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SHORT COMMUNICATIONS

Effect of delivery on serum dopamine-β-hydroxylase activity and urinary vanillyl mandelic acid excretion of normal pregnant subjects

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DOPAMINE-β-hydroxylase (DBH) is an enzyme which is found in the norepinephrine (NE)-containing vesicles in the sympathetic nerve terminals and the chromaffin granules in the adrenal medulla.[1–4] The enzyme catalyzes the conversion of dopamine to NE. In the perfusion experiments, the enzyme is released with catecholamines into the perfusate from the adrenal gland by administration of acetylcholine[5] and from the spleen by splenic nerve stimulation.[6] DBH activity is present in the circulating blood of both man and rat.[7,8] The enzyme activity in rat plasma is elevated under stress by forced immobilization[9] and lowered by treatment with 6-hydroxydopamine,[10] a drug that partially destroys the sympathetic nerve terminals. The plasma DBH activity in man is increased after a submaximal work load.[11] A significant correlation was demonstrated between plasma NE and DBH in individuals with essential hypertension.[12] Cold pressor test and exercise produced small but significant elevations of plasma DBH activity in man.[13] The serum DBH activity among normal human subjects has a great variation, but each subject has a relatively constant level of DBH activity during the course of a day and from day to day.[8,14,15] The elevated serum DBH activity in patients with pheochromocytoma is significantly lowered after complete removal of the tumor with a concomitant decrease in urinary NE, epinephrine (E) and vanillyl mandelic acid (VMA) excretion.[15]

Since it has been demonstrated that the output of both E and NE, especially the latter, remarkably increases with the onset of labor,[16,17] we investigated the changes in serum DBH activity and urinary excretion of VMA of normal pregnant subjects before and after delivery. Blood and urinary samples were taken from 12 primiparas (24–34 years old) about 20 min before and on the fourth day after delivery. In five subjects, blood samples were collected at various time intervals for the 48 hr following delivery and served for the sequential assay of enzyme activity. The patients were not given any analgesics or anesthetics prior to delivery. Blood samples were obtained from a brachial vein and kept at 4° in a refrigerator for at least 30 min. After centrifugation at 2500 rev/min for 10 min at 4°, serum was separated and stored at −20° for the assay of DBH activity. Urine samples collected with a catheter in glass containers during

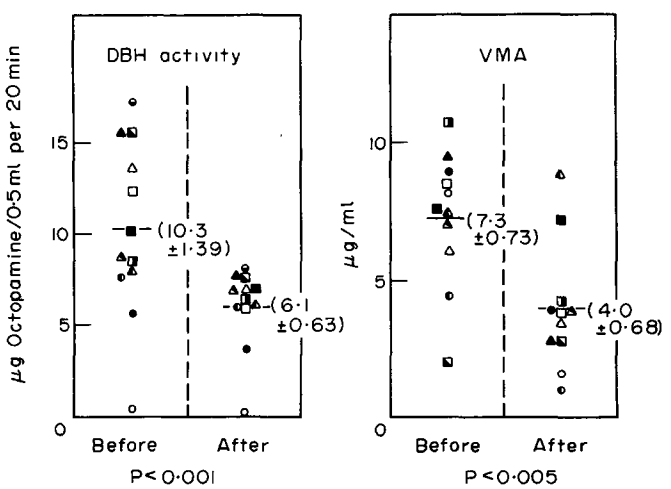

FIG. 1. Serum DBH activity and urinary VMA excretion 20 min before and on the fourth day after delivery. Mean value ± S.E. in parentheses.

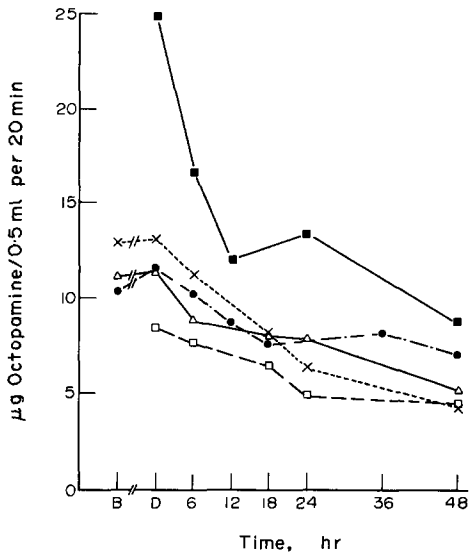

FIG. 2. Time course of serum DBH activity during the 48 hr following delivery of five primiparas. B, Time of onset of labor pain; D, about 20 min before delivery.

delivery and on the fourth day post partum were immediately adjusted to pH 2 with HCl and frozen for the determination of VMA.

