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COMMENTARY

SERUM DOPAMINE-β-HYDROXYLASE AS AN INDICATOR OF SYMPATHETIC ACTIVITY AND PRIMARY HYPERTENSION

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The autonomic nervous system plays an important role in the regulation of the cardiovascular system. In a wide variety of normal and stressful situations, autonomic activity provides support for the circulation, but at the same time may contribute to new or aggravated manifestations of disease. It has been suggested that hyperactivity of the adrenergic system may be an important factor in the development of essential hypertension and other diseases. Although one can monitor acute change in adrenergic activity, no simple method exists for evaluating chronic levels of sympathetic discharge. The demonstrations that the soluble fraction of dopamine-β-hydroxylase (DBH), located in synaptic vesicles of sympathetic neurons [1, 2] is released concomitantly with norepinephrine (NE) [3–6], and that large amounts of the enzyme accumulate in plasma [7, 8], led to the suggestion that plasma DBH concentrations might serve as an accurate index of sympathetic nervous system activity. It is our purpose in this report to evaluate serum DBH concentration as an indicator of sympathetic nerve activity and to discuss the possible use of DBH activity in the study of hypertension.

Measurement of serum DBH

Several different methods have been utilized for the assay of DBH enzyme activity [9]. Most investigators who have compared the different procedures for determining serum or plasma DBH have reported that correlation between the assays is very good. Therefore, it would appear that any of the methods should yield equivalent data if used with appropriate caution regarding endogenous inhibitors.

Another approach has been the measurement of DBH protein by radioimmunoassay (RIA). Rush and Geffen [10] were the first to develop such an assay for DBH. They prepared rabbit anti-sera against purified sheep adrenal DBH and demonstrated the feasibility of RIA for measuring plasma levels of DBH protein. Despite loss of sensitivity because of species specificity, they were able to measure DBH protein in human sera and found the variation among individuals to be much less than the variation in enzymatic activity. In subsequent studies, Geffen *et al.* [11] found a good correlation between hypertension and immunologically reactive DBH. Using anti-sera prepared against bovine DBH, Rush *et al.* [12] found no correlation between enzyme activity and immunologically reactive protein in human plasma and suggested that there are widely variable amounts of enzymatically inactive DBH protein present. However, Ebstein *et al.* [13] prepared anti-sera to DBH obtained from a human pheochronocytoma, and by RIA found a good correlation between enzyme activity and immunologically determined DBH protein; nonetheless, there were sufficient discrepancies to suggest that, in some individuals, enzyme activity was not an accurate measure of DBH protein. Recently, Rush *et al.*, [14], using anti-sera directed against human adrenal DBH, found very close agreement between enzymatic activity and DBH protein over a wide range of values in human sera. Similarly, immunological titration studies in our laboratory also show very close agreement between immunological and enzymatic activity in human sera using anti-sera directed against DBH prepared from a human pheochromocytoma. From these data it may be concluded that in most cases the enzymatic assay and the immunologic assay will provide equivalent data. However, measurement of DBH by both procedures may reveal the presence of abnormal types of DBH.

Source of plasma DBH

It has been established fairly well that DBH is released in proportional amounts with norepinephrine from sympathetic pre-synaptic vesicles [15–17] and enters the circulation, probably via the lymph [18]. While several studies utilizing sibling and twin pairs (monozygote and dizygote) have established that plasma DBH in humans is to a very significant extent genetically determined, [19–21], evidence is accumulating that plasma DBH concentrations also reflect prolonged alterations in sympathetic activity. Increases in plasma DBH have been reported to occur in experimental animals exposed to immobilization [22, 23], chronic swim stress [24], sympathetic stimulation after baroreceptor denervation [18], and in quadraplegic patients during sympathetic mediated hypertensive crises [25,26]. Since adremalectomy does not significantly affect base-line activity of DBH in plasma, but chemical sympathectomy with 6-hydroxydopamine does, it appears that plasma DBH activity is derived mainly from sympathetic nerve terminals.

