the right. The skewness in distribution could be corrected by expressing the values as the square root of DBH activity. To eliminate the possibility that the frequency distribution had been biased by the inclusion of data from siblings, values in one sibling from each set of siblings were chosen randomly and the frequency distribution for serum DBH activity in these 280 unrelated children is shown in Fig. 1. The percentage frequency distribution of serum DBH activity in blood samples obtained from 227 unrelated adult blood donors is also plotted in Fig. 1. The adult control population included 134 men and 93 women. The median age of this population was 32·1 years. 3·1 per cent of these adults had a very low enzyme activity (less than 50 units). The distribution of values for serum enzyme activity in this population was also skewed to the right. Data from the first 317 consecutive control children were analysed, and in this group serum enzyme activity in girls did not change from age 6 through 12 while that in boys increased only approximately 50 units. In the adult population, no change in serum activity with age was detected in either women or men.

A highly significant correlation, $r = 0{\cdot}57$ ($p < 0{\cdot}001$), was found between serum DBH activity in the blood of 94 sibling-sibling pairs included in the first 317 consecutive children examined. This correlation was established in terms of age and sex specific relative deviates about the sex specific regression of the square root of DBH activity on age to correct for the small increase in enzyme activity with age in boys and for the lack of a normal distribution (Fig. 2). There were no differences in the degree of correlation between brother–brother, brother–sister, or sister–sister pairings. When random pairs of single children with no siblings drawn from the same population were generated by using tables of random numbers, no correlation of serum DBH activity was found between members of non-sibling pairs.

To test for the possibility that the differences in serum DBH activity found in different members of a control population were due to differences in circulating

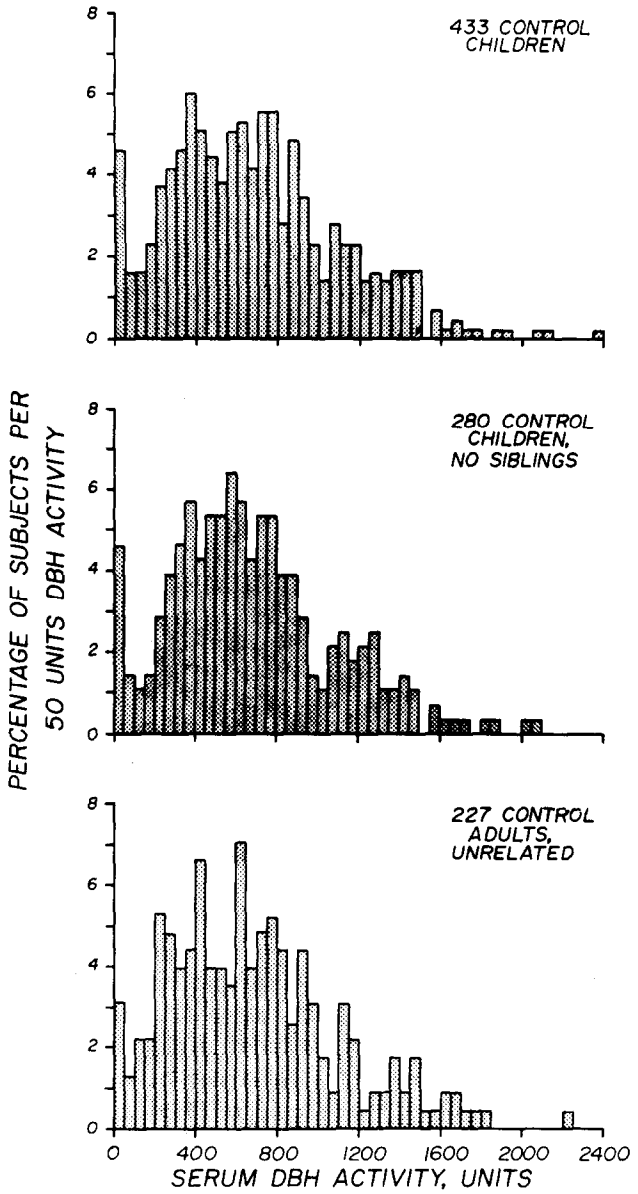

FIG. 1.—Percentage frequency distributions of serum DBH activity. The percentage of subjects with serum DBH activity per 50 unit increments are shown for: top, 433 control children; middle, 280 of the same children with only one child from each family represented; and bottom, 227 control adult subjects.

