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Circulating Catecholamines, Plasma Renin and Dopamine-Beta-Hydroxylase Activity with Postural Stress[1]

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MUELLER, R. A., D. K. MILLWARD AND J. W. WOODS. *Circulating catecholamines, plasma renin and dopamine-beta-hydroxylase activity with postural stress.* PHARMAC. BIOCHEM. BEHAV. 2(6) 757–761, 1974. – Changes in human plasma renin activity, catecholamines and dopamine-beta-hydroxylase activity as a result of head-up tilt and low salt diet are reported. Both salt restriction and head-up tilt increased plasma renin activity and these responses appeared to summate. Head-up tilt increased plasma norepinephrine and epinephrine, but sodium restriction was without effect. Neither stimulus produced significant alterations in plasma dopamine-beta-hydroxylase catalytic activity. No correlation between dopamine-beta-hydroxylase activity and either circulating catecholamine was observed. These results indicate that plasma dopamine-beta-hydroxylase catalytic activity is not a good indicator of acute relatively mild changes in sympathetic activity in humans.

Renin Sodium chloride Dopamine-beta-hydroxylase Norepinephrine Epinephrine Postural stress Sympathetic activity

PLASMA dopamine-β-hydroxylase (DBH) activity (EC 1.14.2.1) has recently been proposed as an index of sympathetic nerve activity [20]. As yet only one study has examined the correlation of plasma DBH with major circulating catecholamines, norepinephrine and epinephrine after an acute sympathetic stress [13]. However, no correlation was observed between serum DBH and either norepinephrine or epinephrine plasma levels of pregnant human females given protaglandin $F_{2\alpha}$ for therapeutic abortion. Since this stimulus to sympathetic activity is unusual, it was decided to examine this correlation using a classical sympathetic stress, head-up tilt.

Renin is another naturally-occurring plasma enzyme protein, and increased plasma renin concentrations are produced by some of the same stimuli which increase circulating catecholamine concentrations [18]. Unlike catecholamines, renin and DBH are large proteins [5,19]. Therefore, though different catabolic factors may be important for inactivating each of these two proteins, their disappearance from plasma is obviously governed by processes far different from those which control catecholamine concentrations.

The present study was designed to examine the correlation of basal and stress-induced circulating catecholamine concentrations with plasma DBH activity, and the correlation of DBH activity with plasma renin activity. The results suggest that after positional stress plasma renin activity responds quickly whereas plasma DBH activity does not change at all, and that DBH activity may not reflect mild sudden changes in sympathetic function.

METHOD

Eight healthy normotensive volunteers, all males, ranging in age from 21–25 years, were studied. None had a history of other than childhood infectious diseases, and none were taking any medication. Each volunteer ate a regular diet containing the usual amount of NaCl prior to Day 1 of the experiment. The 24-hr sodium excretion from Day 0 to Day 1 ranged from 108–297 mEq (Mean = 186). All subjects were admitted to the clinical research unit the day before the first tilt session to assure that the pretilt blood sample obtained at 8:00 a.m. the next morning was indeed basal. No subject was allowed out of bed, and none

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desired to micturate or defecate until the completion of the tilt study. A scalp needle filled with heparinized saline was placed in a large antecubital vein 15 min before withdrawing the first pretilt blood sample. The subject was then transferred from bed to a tilt table with a foot rest, tilted to 80°, and blood samples withdrawn after 15 min for determination of DBH and after 30 min for determination of DBH, renin activity, norepinephrine and epinephrine. If any subject demonstrated a bradycardia, fall in blood pressure, and complained of faintness, he was returned to the horizontal position for 5 min and then retilted for the remainder of the 30 min period. At the conclusion of the tilt experiment each subject was started on a diet containing 10 mEq of sodium per day. All meals for the next four days were prepared and eaten in a clinical research ward, but subjects slept at home. After four days of this diet (Day 5) all but one of the subjects were in sodium balance. The latter's urine contained 32 mEq of sodium for the final 24-hr period. The subjects were readmitted to the clinical research unit the evening of Day 4, and on the morning of Day 5 the subjects were again tilted and blood samples drawn as described above.

For determination of plasma renin activity (PRA) 10 ml of blood was drawn into ice-cold tubes containing 6 mg of sodium edetate (EDTA), immediately centrifuged at 4°C, and the plasma frozen at −20°C. Angiotensin generation was determined by angiotensin I radioimmunoassay using a modification of the method of Haber, Koerner and Page [8]. Incubation was carried out at pH 5.5 for 1 hr at 37°C. All samples from a particular patient were processed in the same assay. Between-assay variability was assessed by analysis of a pooled plasma sample during each assay and averaged 5%. The method is highly specific and can detect levels of 25 picograms of angiotensin I.