Results from other experiments also support the correlation between plasma DBH concentrations and sympathetic activity. It has been shown that while volume depletion causes DBH to rise, volume expansion by infusion of albumin into human subjects

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causes DBH to fall [27]. Finally, the correlation between thyroid function and sympathetic activity has been strengthened by the demonstration that plasma DBH levels are elevated in hypothyroidism, decreased in hyperthyroidism and return slowly to normal during treatment [28, 29]. Although data indicate that DBH levels correlate with chronic sympathetic activity, there is no reason to expect that DBH levels will correlate with blood pressure values *per se* especially if the alteration in blood pressure is mediated other than by the sympathetic nervous system.

Several studies have attempted to correlate acute alterations in sympathetic activity with changes in plasma DBH concentration. The findings have been variable and inconclusive. Freedman *et al.* [30] and Wooten and Cardon [31] found small changes in plasma DBH during the cold pressor test, but Leon *et al.* [32] found no consistent change. In both studies, only about half of the subjects tested showed increases in DBH activity. Wooten and Cardon, [31] and Planz and Palm [33] found increases in plasma DBH after exercise, but no changes were observed in subjects tilted from the horizontal to the upright position [31]. There are several possible reasons why these acute studies have provided equivocal results and contributed to the confusion about the relationship between serum DBH concentration and activity of the sympathetic nervous system. First, unlike norepinephrine, DBH has a biological half-life in terms of at least hours, not minutes [10, 24], and there exists a rather large steady-state plasma pool of this protein. Since relatively insignificant amounts of DBH are released from individual nerves, it would take a marked increase in neuronal discharge over a prolonged period of time before one could expect to observe significant increases in the serum DBH. Second, during stress many physiological changes occur including alterations in organ perfusion, pool sizes of blood in vascular beds, plasma volume, etc. These factors alone obviously could modify DBH plasma concentration. In this regard, Stone *et al.* [34] have shown that during the cold pressor test there are not only transient increases in serum DBH but also transient parallel changes in total serum protein and in a number of specific serum enzymes. These results strongly support the suggestion that factors other than neuronal release of DBH may be important in alterations of serum DBH concentration after acute stresses in man.

Drugs also have been used to stimulate or interrupt sympathetic neuronal firing in an attempt to determine whether DBH plasma activity is altered in the expected direction. Unfortunately, these studies also are difficult to interpret. For example, after administration of chlorisondamine for 3 days to rats, NE was decreased but not plasma DBH. From these studies, the authors concluded that DBH levels did not reflect sympathetic activity [35]. However, chlorisondamine is not purely a ganglionic blocking agent but, in fact, causes sympathoadrenal stimulation as well (T. A. Slotkin, F. J. Seidler, C. Lau, J. Bartolome and S. M. Schanberg, manuscript submitted for publication); and indeed, if treatment is continued for 7–10 days, DBH levels in serum actually increase several-fold. These various studies indicate the need for a more complete understanding of neural release and disposition of circulating DBH.

Fate of circulating DBH

Virtually nothing is known about the metabolism of circulating DBH. Rush and Geffen [10] infused ^{125}I-labeled sheep DBH into sheep and examined the decline of radioactivity in blood over an 8-hr period and the accumulation of radioactivity in kidneys, lungs, liver, spleen, heart, brain, adrenals, vas deferens, blood and urine at 7, 24 and 48 hr. The circulating radioactivity fell with a half-life of 4·4 hr, but at 7 hr they could account for only 8 per cent of the infused radioactivity in blood and tissues examined, and 7 per cent in urine. These studies cannot be considered conclusive. In many studies on the turnover of serum proteins, it has been shown that the disappearance of radio-labeled proteins was at least biphasic, and only after 2–3 days could the decline fit first-order kinetics. For example, with serum albumin, the fast component had a turnover time of 0·4 to 0·6 days, while the slow component had a turnover time of 14–15 days. The fast phase is attributed to redistribution of the administered labeled protein between the intravascular and extravascular spaces, while the slow phase is attributed to the metabolic turnover of the protein itself [26].

After administration of ^{131}I-labeled plasma proteins, the appearance of acid-soluble radioactivity in the urine correlated very closely, after the initial redistribution phase, with the disappearance of radioactivity from the blood. The half-lives determined from the disappearance of ^{131}I-labeled proteins from plasma were identical with the half-lives determined by the accumulation of acid-soluble radioactivity in urine [36]. If this is also true for DBH, then the data of Rush and Geffen [10] indicate a half-life for circulating DBH of at least 2 days in the sheep.

