Plasma Dopamine-β-Hydroxylase Activity in Normal Young Men: Its Responsiveness to Manipulation of Sodium Balance and Upright Posture

SHUICHI TAKISHITA, KOSHIRO FUKIYAMA, KENSHI KUMAMOTO, YASUHIRO NODA, TERUKAZU KAWASAKI, AND TERUO OMAE

Dopamine-β-hydroxylase (DBH) is the enzyme that catalyzes the conversion of dopamine to norepinephrine. It is localized in both the noradrenergic vesicles of sympathetic nerves and the chromaffin granules of the adrenal medulla. Since experimental studies revealed that DBH was released via the process of exocytosis together with norepinephrine, it has been considered that plasma or serum DBH activity might serve as an index of sympathetic activity. However, the usefulness in man remains controversial.

The present study was done to determine whether DBH activity might reflect sympathetic activity in young healthy adults.

MATERIALS AND METHODS

Subjects for the study were 9 healthy male volunteers of medical students, aged 19–25 years. Prior to the study, they were examined at the out-patient clinic by history taken, physical examinations, routine laboratory test including urinalysis, blood chemistries, ECG, and chest x-ray. None of them showed abnormality. The blood pressure was below 140/90 mmHg.

They were hospitalized for 18 days and fed with special diet for the study. Throughout the hospitalization, the study was separated into 4 phases as follows:

Phase I. The subjects were on a salt-free diet for four days which, however, still contained daily sodium of 35 mEq. To get negative sodium balance, they were given orally 120 mg of furosemide in the morning on the first day.

Phase II. In this period of 4 days, they were given a high salt diet containing sodium of 280 mEq and further, sodium capsules of 120 mEq were added.

Phase III. This was control period and dietary sodium of 175 mEq was given for 4 days.

Phase IV. Sodium deprivation was produced by administration of furosemide in this period. A dietary sodium restricted to 100 mEq and the dose of furosemide was 120 mg per day for 3 days. One of the subjects dropped out in the period for personal reasons.

Body weight was checked every morning after urination. Urine was collected for 24 hours and urinary sodium excretion was detected every day.

At the end of each phase, venous blood samples were taken at 8:00 a.m. at recumbent position after overnight fasting for measurement of hematocrit, DBH activity and plasma renin activity (PRA). And then, the subjects were kept in upright position, followed by blood sampling at 1st, 2nd, and 4th hour in phase I, III, IV. In phase II, furosemide was given at an oral dose of 80 mg immediately after exsanguination at 1st hour in upright position and again blood samples were taken another 2 hours later in upright position.

Blood pressure and pulse rate were measured just before each blood sampling. Blood pressure was determined with a sphygmomanometer and pulse rate was counted from radial pulse.

Responses to angiotensin II analogue (1-Sar-8-Ile-angiotensin II) were determined at the end of phase either I or IV in all subjects. Following

---

Key Words:
Dopamine-β-hydroxylase
Sympathetic activity
Normal subject
Plasma renin activity

2nd Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, Japan
This paper was presented at the VI Conference on the Pathogenesis of Hypertension, November 21, 1976, Tokyo.

Japanese Circulation Journal Vol. 41, August 1977 895
intravenous infusion of isotonic saline, the analogue was infused at rate of 600 ng/kg/min in a total volume of 30 ml for 60 min. Blood pressure and pulse rate were monitored every 5 minutes during the infusion.

Plasma DBH activity was determined by the method of Nagatsu and Udenfriend.\(^4\) DBH activity was expressed as international units per liter (I.U./L.). One international unit of DBH activity is defined as the amount of activity yielding 1 μmole of octopamine from tyramine per minute. Two 20 μl aliquots of each plasma sample were assayed and the mean value was used in calculation. The coefficients of variation (C. V.) for analyses of 30 samples from pooled plasma with moderate DBH activity were 2.2%. The interassay reliability (C. V.) was 6.6% for a pool (n = 11). All samples for a given individual were assayed together. When diluted pooled plasma was used, DBH activity as low as 0.5 I. U./L was detected confidently in our laboratory.

PRA was determined by radioimmunoassay using the kits supplied by CEA-IRE-SORIN.

The data were analyzed statistically by paired t-test. Significant differences were those with \( p < 0.05 \).