For determination of plasma catecholamines and DBH, blood samples of 25 ml were removed drom the catheter into heparinized, chilled, polypropylene syringes, and the blood immediately transferred to polycarbonate centrifuge tubes kept on ice. All samples were centrifuged within 5 min at 12,000 Xg for 10 min to sediment red cells, and 1.0 ml plasma removed for DBH assay, and 9–11 ml plasma placed in glass vials containing 25 mg sodium metabisulfite, 6,000 dpm dl-7-^{3}H norepinephrine, and 6,000 dpm dl-7-^{3}H epinephrine (Amersham/Searle, 11.3 c/mM and 7.1 c/mM respectively).

Plasma samples for catecholamine analysis were frozen at −20°C until assay by the Siggers, Salter and Toseland modification [15] of the Engelman, Portnoy and Lovenberg double-isotope derivation assay for norepinephrine and epinephrine [4]. All values for norepinephrine and epinephrine were computed using the verified specific activity of the tritium labeled internal standards, the S-adenosylmethionine-^{14}C, and the dpm of ^{3}H and ^{14}C in each sample area on the electrophoresis strip, and additional standard samples [12].

The plasma samples for assay of DBH activity were diluted 10-fold with water after adjusting to pH 6.0. DBH activity was determined on 100 or 200 μl of diluted plasma, (10 or 20 λ of original plasma) using the Method of Weinshilboum and Axelrod employing bovine phenylethanolamine-N-methyl transferase. However, in this second reaction EDTA (10 mM) was added, as suggested by Coyle and Axelrod [2]. Each sample was assayed using at least 3 concentrations of cupric ion, and appropriate blank values were obtained using boiled diluted plasma. Known amounts of octopamine were added to appropriate blank tubes to facilitate calculation of amount of product formed by DBH. All values are expressed as μmoles octopamine produced/20 min/liter plasma.

RESULTS

The PRA noted in the volunteers after an overnight fast and before arising was 2.21 ± 0.32 ng/ml/hr (Fig. 1). Thirty min of tilt at 80° increased this activity to 5.99 ± 0.57. Vasovagal responses occurred in 4 of the 8 subjects, and the PRA values obtained after returning the patient to the horizontal position were significantly higher ($p<0.05$) in those who experienced this reaction (6.05 ± 0.66) than in those who did not (3.93 ± 0.56). After 4 days of salt restriction these same 4 subjects increased their basal PRA only 0.95 ± 0.77, whereas those who tolerated the first tilt increased their PRA by 5.48 ± 1.7 ($p<0.05$). However, when the tilt exposure was repeated, only one of the 4 had a repeated vasovagal response, and their incremental changes in PRA were then not significantly greater (9.4 ± 7.0) than those of the 4 subjects who tolerated both tilt exposures well (+4.78 ± 2.3). The average increase in supine PRA in the group as a whole due to salt restriction was 5.43 ± 1.08 ($p<0.01$) ng/ml/hr, and this increased significantly with tilting to 12.6 ± 3.5 ($p<0.05$).

Since a wide range of basal norepinephrine (85–451 ng/l) and epinephrine (2–40 ng/l) was observed, the effects of sodium restriction and tilt were analyzed by means of a *t*-test for paired observations. As demonstrated in Table 1, the head-up tilt for 30 min produced a significant increase in norepinephrine. This change was present whether the subject was on a normal sodium intake (+ 36%) or on a restricted sodium diet (+ 69%). The average increase in epinephrine concentration after tilting was more than two-fold on both diets. However, only the increase on a low salt diet was significantly elevated because of large variability in plasma concentrations of this amine.

Serum DBH values showed a wide range in our subjects whether measured on a normal diet (8-267, mean = 138 ± 32 μmoles/20 min/l.) or a low salt diet 6-321, mean = 152 ± 36) (Fig. 1). The 10 per cent increase in DBH activity with salt restriction was not a significant change (paired *t*-test). Tilting of the subjects on a normal diet did not change the DBH activity after either 15 min (98 ± 10% of initial flat DBH activity) or 30 min (109 ± 8%). This response was not altered after 4 days on 10 mEq sodium intake (15 min = 97 ± 6%, 30 min = 105 ± 4%).