Little is known about the factors responsible for the removal of circulating proteins. Minor alterations in protein structure can markedly affect the turnover of administered homologous or heterologous proteins. The turnover time of several iodinated proteins has been shown to depend upon the amount of iodine incorporated [36], and Morell *et al.* [37–39] have shown that removal of sialic acid residues from many glycoproteins drastically shortens their circulating half-life. These and other studies also suggest that protein molecules are not metabolized in the circulation but disappear intact [40]. This is consistent with the finding that plasma DBH enzyme activity correlates with DBH immunologic reactivity over a wide range of values. Moreover, we have found that DBH added to whole blood or serum is completely stable for at least 8 hr when incubated at 37°.

Several other approaches have been utilized in an attempt to measure the half-life of circulating DBH. After rats were subjected to repeated immobilization stress, the plasma DBH levels increased about 2-fold and required about 5 days after cessation of stress to return to normal levels [23]. Implantation of a neuroblastoma in mice elevated serum DBH activity, and after removal of the tumor the DBH activity in plasma decreased with a half-life of approximately 6 hr [41]. Similarly, after removal of a pheochromocytoma

from a human subject there was a decrease in plasma DBH with a half-life of approximately 8 hr [42]. However, in another subject no decrease in plasma DBH was found after removal of the tumor (unpublished observation). Although urinary excretions of vanillmandelic acid (VMA) and catecholamines were highly elevated in this subject, the release of DBH into plasma apparently was too low to raise the circulating amount significantly.

Clinical studies

A large number of observations suggest that the sympathetic nervous system plays a role in the pathogenesis of various forms of hypertension [43–49]. These observations include the recognition of similarities between patients with pheochromocytomas and those with primary hypertension, the description of experimental forms of hypertension induced in animals which appear to be mediated via neurogenic factors [23, 49–51], and the long-recognized efficacy of sympatholytic agents in the treatment of hypertension [44]. Significant differences in sympathetic function have been demonstrated between some hypertensive subjects and normotensive controls, including the hemodynamic response to various types of stress [43, 44, 52], urinary excretion of catecholamines, [43], and the plasma concentration of norepinephrine [46–48. Despite indirect evidence that the sympathetic nervous system may be involved in the pathogenesis of "primary" hypertension, it has been difficult to document relative differences between the level of sympathetic nervous system activity in normal man and hypertensive man [43]. Similarly, the results of early studies of plasma DBH in patients with hypertension are conflicting [11, 53, 54]. A wide range of plasma DBH levels has been reported to occur in apparently healthy subjects [10, 53, 55, 56]. More recently, our laboratory also reported a similar wide range of values in 82 apparently healthy subjects, but the data indicated non-unimodal distribution. Sixty-two subjects had values below 35 units/liter (average 18 ± 1 unit/liter), while 13 of the remaining 20 subjects had values over 60 units/liter (average 80 ± 5 units/liter). DBH activity was constant from day to day in each individual. Those with "low" DBH activity had lower values for urinary catecholamine excretion (31 ± 3 μg/day), while those with "high" DBH activity had higher values for urinary catecholamine excretion (72 ± 6 μg/day). There was a good correlation between plasma DBH activity and urinary catecholamines (linear correlation coefficient of 0·93). This degree of correlation between basal 24 hr urinary catecholamine excretion and plasma DBH activity supports the notion that each of these parameters reflects sympathetic nervous system activity and not just individual differences in the catabolism of these substances. It was also of great interest that arterial blood pressure was stable from day to day in subjects with "low" DBH activity, while individuals with "high" DBH activity exhibited a far greater lability of blood pressure from day to day and frequently had values greater than 135/85 mm Hg [57, 58].

