Biochemical Pharmacology, Vol. 26, pp. 986–987. Pergamon Press, 1977. Printed in Great Britain.

Rapid changes and half life of dopamine-β-hydroxylase; effect of glucagon

(*Received* 8 *September* 1976; *accepted* 28 *October* 1976)

Serum dopamine-β-hydroxylase (EC 1.14.2.1) (DBH) activity has been measured by many workers to detect change in the activities of the sympathoadrenal system. A number of reports were recently summarized in the considerate commentaries by Laduron [1] and Schanberg and Kirshner [2]. These measurements were based on the fact that this enzyme is specifically localized in granules or vesicles containing catecholamines and is secreted with catecholamines by the mechanism of exocytosis from adrenomedullary cells and sympathetic nerve endings [3–7]. In theory it should be possible to measure the level of circulating catecholamines, but this is very difficult since they are metabolized very rapidly and also taken up to various tissues. Therefore the level of serum DBH was measured instead.

The serum DBH activity has been measured in many pathological states, such as familial dysautonomia, Parkinsonism, Down's syndrome and hypertension and in patients with catecholamine-secreting tumors and results have been compared with those in normal individuals [8]. It has also been reported that the serum DBH level changes rather fast after a load of physical work [9–11].

In a clinical survey of the serum DBH level in a patient with the Shy-Drager syndrome, we observed a rapid change of its activity. Accordingly we re-examined the fate of serum DBH in experimental animals and found that the half life of injected DBH was less than 30 min, due to very rapid degradation of DBH.

Measurement of serum DBH activity. Serum DBH activity was measured by the formation of octopamine from tyramine following to the original method of Pisano *et al.* [12]. The incubation mixture consisted of 10 mM tyramine, 10 mM ascorbate, 10 mM fumarate, 50 mM acetate buffer (pH 5.0), 0.1 mM *p*-chloromercuribenzoate and 0.5 ml of serum in a final vol. of 5.0 ml. Incubation was carried out at 37° for 30 min and the reaction was stopped by adding 1.5 ml of 2 N perchloric acid. As blanks, the sera were incubated under the same conditions except that the tyramine was added after perchloric acid.

Preparation of DBH from bovine adrenal medulla. DBH was prepared from chromaffin granules isolated by the millipore filter, as described previously [13]. The isolated granules were lysed in distilled water and the lysate was centrifuged at 105,000 g, for 1 hr. Under these conditions, about 50 per cent of the DBH was recovered in the supernatant, the rest being bound to the stroma of the granules. Then the supernatant was adjusted to 35 per cent saturation of ammonium sulfate. In this way about 80 per cent of the DBH activity in the supernatant was precipitated. The precipitated material was dissolved in Ringer's solution and the ammonium sulfate was removed by passage through a Sephadex G-25 column. The DBH preparation obtained was injected into rats to measure the half life of DBH.

Determination of the half-life of injected DBH. Samples of the DBH preparation from bovine adrenal medulla (with activity to form 80 nmoles of octopamine/mg protein/min) were injected into the tailvein of Wistar strain rats. The animals were killed at intervals for 60 min after the injection by bleeding and the blood was collected in flasks, to which had been added 2 ml of Ringer's solution containing 500 units of heparin. The serum was separated by centrifugation and the DBH activity was measured as described above. In some experiments, purified *m*-GOT from pig heart was injected with the DBH preparation to compare the half-lives of the two enzymes. The activity of *m*-GOT was estimated by the method of Karmen [14].

Results and discussions. In a patient with the Shy-Drager syndrome showed typical orthostatic hypotension (blood pressure: 140/90 mmHg supine and 70/48 mmHg standing), the serum DBH level increased very rapidly when the patient tilted from the horizontal to the upright position and then decreased very rapidly within 20 min to a steady level (Fig. 1.A). Normal volunteers did not show such an increase in the DBH level when they stood up.

The elevation of serum DBH level in the patient with the Shy-Drager syndrome by standing up may be due to increased activity of the sympathoadrenal system as a compensatory reaction to the rapid onset of hypotension. Assuming that catecholamines were liberated into circulation with DBH the inadequate response of α-receptor to catecholamines seems to underlie in this case.

When glucagon (1 mg, i.v.) was administered to normal volunteers, the serum DBH level showed a prompt increase and it returned to the initial level 60 min later (Fig. 1.B). Such an increase of serum DBH level by glucagon was not reported previously. Their serum lactate dehydrogenase level did not show any change after injecting glucagon.

Another interesting problem is the mechanism of rapid recovery of serum DBH to a steady level. It seems most unlikely that, like catecholamine, DBH was taken up again at the sites from which it was secreted, because its mol. wt is known to be more than 2×10^5. There seems to be two possible mechanisms for the rapid decrease in DBH. The first is the presence of sulfhydryl compounds

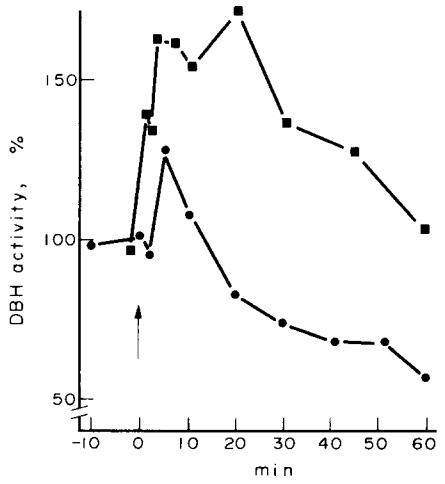

Fig. 1. Changes of serum DBH level in a case of Shy-Drager syndrome upon assuming the upright position and in a normal volunteer after injection of glucagon.