DISCUSSION

The secretion of renin from the juxtaglomerular cells is governed by the renal artery pressure, the sodium load in the tubule lumen, and renal sympathetic nerve activity [18]. The present results confirm the widely reported increase in plasma renin activity which occurs with assumption of the erect position, probably as a result of the latter two mechanisms [1]. The relative increase in plasma renin activity observed in this study was similar before and after four days of low salt diet.

Both renin and DBH are proteins, and may have similar mechanisms of inactivation. Elevations of plasma renin activity occurred in association with an increase in sympathetic activity. This would suggest that the inability to detect more DBH activity during acute sympathetic discharge may reflect a difficulty of penetration of this

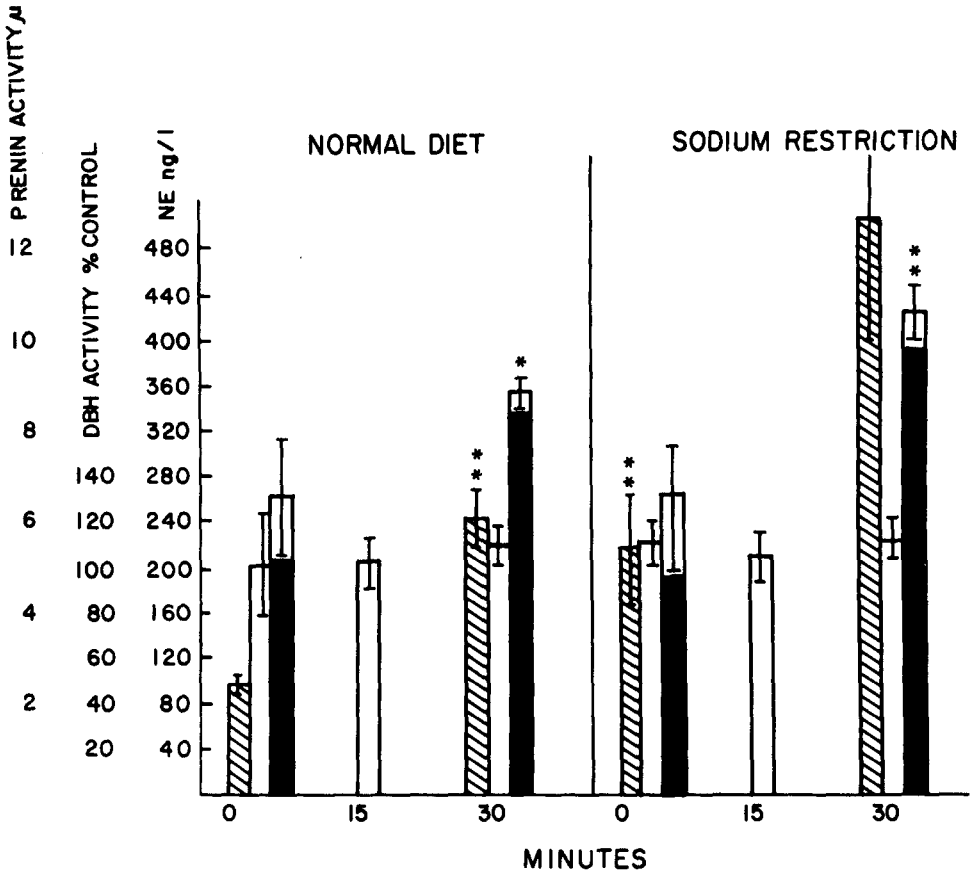

FIG. 1. Volunteers were changed from supine to 80° head-up tilt at zero time. Blood was removed for determination of PRA (cross hatched), DBH (open) and norepinephrine (black), before and after 15 and 30 min of position change. Brackets indicate S.E.M. *$p<0.05$, ‡$p<0.01$, using t-test for paired observations, when compared to pretilt or pretilt normal diet values.

TABLE 1

EFFECT OF SODIUM RESTRICTION AND POSITIONAL CHANGE ON PLASMA CATECHOLAMINES

Number of Subjects	Plasma Catecholamines (ng/l)			
	Normal Diet		Low Sodium Diet	
	NE	E	NE	E
	Volunteers before tilt			
8	261 ± 50*	20 ± 5*	251 ± 55*	24 ± 4*
	Increase with tilt			
8	+95§ ± 39†	+25 ± 18†	+172§ ± 47†	+28‡ ± 11†
	% Change			
	+36	+125	+69	+116

*Standard error of mean
†Standard error of difference
‡$p<0.05$ for paired observations
§$p<0.01$ for paired observations

protein into the vascular space, rather than a rapid inactivation of released enzyme. Certainly the special orientation of the juxtaglomerular apparatus to the anteriolar lumen of the kidney suggests direct release of renin into the vascular space [11]. On the other hand, DBH released at other arterioles would have to either penetrate several wall layers or utilize lymphatic drainage channels to enter the blood. Moreover, the large size of DBH [5] would make its penetration more difficult than that of the smaller renin molecule [19]. While an individual's DBH activity is remarkably constant, rather large variations are observed within the general population [7, 20, 23].