Fig.1. DBH activities in individuals during manipulation of sodium balance and upright posture. Phase I: salt-free diet, Phase II: sodium load, Phase III: control, Phase IV: sodium depletion. Abbreviation: 1 hr-up means upright posture for 1 hour.

Fig.2. Changes in DBH activities induced by manipulation of sodium balance and upright posture. DBH activity is expressed as mean ± S.E.M. *: significant change when compared with the previous value in each phase. △: phase I, ○: phase II, ●: phase III, ●: phase IV. F: furosemide 80 mg p.o., 2hr before.
RESULTS

1. DBH activity.

Plasma DBH activities at rest in phase III (control values) ranged from near 0 to 44 I. U./L with a mean value of 22.6 ± 5.1 (mean ± S.E.M.) (Fig. 1, 2). The activities were lowered with sodium loading (phase II) and elevated by sodium depletion (phase IV). The values were 19.9 ± 4.4 (8.3% decrease from the control), and 29.3 ± 6.2 (36.6% increase), respectively. The activities in phase I were 22.8 ± 5.0 and not significantly different from the control.

DBH activities were elevated significantly by erect posture in all 4 phases. The increases in 1 hour-upright position were 3.7 ± 1.3 I. U./L for phase I, 5.1 ± 1.1 for II, 2.8 ± 0.5 for III, and 2.6 ± 0.7 for IV. Once elevated, DBH activities remained almost constant during upright posture. Those were not significantly different among 1 hr-, 2 hr- and 4 hr-upright posture in phase I, III, IV. In phase II, DBH activity for 1 hr-upright was 25.0 ± 5.2 I. U./L, which was further increased by 2.1 ± 0.8 following furosemide administration and 2 hr-duration of erect posture. DBH activity at rest in phase IV was significantly higher than that of 4-upright in phase III. The values of activity in each individual were compared throughout the study. The biggest of variances in the activity was ranged from 0.4 to 20 I. U./L with an average of 11.8 ± 6.6, and was directly correlated to the lowest activity (basal) in each subject (Fig. 3).


Cumulative sodium balance was negative in phase I and IV, and positive in II and III. Hematocrits in recumbent position were significantly higher in phase I and IV, and lower in phase II than those in the control; per cent changes from the control were +6.2%, +11.0, and −3.6, respectively. The changes in hematocrit were inversely correlated with the changes in body weight.


Resting systolic pressure in phase I, II, IV, and control, was as follows: 113 ± 2 mmHg, 110 ± 3, 113 ± 3, and 106 ± 2. The values in phase I and IV were significantly higher than the control. When erected, systolic blood pressure fell significantly in phase IV, but no significant changes was observed in other phases.

Diastolic blood pressures at rest were 58 ± 3 mmHg in control, 72 ± 3 in phase I, 67 ± 2 in II and 75 ± 3 in IV. The presures in phase I, II, IV were significantly higher than the control. Upright posture of 1 hr-duration caused a rise in diastolic blood pressure in phase I and III by 15 ± 4 mmHg and 20 ± 5, respectively. No further increase in pressure was observed thereafter.

Resting pulse rate in each phase was as follows; 57 ± 3 beats/min in control, 65 ± 2 in phase I, 61 ± 2 in II, and 66 ± 2 in IV. Pulse rate in phase I and IV was significantly higher than the control. An increase in pulse rate was induced by upright posture for 1 hour in all phases, kept constant thereafter but in IV where it was reduced slightly at 2 hr-upright posture.

4. DBH activities with respect to other parameters.

Per cent changes of resting DBH activities were directly correlated with % changes in hematocrits (r = 0.60, p < 0.005), and inversely with % changes in body weight (r = −0.65, p < 0.001), as shown in Fig. 4. An augmentation in DBH activity by upright posture was accompanied with an increase in blood pressure and pulse rate. However, no interrelationship was found among them.

PRA at rest was elevated in phase I and IV, and suppressed in II compared with control. Upright posture caused an increase in PRA in all phases. A significant correlation was found between changes in DBH activity and in PRA induced by manipulation of sodium balance (r = 0.64, p < 0.001). With 1 hr-upright posture, DBH activity increased more than PRA in phase II, and vice versa in phase IV. In phase I and III, however, changes in DBH activity significantly correlated with those in PRA (r = 0.51, p < 0.05).
Fig. 4. Correlation between changes in DBH activity and those in hemato-
crit (left), and in body weight (right).