Additional observations were made on 72 patients with various forms of hypertension [59]. Utilizing established clinical and laboratory criteria, these patients were assigned to one of six categories: (a) "primary" labile hypertension; (b) "primary" sustained hypertension; (c) "primary" hypertension with secondary renal abnormalities; (d) renovascular hypertension; (e) hypertension due to renal parenchymal disease; and (f) adrenocortical hypertension. Plasma DBH activity was found to be "high" in patients with all forms of "essential" hypertension, and it differed significantly from the lower values that were observed in patients with "secondary" forms of hypertension. Values were highest in patients with "primary" labile hypertension (average 71 ± 2 units/liter), and in the same range as those observed in apparently healthy subjects with labile blood pressure (see above). For the most part, patients with "labile" hypertension were younger (average age: 26 years) and their history of labile high blood pressure was of shorter duration (average: 1 year) than that observed in other groups. In patients with "primary" hypertension and in patients with "primary" hypertension who also had secondary renal involvement, the plasma DBH levels (55 ± 3 and 40 ± 2 units/liter, respectively) were significantly greater than those of subjects with normal stable blood pressure, but significantly less than those patients with "labile" hypertension. The patients in the latter two groups were older than those with "primary labile" hypertension and the known hypertension had been of much longer duration. Plasma DBH activity was even lower in patients with secondary forms of hypertension (renovascular: 17 ± 2 units/liter; renal hypertension: 11 ± 2 units/liter; adrenocortical: 14 ± 7 units/liter). The range of DBH activity in the apparently healthy subjects with labile blood pressure is almost identical to that observed in the patients with "primary" labile hypertension, while patients with "secondary" forms of hypertension had plasma DBH activities in the same range as that observed in the persistently normotensive control subjects. We wish to emphasize that high levels of DBH activity do not necessarily indicate the presence of clinical manifestations of essential or labile hypertension. We, as well as others, have found that some individuals with high levels of plasma DBH have normal stable blood pressures. As a parallel to this, one may consider the fact that many individuals who have abnormal glucose tolerance tests and carry the genetic trait for diabetes do not have clinical symptoms of the disease. On the other hand, all of the patients studied in our laboratories who were diagnosed to have essential or labile hypertension had elevated DBH levels. Thus, the value of the plasma assay is not as an absolute indicator, but rather as an aid in the differential diagnosis of hypertension.

Our results are in closest agreement with those of Wetterberg *et al.* [54] who found elevated levels of plasma DBH in a group of hypertensive patients and with those of Geffen *et al.* [11]. Our results differ most from those of Horowitz *et al.* [53] who failed to detect "any alteration" of plasma DBH activity in a large group of patients with "essential" hypertension. The reasons for these differences are not apparent at the present time but may in part be ascribed to methodological differences in the assay procedure and to the more complete clinical evaluation performed in our studies which led to a more defined clinical classification of patients. In support of our

findings, a double-blind study of clinical diagnosis and serum DBH values was performed on subjects at Temple University School of Medicine [60]. These data confirm the findings of elevated serum DBH activity and norepinephrine concentrations in subjects with essential hypertension.

It is also worth noting that a recent report indicates that serum DBH activities in spontaneously hypertensive rats are twice that of their normal Kyoto-Wistar controls during the first weeks after birth, at a time when the rats appear normotensive, but as their hypertension develops, their serum DBH values decrease to those of the controls [61].

These various studies indicate that measurements of serum DBH activities could prove extremely useful in the diagnostic evaluation of patients with different types of hypertension, as well as providing a means to further our understanding of sympathetic mechanisms involved in these diseases. However, it is clear that a complete understanding of the systems and factors which influence plasma DBH accumulation and degradation is essential before the various observations can be interpreted definitively.