The total sympathetic response, as reflected in urinary excretion of catecholamines [17] or changes in plasma circulating catecholamines [9] is also increased by acute posture alteration. The results of the present study confirm that an 80° head-up tilt from the recumbent position will produce an increase in circulating norepinephrine. The maximum concentration noted by Hickler, Hamlin and Wells [10] in a similar population occurred at 10 min. These peak levels were already declining at 30 min, the time period chosen for the present study. While quite different procedures were used in the above study to quantitate the absolute amount of amines present in blood, the relative changes observed here agree well with those previously reported at this time interval.

Though the turnover rate of cardiac norepinephrine in rats given a low salt diet is decreased [3] little data is available about the effects of salt restriction on the turnover or levels of circulating catecholamines in the blood of humans who are given a low sodium diet. The present study indicates that salt restriction sufficient to double plasma renin activity does not alter basal plasma norepinephrine or epinephrine concentrations.

Although the expected effects of head-up tilt on both plasma renin activity and plasma norepinephrine were observed in this study, no change in plasma DBH was observed during this 30-min stress period. Similar results were obtained in patients experiencing abdominal pain induced by infusing protaglandin $F_{2\alpha}$ to induce therapeutic abortion, i.e. an increase in circulating norepinephrine occurred without a significant change in DBH activity [13]. Wooten and Cardon have recently concluded that "no significant change occurred during tilting," when plasma DBH activity of 6 normal volunteers was measured [23]. However, these workers did not measure circulating catecholamines to verify that a significant sympathetic response had been produced. The present results demonstrate that a significant elevation in plasma DBH acitivty does not occur after a tilt-stress sufficient to double circulating catecholamine concentrations. Wooten and Cardon measured DBH by a similar method and at much shorter intervals (2–3 min) but only for as long as 15 min after tilting [10]. Thus it would seem that significant early elevations in DBH activity do not occur, and the 30 min interval of the present study also failed to indicate a change in DBH activity. Although we cannot rule out some change in DBH activity between 15 and 30 min after starting the tilt exposure such changes would seem unlikely.

DBH appears to be released from in vitro tissue preparations as a result of sympathetic nerve stimulation, [22] possibly by exocytosis of the amine storage vesicle contents [6]. In rats circulating DBH is thought to be released from sympathetic nerves rather than from the adrenal medulla, since chemical sympathectomy with 6-hydroxydopamine lowers rat plasma DBH but adrenal medullectomy has no effect [21]. If the human enzyme release paralleled that in the rat, one would expect a better correlation of DBH with plasma norepinephrine than epinephrine. Our results, however, demonstrate no significant correlation of DBH with either amine, either at rest or during 80° upright tilt and regardless of sodium blaance. On this basis it might appear that plasma DBH catalytic activity is a poor reflection of acute changes in sympathetic activity. However, Wooten and Cardon did find small but significant increases in plasma DBH activity after exercise or cold pressor test [23]. Moreover, Naftchi, Wooten, Lowman and Axelrod have demonstrated that quadriplegics experiencing hypertensive crisis have an increased urinary excretion of catecholamine metabolities as well as an elevation of serum DBH activity [14]. The present work, however, emphasizes that appreciable changes in sympathetic activity may occur and yet not be reflected in values of serum or plasma DBH catalytic activity. It is possible that a much better correlation would appear if immunologically-reactive DBH were measured rather than enzyme activity. Geffen, *et al.* have recently reported that unlike DBH catalytic activity, DBH immunologically reactive protein in the serum correlates well with plasma norepinephrine in patients with essential or labile hypertension [7].

It is hoped that the availability of such immunologic methods to measure DBH protein content rather than DBH catalytic activity as in the present study will facilitate the determination of sympathetic activity in the future. However, it should be emphasized that mild, acute changes in sympathetic activity may be overlooked if the determination of DBH catalytic activity is used as the sole indicator of sympathetic function.