A: The patient stood up at zero time, indicated by the arrow (●——●). 100%: 9 μmoles/l/min (i.u.).

B: The normal volunteer was injected i.v. with 1.0 mg of glucagon at zero time (■——■). 100%: 26 μmoles/l/min (i.u.).

At the times indicated, blood samples were taken from the antecubital vein.

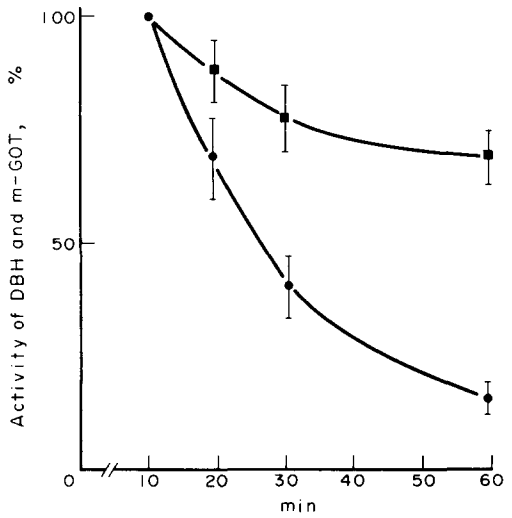

Fig. 2. Decreases of DBH and *m*-GOT injected into rats. DBH from bovine adrenals and *m*-GOT from pig heart were injected into rats together i.v., and their changes in activity in the serum were followed with time. Values are the mean averages and standard deviations (expressed by vertical bars) from five experiments. The one hundred percent means the enzyme level after 10 min of injection. 100%: DBH (400 μmoles/l/min i.u.) (●——●) *m*-GOT (3000 i.u.) (■——■).

in the blood, which are known to mask DBH activity. However, from experiments using *p*-chloromercuribenzoate to block SH-groups in the serum during estimation of DBH activity, this mechanism seems unlikely. Moreover when the DBH preparation from bovine adrenal medulla was incubated with fresh whole blood from humans or rats, it did not show any decrease in activity (data not shown). These findings clearly indicate that the change of serum SH-compounds level is not involved in the rapid decrease of serum DBH level in these experiments.

The second possibility is the existence of a specific mechanism to inactivate or destroy the DBH secreted into the circulating system. As shown in Fig. 2, after injection of bovine adrenal DBH and pig *m*-GOT into rats, the activity of DBH decreased quite rapidly, whereas the activity of *m*-GOT decreased very slowly. The half-life of injected DBH was calculated to be less than 20 min, which is in good accordance with the rate of the changes in the serum DBH level shown in Fig. 1. These results suggest that DBH in the serum is inactivated very rapidly and that the mechanism of its inactivation is relatively specific, because *m*-GOT was inactivated much slower.

The steady level of serum DBH must represent the balance between the rate of its secretion from the sympathoadrenal system and the rate of its degradation. However, the serum DBH level changes very rapidly, so it is rather questionable how nearly the steady level of serum DBH reflects changes of sympathoadrenal activity.

Based on these results we consider that statistical comparisons of the steady state levels of DBH in pathological and normal states do not provide exact representations of the levels of sympathoadrenal function. Thus we would like to propose that serum DBH should be determined by monitoring rapid changes in individual subjects after various stimuli. The estimation of the rate of rise and decay of serum DBH level after various stimuli might be valid to detect the function of sympathoadrenal system and its regulation.

Acknowledgement—We thank Dr. H. Kagamiyama for his kindly gift of purified pig heart *m*-GOT.

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REFERENCES

1. P. Laduron, *Biochem. Pharmac.* **24**, 557 (1975).
2. S. M. Schanberg and N. Kirshner, *Biochem. Pharmac.* **25**, 617 (1976).
3. P. Banks and K. Helle, *Biochem. J.* **97**, 40 (1965).
4. W. W. Douglas, *Br. J. Pharmac.* **34**, 451 (1968).
5. L. B. Geffen, B. G. Livett and R. A. Rush, *J. Physiol.* **204**, 58 (1969).
6. W. P. De Potter, A. F. De Schaepdryver, E. J. Moreman and A. D. Smith, *J. Physiol.* **204**, 102 (1969).
7. R. M. Weinshilboum and J. Axelrod, *New Engl. J. Med.* **285**, 938 (1971).
8. L. S. Freedman, M. Roffman and M. Goldstein, in *Frontiers in Catecholamine Research*, p. 1109 (1973).
9. G. F. Wooten and P. V. Cardon, *Archs Neurol.* **28**, 103 (1973).
10. G. Planz and D. Palm, *Eur. J. clin. Pharmac.* **5**, 255 (1973).
11. Y. Hashimoto, Y. Kurobe and K. Hirata, *Biochem. Pharmac.* **23**, 2185 (1974).
12. J. J. Pisano, C. R. Creveling and S. Udenfriend, *Biochim. biophys. Acta* **43**, 566 (1960).
13. M. Oka, T. Ohuchi, H. Yoshida and R. Imaizumi, *Life Sci.* **5**, 427 (1966).
14. A. Karmen, *J. clin. Invest.* **34**, 131 (1955).