Dopamine-Beta-Hydroxylase Effectors in Neuroblastoma

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ABSTRACT

Investigation of dopamine-beta-hydroxylase (DBH) serum activity as a potential diagnostic/prognostic tool for neuroblastoma did not confirm previous reports. However, age-dependent differences in normal children’s DBH “activation” by water dilution ± copper addition was revealed. These findings of age-related DBH effector differences suggest that the strong age relationship of DBH activity may be due to alterations in effectors. Additionally, these effectors may be variably affected by assay methodology, influencing DBH results obtained in neuroblastoma and other disease states.

Neuroblastoma, a very common malignant solid tumor in childhood, is unique in that it appears to have a very high incidence of spontaneous regression, theorized to be influenced by immunologic host defense mechanisms and/or by poorly understood maturational factor(s). This enigmatic tumor has familial, age, and etiologic agent relationships.

Neuroblastoma is generally detected only after metastatic involvement has produced symptoms. Although most of these tumors are hormonally active, secreting catecholamine by-products, intra-tumor catabolism of non-epinephrine generally prevents catecholamine-related symptoms. Neuroblastoma tissue contains tyrosine hydroxylase and dopamine-B-hydroxylase (DBH) activities roughly equivalent to adrenal medulla tissue but produces much less epinephrine than adrenal tissue owing to lower activity of the nor-epinephrine enzymatic step.

Elevation of the catecholamine by-products (homovanillic (HVA) and vanillylmandelic (VMA) acids) in urine has been the most reliable biochemical marker of this tumor. Serial estimation of both have proven useful in evaluating therapeutic response, and there is a significant association between their excretion and survival. Schweisguth, however, demonstrated that 11 percent of neuroblastoma cases are not accompanied by elevated excretion of either metabolite.

Goldstein et al reported that 10 of 22 patients with neuroblastoma had elevated serum DBH activity not correlated with HVA excretion, while Rockson et al reported one more patient with elevated DBH and normal catecholamine metabolite excretion.
These studies stimulated interest in this enzyme as another possible diagnostic/prognostic tool. This serum enzyme was initially assayed by us in neuroblastoma patients to provide more data on this point. Variable response to two different methodologies for counter acting endogenous DBH inhibition provoked additional studies involving DBH effectors. These preliminary findings and their possible significance are reported.

Methods

Serum DBH activity was measured by the photometric assay of Nagatsu and Udenfriend\textsuperscript{23} as modified by Schanberg et al,\textsuperscript{26} ±10 \times water dilution of sera as proposed by Harrelson and Brown\textsuperscript{10} and also ±7.5 n moles CuSO\textsubscript{4} addition to the 0.95 ml reaction mixture. All assays were performed in duplicate.

Results and Discussion

Mean activities (and range) of DBH are shown in the table, separated by age group into normal and neuroblastoma (active and remission) categories. The standard assay of Nagatsu and Udenfriend (undiluted sera-Cu) reveals the strong age dependence of DBH activity in normals, as reported by others.\textsuperscript{25} No elevations above these age-related normal values were seen in these 11 neuroblastoma patient sera. Serially measured DBH activity was previously reported by us in three of these patients, one patient showing wide fluctuation of DBH from diagnosis through therapy and three years remission and two patients showing remarkable constancy from diagnosis until demise after one year.\textsuperscript{3}

During this study, Harrelson and Brown reported that not all endogenous inhibitors in plasma are counteracted by the N-ethylmaleimide (NEM) incorporated in this DBH assay, and proposed plasma dilution as a means of counteracting these inhibitors.\textsuperscript{10} Our DBH assay results confirmed this report in that greater DBH activity was measured with diluted adult sera (1.6 to 4.5 fold). Extending this approach to sera of children revealed that the expected activation by dilution did not occur in normal children and was more variable in children with neuroblastoma. This finding suggested that endogenous DBH inhibitors and/or activators (effectors) change with age and that assay methodology may or may not reflect these effectors. Weinshilboum et al\textsuperscript{29} have shown a good correlation between immunoreactive DBH and DBH activity when plasma is diluted in the presence of substrate.

Copper is a known endogenous effector of DBH activity, with maximal stimulation reported at 3 to 5 \times 10^{-6} M and rapid inactivation occurring at greater concentrations.\textsuperscript{23} As the optimal copper concentration appears to be dependent upon components of the reaction mixture, an investigation was made of the effect of added copper upon diluted and undiluted serum DBH activities (estimated final concentration of 10 to 25 \times 10^{-6} M diluted and 8 \times 10^{-6} M diluted). As seen in the table, subject age exerts a strong influence upon the copper effect in undiluted sera. Copper inhibited DBH above age four but did not at younger ages of normal children. There appears to be an activation of DBH in the young children with neuroblastoma, although more data is needed to confirm this impression. Copper added to reaction mixtures containing diluted sera stimulated DBH activity to very high levels in all age groups in both normal and neuroblastoma categories.

These two findings of striking dependence of \textit{in vitro} DBH behavior upon subject age indicate that assay methodology may well influence results obtained in neuroblastoma and other disease states. DBH is a complex mixed-function oxidase containing disulfide bonds and requiring
bound copper, ascorbate, oxygen and fumarate as co-factors. Endogenous inhibitors include copper chelators, sulfhydryl-reactive compounds, and perhaps others. One must question whether in vitro DBH activity truly reflects tissue content and/or output, and must also question whether the age-related increase is due to a decrease in measured inhibition.

Summary

Initial studies to determine the value of serum DBH as a diagnostic/prognostic indicator of neuroblastoma have led us to evidence that the factors determining DBH activity in vitro are normally different in younger children and adults. These preliminary findings suggest that DBH of children with neuroblastoma may be more “adult-like.” Investigation of the regulation of these endogenous effectors, especially in the zero to two year age group, may provide insight into the neuroblastoma’s great tendency to spontaneously regress, its predilection for the very young child and its genetic relationship.

References