REFERENCES

1. L. T. Potter and J. Axelrod, *J. Pharmac. exp. Ther.* **142**, 299 (1963).
2. W. P. De Potter, A. F. De Schaepdryver, E. J. Moerman and A. D. Smith, *J. Physiol. Lond.* **204**, 102 (1969).
3. O. H. Viveros, L. Arqueros and N. Kirshner, *Life Sci.* 7, 609 (1968).
4. L. B. Geffen, B. G. Livett and R. A. Rush, *J. Physiol., Lond.* **204**, 58 (1969).
5. G. P. Gewirtz and I. J. Kopin, *Nature, Lond.* **227**, 406 (1970).
6. A. D. Smith, W. P. De Potter, E. J. Moerman and A. F. De Schaepdryver, *Tissue Cell* **2**, 547 (1970).
7. R. M. Weinshilboum and J. Axelrod, *Circulation Res.* **28**, 307 (1971).
8. M. Goldstein, L. S. Freedman and M. Bonnay, *Experientia* **27**, 632 (1971).
9. P. Laduron, *Biochem. Pharmac.* **24**, 557 (1975).
10. R. A. Rush and L. B. Geffen, *Circulation Res.* **31**, 444 (1972).
11. L. B. Geffen, R. A. Rush, W. J. Louis and A. E. Doyle, *Clin. Sci.* **44**, 617 (1973).
12. R. A. Rush, P. E. Thomas, T. Nagatsu and S. Udenfriend, *Proc. natn. Acad. Sci. U.S.A.* **71**, 872 (1974).
13. R. P. Ebstein, D. H. Park, L. S. Freedman, S. M. Levitz, T. Ohuchi and M. Goldstein, *Life Sci.*, in press.
14. R. A. Rush, P. E. Thomas and S. Udenfriend, *Proc. natn. Acad. Sci. U.S.A.* **72**, 750 (1975).
15. L. Cubeddu, S. Z. Langer and N. Weiner, *J. Pharmac. exp. Ther.* **188**, 368 (1974).
16. L. X. Cubeddu, E. M. Barnes, S. Z. Langer and N. Weiner, *J. Pharmac. exp. Ther.* **190**, 431 (1974).
17. G. Wooten, N. B. Thoa, I. J. Kopin and J. Axelrod, *Molec. Pharmac.* **9**, 178 (1973).
18. S. H. Ngai, W. Danman, M. Marchelle and S. Spector, *Life Sci.* **14**, 243 (1974).
19. S. B. Ross, L. Wetterberg and M. Myrrhed, *Life Sci.* **12**, 529 (1973).
20. F. Lamprecht, R. J. Wyatt, R. Belmaker, D. L. Murphy and W. Pollen, in *Frontiers in Catecholamine Research* (Eds. E. Usdin and S. Snyder), p. 1123. Pergamon Press, New York (1973).
21. R. M. Weinshilboum, F. A. Raymond, L. R. Elveback and W. H. Weidman, *Science, N.Y.* **181**, 943 (1973).
22. R. M. Weinshilboum, R. Kvetnansky, J. Axelrod and I. J. Kopen, *Nature New Biol.* **230** 287 (1971).
23. F. Lamprecht, R. B. Williams and I. J. Kopin, *Endocrinology* **92**, 953 (1973).
24. M. Roffman, L. S. Freedman and M. Goldstein, *Life Sci.* **12**, 369 (1973).
25. N. E. Naftchi, G. F. Wooten, E. M. Towman and J. Axelrod, *Fedn. Proc.* **32**, 708 (1973).
26. N. E. Naftchi, G. F. Wooten, E. W. Lowman and J. Axelrod, in *Frontiers in Catecholamine Research* (Eds. E. Usdin and S. Snyder), p. 1143. Pergamon Press, New York. (1973).
27. W. L. Miller, F. Lamprecht, P. Cardon and F. C. Bartter, *J. clin. Invest.* **52**, 57a (1973).
28. R. H. North and S. W. Spaulding, *J. clin. Endocr. Metab.* **39**, 614 (1974).
29. Y. Nishizawa, N. Hamada, S. Fujii, H. Morii, K. Okuda and M. Wada, *J. clin Endocr. Metab.* **39**, 599 (1974).
30. L. S. Freedman, R. P. Ebstein, D. H. Park, S. M. Levitz and M. Goldstein, *Res. Commun. Chem. Path. Pharmac.* **6**, 873 (1973).
31. G. F. Wooten and P. V. Cardon, *Archs Neurol.* **28**, 103 (1973).