Infusion of angiotensin II analogue caused a fall in mean blood pressure of 4 subjects by 4 to 12 mmHg in phase IV. The same procedure performed in 5 subjects at the end of phase I failed to produce a fall in pressure except in one. Changes in blood pressure seemed to correlate with changes in resting DBH activity (Fig. 5).

DISCUSSION

The significance of plasma DBH activity as an index of sympathetic function has been controversial, because of a wide range of normal values\(^5,6\) the small changes\(^5,7\) induced by acute stresses which are thought to modify sympathetic activity, and genetic influences\(^8,9\).

It was confirmed in our study that DBH activity in the subjects at rest had a wide range. It was increased significantly by upright posture. Sodium depletion produced by administration of furosemide under slight dietary sodium restriction caused an increase in DBH activity. In contrast, the activity was significantly suppressed when sodium balance was positive with sodium loading. Thus, DBH activity was showed to respond well to manipulation of sodium balance, and to upright posture.

Two possibilities would be considered for a change in DBH activity: 1. hemocoencentration or hemodilution, 2. change in sympathetic activity. Hematocrit was decreased, but serum protein and lactate dehydrogenase were not changed signifi-

*Japanese Circulation Journal*  Vol. 41, August 1977
significantly after sodium loading in hypertensive patients who had been investigated on the same protocol as in the present study. On sodium depletion, serum total protein was increased to the same extent as hemocrit, but lactate dehydrogenase was not changed (unpublished data). Since per cent change in DBH activity surpassed that in hemocrit in present study, the change in DBH activity at rest could not be completely attributed to hemoconcentration or hemodilution.

It is reasonable to consider that the DBH activity represents the sympathetic nerve activity. The sympathetic nerve activity can be influenced by body fluid balance. In animal experiments, it was demonstrated electrophysiologically that a change in circulatory volume elicited an alteration in sympathetic nerve discharges by affecting baroreceptor reflex. Reports were published that dietary sodium loading in man decreased urinary excretion of norepinephrine and that acute supplement of saline suppressed plasma DBH activity and urinary norepinephrine and sodium depletion increased urinary excretion of norepinephrine.

Sympathetic outflow is increased when one takes erect-position. If the change in DBH activity corresponds to physiologic stimuli, DBH activity may be enumerated as an index of the function of sympathetic nervous system. In the present study DBH activity was significantly increased when the subjects took upright posture for 1 hour in each phase. The finding is consistent with that reported by Okada, et al. in which diurnal rhythm in DBH activity was shown, higher at daytime and lower at night, suggesting due to increased physical activity or an erect posture during daytime. High DBH activity was also shown on exercise. The increase was assumed to be related amount of work done and to level of plasma catecholamine. Our data showed no difference in the activity among the plasma samples taken at 1st, 2nd, and 4th hour after ambulation in each phase. Blood pressure and pulse rate were increased significantly during upright posture. However, no correlation between increase in DBH activity and that in blood pressure or pulse rate was found. For better understanding, physiological role and kinetics of plasma DBH should be investigated.

PRA was augmented by sodium depletion and ambulation, and suppressed by sodium loading. Although Noth and Mulrow observed that dietary sodium restriction could not produce significant relationship between increases of DBH activity and PRA, our data showed that a change in DBH activity significantly correlated with that of PRA in sodium balance manipulation or ambulation.

It was interesting that a change in blood pressure induced by angiotensin II analogue had a tendency to correlate with a change in resting DBH activity following sodium balance manipulation. The fact might suggest that circulating angiotensin has a role to maintain blood pressure under condition of sodium depletion in normal young adults, and also sympathetic nerves are stimulated in the circumstance where renin-angiotensin system is activated. It should be recognized that interaction between renin-angiotensin system and sympathetic nervous system has been well studied in experimental animals. It was clearly demonstrated here that there was a wide range of DBH activity, of which level could be reduced or increased by body fluid manipulation and postural changes. Longitudinal study of changes in plasma DBH activity in individuals may be useful for estimation of sympathetic activity.