REFERENCES

1. Cohen, E. L., D. R. Rovner and J. W. Conn. Postural augmentation of plasma renin activity. *J. Am. med. Ass.* **197:** 973–987, 1966.
2. Coyle, J. W. and J. Axelrod. Dopamine-β-hydroxylase in the rat brain: developmental characteristics. *J. Neurochem.* **19:** 449–459, 1972.
3. de Champlain, J., L. R. Krakoff and J. Axelrod. Relationship between sodium intake and norepinephrine storage during the development of experimental hypertension. *Circ. Res.* **23:** 479–491, 1968.
4. Engelman, K., B. Portnoy and W. Lovenberg. Sensitive and specific double-isotope derivative method for the determination of catecholamines in biological specimens. *Am. J. med. Sci.* **255:** 259–268, 1968.
5. Friedman, S. and S. Kaufman. 3,4-dihydroxyphenylethylamine-beta-hydroxylase. *J. biol. Chem.* **240:** 4763–4773, 1965.
6. Geffen, L. B. and B. G. Livett. Synaptic vesicles in sympathethic neurons. *Physiol. Rev.* **51:** 98–157, 1971.
7. Geffen, L. B., R. A. Rush, W. J. Louis and A. E. Doyle. Plasma dopamine-beta-hydroxylase and noradrenaline amounts in essential hypertension. *Clin. Sci.* **44:** 617–620, 1973.
8. Haber, E., T. Koerner, L. B. Page, B. Kliman and A. Purnode. The application of a radioimmunoassay for angiotensin I to the physiologic movements of plasma renin activity in normal human subjects. *J. clin. Endocr. Metab.* **29:** 1349–1355, 1969.

9. Hickler, R. B., J. T. Hamlin and R. E. Wells, Jr. Plasma norepinephrine response to tilting in essential hypertension. *Circulation* **20:** 422–426, 1959.
10. Hickler, R. B., R. E. Wells, Jr., H. R. Tyler and J. T. Hamlin. Plasma catecholamine and electroencephalographic responses to acute postural change. *Am. J. Med.* **26:** 410–423, 1959.
11. Latta, H., A. B. Maunsbach and M. L. Cook. The juxtaglomerular apparatus as studied electron microscopically. *J. Ultrastruct. Res.* **6:** 540–561, 1971.
12. Louis, W. J. and A. E. Doyle. The use of a double isotope derivative plasma assay for noradrenaline and adrenaline in the diagnosis and localization of phaeochromocytoma. *Aust. N. Z. J. Med.* **3:** 212–223, 1971.
13. Mueller, R. A., J. I. Fishburne, W. E. Brenner, J. T. Braaksma, L. G. Staurovsky, J. L. Hoffer and C. H. Hendricks. Changes in human plasma catecholamines and dopamine-beta-hydroxylase produced by prostaglandin $F_{2\alpha}$. *Prostaglandins* **2:** 219–225, 1972.
14. Naftchi, N. E., G. F. Wooten, E. W. Lowman and J. Axelrod. Concomitant changes in dopamine-β-hydroxylase (DBH) activity and hemodynamics during hypertensive crises in quadriplegia. *Fedn. Proc.* **32:** 708 Abs, 1973.
15. Siggers, D. C., C. Salter and P. A. Toseland. A double isotope dilution (sic) method for differential determination of adrenaline and noradrenaline in plasma. *Acta Clin. Chim.* **30:** 373–376, 1970.
16. Steele, R. G. D. and J. H. Torrie. *Principles and Procedures of Statistics.* New York: McGraw-Hill Book Co., 1960.
17. Sundin, T. The influence of body posture on the urinary excretion of adrenaline and noradrenaline. *Acta med. scand.* **154:** Suppl. 313, 9–56, 1956.
18. Vander, A. J. Control of renin release. *Physiol. Rev.* **47:** 359–382, 1967.
19. Waldhausl, W. K., C. P. Lucus, J. W. Conn, J. H. Lutz and E. L. Cohen. Studies on the partial isolation of human renin. *Biochim. Biophys. Acta* **221:** 536–548, 1970.
20. Weinshilboum, R. and J. Axelrod. Serum dopamine-beta-hydroxylase activity. *Circ. Res.* **28:** 307–315, 1971.
21. Weinshilboum, R. M. and J. Axelrod. Serum dopamine-beta-hydroxylase: decrease after chemical sympathectomy. *Science* **173:** 931–934, 1971.
22. Weinshilboum, R. M., N. B. Thoa, D. G. Johnson, I. J. Kopin and J. Axelrod. Proportional release of norepinephrine and dopamine-β-hydroxylase from sympathetic nerves. *Science* **174:** 1349–1351, 1971.
23. Wooten, G. F. and P. V. Cardon. Plasma dopamine-β-hydroxylase activity. *Arch. Neurol.* **28:** 103–106, 1973.