32. A. S. Leon, P. E. Thomas, E. Sernatinger and A. Canlos, *J. clin. Pharmac.* in press.
33. G. Planz and D. Palm, *Eur. J. clin. Pharmac.* **5**, 255 (1973).
34. R. A. Stone, N. Kirshner, J. C. Gunnells and R. R. Robinson, *Life Sci.* **14**, 1797 (1974).
35. J. L. Reid and I. J. Kopin, *Proc. natn. Acad. Sci. U.S.A.* **71**, 4392 (1974).
36. S. Cohen, R. C. Holloway, C. Matthews and A. S. McFarlane, *Biochem. J.* **62**, 143 (1956).
37. A. G. Morell, S. Gregniadis, I. H. Schernberg, J. Hickman and G. Ashwell, *J. biol. Chem.* **246**, 1461 (1971).
38. G. Gregniadis, A. G. Morell, I. Steinlieb and I. H. Schernberg, *J. biol. Chem.* **245**, 5833 (1970).
39. A. G. Morell, R. A. Irvine, I. Steinlieb, I. H. Schernberg and G. Ashwell *J. biol. Chem.* **243**, 155 (1968).
40. H. S. Anker, in *The Plasma Proteins* (Ed. F. W. Putnam), Vol. II, p. 267. Academic Press, New York (1960).
41. L. S. Freedman, M. Roffman, K. P. Lele, M. Goldstein, J. L. Bidler, B. A. Spengler and L. Helson, *Fedn. Proc.* **32**, 708 (1973).
42. W. Lovenberg, E. A. Bruckwick, R. W. Alexander, D. Horowitz and H. Kerser, in *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes* (Ed. E. Usdin), p. 121. Raven Press, New York (1974).
43. P. J. Nestel and M. D. Esler, *Circulation Res.* **26** and **27** (suppl. II), 75 (1970).
44. J. H. Windesheim, G. M. Roth and E. A. Hines, *Circulation* **12**, 878 (1955).
45. A. E. Doyle and F. H. Smirk, *Circulation* **12**, 543 (1955).
46. K. Engleman, B. Portnoy and A. Sjoerdsma, *Circulation Res.* **26** and **27**, (suppl. I), 141 (1970).
47. W. J. Louis, E. Doyle and S. Anavekar, *New Engl. J. Med.* **288**, 599 (1973).
48. V. DeQuattro and S. Chan, *Lancet* 806 (1972).
49. R. B. Williams, F. Lamprecht and I. J. Kopin, *J. clin. Invest.* **51**, 104a (1972).
50. F. Lamprecht, B. S. Eichelman, R. B. Williams, F. Wooten and J. Kopin, *Psychosom. Med.* **36**, 298 (1974).
51. A. Ito and S. M. Schanberg, *J. Pharmac. exp. Ther.* **181**, 65 (1972).
52. M. D. Esler and P. J. Nestel, *Clin. Sci.* **44**, 213 (1973).
53. D. Horowitz, R. W. Alexander, W. Lovenberg and H. R. Keiser, *Circulation Res.* **28**, 594 (1973).
54. L. Wetterberg, H. Aberg and S. B. Ross, *Scand. J. clin. Lab. Invest.* **30**, 283 (1973).
55. L. S. Freedman, T. Chuchi, M. Goldstein, F. Axelrod, I. Fish and J. Dancis, *Nature, Lond.* **236**, 310 (1972).

56. T. Nagatsu and S. Udenfriend, *Clin. Chem.* **18**, 980 (1972).
57. S. M. Schanberg, R. A. Stone, N. Kirshner, J. C. Gunnells and R. R. Robinson, *Science, N.Y.* **183**, 523 (1974).
58. S. M. Schanberg, R. A. Stone, N. Kirshner, J. C. Gunnells and R. R. Robinson, in *Neuropsychopharmacology of Monoamines and Their Regulatory Enzymes* (Ed. E. Usdin), p. 129. Raven Press New York, (1974).
59. R. A. Stone, J. C. Gunnells, R. R. Robinson, S. M. Schanberg and N. Kirshner, *Circulation Res.* **34** and **35**, (suppl. I), 47 (1974).
60. C. T. Bello, M. M. Reidenberg and S. M. Schanberg, *Proc. Soc. exp. Biol. Med.*
61. T. Nagatsu, T. Kato, Y. Numata, K. Ikuta, H. Vemezawa, M. Matsuzaki and T. Takeuchi, *Nature, Lond.* **251**, 630 (1974).