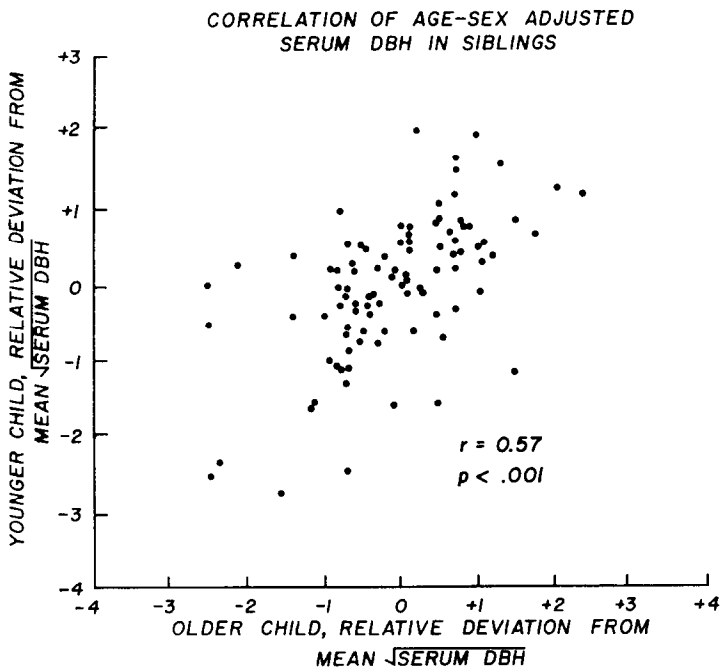

FIG. 2.—Sibling-sibling correlation of serum DBH activity. The correlation of specific relative deviates of the square root of serum DBH activity in sibling pairs is shown. $r = 0{\cdot}57$, $p < 0{\cdot}001$.

inhibitors of the enzyme, purified bovine adrenal DBH activity was added to samples of blood assayed under the standard assay procedure. In multiple samples from different individuals with either very low activity (less than 50 units), low normal activity (200–350 units), or high normal activity (850–1000 units), there was no demonstrable inhibition of exogenously added purified bovine adrenal DBH activity. The wide range in serum DBH activity found in control human subjects could not be accounted for on the basis of different levels of circulating inhibitor(s) of the enzyme.

These data demonstrated a familial correlation of enzymatic DBH activity in the blood of control human subjects. It was not possible to determine how much of this correlation was due to the effects of shared environment and how much was due to factors of heredity. Ross and his co-workers in Sweden have studied serum DBH activity in monozygotic and dizygotic twins (Ross *et al.*, 1973). They found a correlation coefficient for monozygotic twins of 0·96 and for dizygotic twins of 0·75. Both of these populations showed significant correlation between members of twin pairs, but a significantly higher degree of correlation was found in enzyme activity in monozygotic than in dizygotic twins. Their interpretation of these results was that heredity was the major factor which contributed to the familial correlation of serum DBH activity.

CONCLUSION

There is a wide range of serum DBH activity in control human populations. Circulating enzyme activity levels change dramatically during the first years of life.

After that period of time, changes in serum DBH activity in response to stress are relatively small unless the stress is severe. Familial factors contribute significantly to the determination of baseline circulating DBH activity. Heredity appears to be responsible in large part for the familial correlation in serum DBH activity. The mode of inheritance, whether single gene or polygenic, is not known. Studies of a large group of control children and adults suggest that a subgroup of subjects with very low serum DBH activity may exist. Familial factors might affect any one of several variables which influence serum DBH activity including (a) the quantity of DBH available for release, (b) the rate of release of the enzyme, (c) the access of enzyme protein to the circulation, or (d) the rate of clearance of the enzymatic activity from the blood.

These findings make it less likely that an isolated determination of serum DBH activity which falls within the wide range of normal values will be useful in the determination of the levels of sympathetic nervous system function in a given individual. This does not mean that the determination of enzyme activity in serial samples from an individual might not be of value clinically, or that the comparison of values determined in blood from a group of patients suffering from a disease with those determined in an appropriate and large control population might not be useful clinically. Furthermore, the existence of a group of control subjects with very low serum DBH activity suggests that previous reports of very low enzyme activity in patients with diseases such as familial dysautonomia and Down's syndrome (WEINSHILBOUM and AXELROD, 1971c; WETTERBERG *et al.*, 1972) must be interpreted with caution.

It is not possible to predict the ultimate clinical usefulness of either the enzymatic measure of serum DBH activity or the immunochemical radioimmunoassay of DBH protein as measures of the level of function of the sympathetic nervous system in man. Until the factors which control both the enzymatic activity and immunoreactive DBH are better understood, clinical studies in which these measures of the rate of release of transmitter from sympathetic nerve are assayed will have to be interpreted with caution. Whether the measurement of serum DBH activity and serum immunoreactive DBH protein in man will eventually prove to be unrelated to sympathetic nervous system function, will prove to be merely a different way of obtaining the same information provided by the accurate determination of blood catecholamines, or will provide us with new and different insights into neural function remain to be investigated. Today the clinician dealing with a patient with hepatic disease measures not only the bilirubin levels in the blood but also the levels of glutamic oxaloacetic transaminase activity and alkaline phosphatase activity. The time may be approaching when the clinician will have available several procedures by which the function of the sympathetic nervous system may be monitored.

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