Serum DBH activity was assayed by a simple enzymatic procedure which depends on the β-hydroxylation of tyramine to octopamine.[15] The serum, 0·5 ml, was transferred to a tube containing 50 μM tyramine, 50 μM ascorbic acid, 25 μM maleate buffer, pH 4·6, and 0·5 μM *p*-chloromercuribenzoic acid. The reaction mixture was adjusted to pH 4·6 with diluted NaOH and added distilled water to make a final volume of 5·0 ml. The reaction mixture was incubated for 20 min at 37°. The reaction mixture with boiled serum was used as a blank. After incubation, the reaction was stopped by the addition of 1·5 ml of ice-cold perchloric acid and the mixture was centrifuged at 3000 rev/min for 10 min at 4°. The supernatant containing octopamine was passed over a Dowex 50 column (H^+-type, 0·4 × 4·0 cm, 100–200 mesh) and washed with 10 ml of distilled water. Octopamine was eluted with 3 ml of 3 N NH_4OH The octopamine in the column eluate was oxidized with 0·2 ml of 2% $NaIO_4$ to *p*-hydroxybenzaldehyde. After 3 min, the oxidation was stopped with 0·2 ml of 10% $Na_2S_2O_5$ The absorbance at 330 nm due to *p*-hydroxybenzaldehyde was measured in a spectrophotometer. The serum DBH activity was expressed as μg octopamine/0·5 ml of serum/20 min of incubation. The urinary VMA was estimated by the method of Pisano *et al.*[18]

The results are shown in Figs. 1 and 2. The DBH activity in each subject was increased before delivery. The magnitude of change varied from subject to subject; however, the mean DBH activity before delivery was significantly higher than that of the fourth day post partum. The sequential assay of the enzyme activity in five subjects showed that the elevated enzyme activity gradually decreased and returned to the individual baseline levels at 48 hr post partum. To confirm that the change in enzyme activity resulted from the increase in release of DBH from the sympathetic nervous system, additional studies were undertaken. The serum of a given normal female subject was mixed with that of a pregnant woman during delivery and served for the assay of DBH activity. The DBH activity of the normal subject was not increased by this procedure. Therefore, it is unlikely that there is any change in serum concentration of enzyme activators during delivery. The mean DBH activity in umbilical cord blood of ten subjects was estimated to be 15·4 ± 2·8 per cent of that in maternal blood during delivery. No correlation was found between the enzyme activity of umbilical cord and maternal blood. This suggests that the DBH activity of umbilical cord blood had no important influence upon the increase of maternal enzyme activity, even if the enzyme was transferred freely between umbilical cord and maternal blood From these findings, we strongly suggest that the stress of labor produced the increase in release of DBH from the sympathetic nervous system.

The mean urinary excretion of VMA was significantly increased before delivery. Therefore, a gross relation was observed between the increase in DBH activity and urinary excretion of VMA in pregnant women before delivery. In three subjects no increase in urinary output of VMA was observed, but in two

of them the DBH activity was increased significantly. The magnitude of increase in urinary excretion of VMA was not directly related to that of the increase in serum DBH activity when compared in each subject. Since recent studies demonstrate the stoichiometric release of DBH and NE,[19] and the considerable intersubject variation of urinary excretion of NE and E before and after delivery,[17] the discrepancy in our results seems to be due to intersubject variation in the enzymatic conversion of NE and/or E to VMA and in the rate of elimination of VMA from the kidney.

The present study demonstrates that the emotional and physiological stress of delivery produced the increase in serum DBH activity and urinary excretion of VMA, and that the increase in serum DBH activity was the result of increased release of DBH from the sympathetic nervous system. This study is compatible with other data[9] and again suggests that, if compared in individual subjects, the increase in enzyme activity might be an index of the increase in release of NE and/or E from the sympathetic nervous system.

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Hepatocyte suspensions as a model for demonstration of drug hepatotoxicity

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PREVIOUS studies in this laboratory have attempted to test the hypothesis that some instances of drug-induced hepatic injury, which appear to be the result of hypersensitivity to the respective drugs, may reflect