SUMMARY

Plasma dopamine-β-hydroxylase (DBH) activity was examined in nine healthy young adults. DBH activity at rest had a wide range, near 0 to 44 I.U./L. When sodium depletion was performed by dietary sodium restriction and diuretic, the activity was significantly increased. With dietary sodium loading, it was decreased. The change in DBH activity was significantly correlated with that in hemocrit. When subjects were erected, the enzyme activity was elevated in situations regardless of sodium balance. Blood pressure and pulse rate were changed by manipulation of sodium balance and postural change. However, the change in DBH activity did not correlate with them. Plasma renin activity (PRA) was determined concomitantly in the same plasma with DBH. The change in PRA had a direct correlation with that in DBH activity. Furthermore, the change in mean arterial pressure induced by infusion of angiotensin II analogue seemed to correlate with DBH activity change by sodium depletion. When the values of DBH activity in each individual were compared throughout the study, it was observed that the biggest variance in the activity of each individual was significantly correlated with the basal acti-
vity.
In longitudinal study of individuals, plasma DBH activity could be a useful index for estimation of sympathetic activity.

REFERENCES


Discussion

Chairman: Dr. K. Aoki (Nagoya City Univ.)

CHAIRMAN: Now this session is opened for discussions and comments.

Dr. Z. MASUYAMA (Wakayama Med. College): We would like to present our results. The levels of serum DBH activity in human individuals vary widely and seems to be influenced by genetic factor. The activity gradually decreases with age between the third to eight decades. The serum DBH activity elevates in patients with essential hypertension, compared with age-matched normotensive controls. The levels of the activity elevate slightly, but not significantly, after the stresses such as sodium depletion, upright posture and walking. However, those stresses are milder than your stress to men. It may be recommended that the levels of plasma catecholamines is a much better indicator for the sympathetic response to the stress than the levels of serum DBH activity, because the changes of plasma catecholamine levels by stress is larger than that of serum DBH activity by sodium depletion and furosemide is larger than that by upright and walking, is it right?

Dr. S. TAKISHITA (Kyushu Univ.): Yes, it is. The change of serum DBH activity is more depending on circulating fluid volume than that by upright and walking stress.

Dr. O. IMURA (Sapporo Med. College): The DBH activity and urinary noradrenaline had also been measured in the patients with essential hypertension in our laboratory, and following results were obtained. Both serum DBH activity and urinary noradrenaline decreased following two weeks rest after hospital admission in labile hypertension and increased after salt restriction for five days in majority of the patients. Following two hours tilting, we found percent change of the DBH activity is well correlated with the increased amount of noradrenaline in urine. On the other hand, it was generally recognized that the half-life time of DBH is longer than that of catechol-

Japanese Circulation Journal Vol. 41, August 1977
amine in plasma.

Thus, I would like to support the DBH activity should be measured as an indicator of sympathetic nerve activity.

Dr. S. TAKISHITA: I would like to say, the exercise on full speed running for 100 m elevates serum DBH activity by approximately 15%, however the exercise on running for one Km elevates the activity levels only by approximately 10%, and the activity in the individual is higher in the daytime than that in the nighttime, so that the levels of the DBH activity would provide a index of sympathetic neural activity. On the other hand, the DBH activity in the patients with acute myocardial infarction within 3 hours after the onset is higher than that in the patients after disappearance of the symptoms of myocardial infarction, and the activity also elevates in the patients with acute hepatitis. From the points of view, the levels of serum DBH activity participate in not only the function of sympathetic nerve but the pathophysiological conditions in the individuals. The levels of the activity in patients with labile essential hypertension is slightly higher tendency than in normotensive control. And, the levels in established essential hypertension is slightly lower but the levels is within normal values.

CHAIRMAN: I would like to point out that the DBH activity is present in plasma, but its levels vary widely between individuals. This variation seems to be related more to genetic factors than to sympathetic nerve activity. Also, the change of the levels of the DBH activity by the stresses is considerably small. Several laboratories reported on the relation between serum DBH activity and hypertension. Now, the conflicting results led to considerable confusion (Ann. Intern. Med. 85: 211–223, 1976). Thank you very much.