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Inhibitors of Dopamine-β-Hydroxylase in Human Plasma[1] (38870)

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(Introduced by J. L. Wood)

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Dopamine-β-hydroxylase {3,4-dihydroxyphenethylamine, ascorbate-O_2 oxidoreductase (hydroxylating), EC 1.14.2.1} (DBH) activity in most tissues, including blood plasma (1–6) is inhibited *in vitro* by endogenous inhibitors. In rat adrenal, brain, and other tissues, the inhibitors are apparently sulfhydryl-containing material (1, 4, 5). When crude tissue extracts are assayed for activity, sulfhydryl reagents such as copper ions or *N*-ethylmaleimide are incorporated into reaction mixtures to counteract the inhibitors. Such reagents must be used with caution, since they may cause side reactions which interfere with the assay (7). In this paper, we present evidence for the presence of two types of endogenous inhibitors in human plasma. One is copper (or *N*-ethylmaleimide)-sensitive and dialyzable; the other is insensitive to copper or maleimide, and is not dialyzable. The inhibitory effect of both can be counteracted by diluting the plasma, thus negating the need for inactivators of dialysis.

Materials and Methods. Materials used in this study have been previously described (7). Fasting blood was drawn into heparinized syringes and centrifuged. Dilutions were made with H_2O. Plasma dopamine-β-hydroxylase was assayed by the procedure of Friedman and Kaufman (8) as modified by Foldes *et al.* (9), except that the reaction mixture was modified slightly. It contained sodium acetate buffer pH 5.0, 30 mmole; fumarate, pH 5.0, 12.5 mmole; pargyline, 0.15 mmole; catalase, 1500 Sigma units; ascorbate, 1.25 mmole, and ^{3}H-tyramine, 1.0 mmole (1.0 μCi); and various quantities of plasma in a total volume of 250 μl. Unless otherwise specified, incubations were carried out for 30 min at 37° after the addition of enzyme. The reaction was stopped by the addition of 4.0 *M* NH_4OH (1 mole) and the octopamine formed was oxidized by the addition of periodate (6 mmoles) for exactly 4 min. Excess periodate was destroyed by adding sodium bisulfite (14 mmoles) and the mixture was acidified with HCl (1.1 moles). The *p*-hydroxy benzaldehyde was extracted into toluene and the radioactivity determined. The total volume prior to extraction was 0.85 ml. ^{3}H-Octopamine incorporated into the reaction mixture in the absence of enzyme, was used to devise standard curves which were linear with from 10 to 250 nmoles of octopamine. ^{3}H-Octopamine, added to plasma in concentration of from 10 to 240 nmoles, gave recoveries of 98.5 ± 3.6% (±SE, n = 4).

Results and Discussion. Figure 1A shows that DBH activity in undiluted human plasma (100 μl) decreases with time. Under these assay conditions, plasma DBH was inhibited by about 90% (Table I), as was partially purified DBH from bovine adrenal medulla when it was added to plasma. Addition of optimal concentrations of copper caused a 2-fold increase in activity (8, 9), but did not completely nullify the inhibition. Judging from the recovery of added DBH, which can be estimated by subtracting the observed rate from an extrapolation of the initial rate (Fig. 1A), a major portion of the inhibition occurs in the first 10 min of reaction.

The time-dependent inhibition of DBH by plasma decreased with increasing dilution of the latter (Fig. 1B). At dilutions of from 5- to 10-fold, depending upon the individual, product formation became a linear function of concentration (10), and of time up to at least 60 min. Subsequent experiments on diluted plasma led to several interesting observations, heretofore unreported. 1. Neither the addition of copper nor *N*-ethylmaleimide is necessary for optimum activity. In fact, the addition of copper, but

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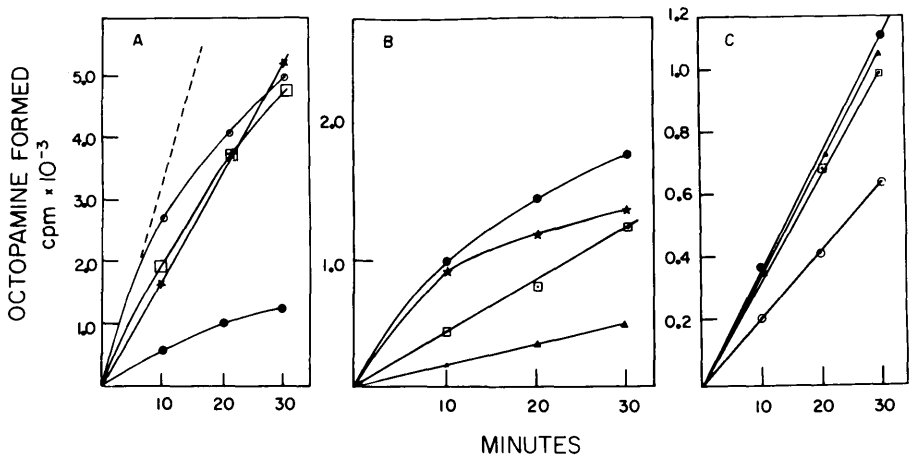

FIG. 1. DBH activity of plasma. A. DBH activity in 100 μl of whole plasma. Additions were: ●—●, none; □—□, 13 nmoles $CuSO_4$ or 26 μmoles of *N*-ethylmaleimide. This curve also represents the activity in 100 μl of plasma which contained no additives, but which had been dialyzed overnight against 0.2 *M* acetate, pH 5.0 (1000 vol); ⊙—⊙, 13 nmoles $CuSO_4$ and 2.6 μg of DBH (sp act, 45.4 μmoles of octopamine produced per mg per hour). The line --- represents an extrapolation of the initial rate. The activity of 2.6 μg of DBH (*—*) is shown in the absence of plasma or other additives. B. DBH activity in 100 μl of whole or diluted plasma plus 13 nmoles $CuSO_4$. Dilutions were: ●—●, none; *—*, 1:2; ⊡—⊡, 1:4, ▲—▲, 1:8. C. DBH activity in 100 μl of diluted plasma (1:8). Additions were: ●—●, none or 26 μmoles of *N*-ethylmaleimide. ▲—▲, 0.25 nmole, $CuSO_4$; ⊡—⊡, 1.3 nmoles $CuSO_4$; ⊙—⊙, 13 nmoles $CuSO_4$. Experiments in A, B, and C were done on plasma freshly drawn from different individuals.

TABLE I. COMPARISON OF DBH ACTIVITY AND INHIBITORS IN PLASMA FROM DIFFERENT INDIVIDUALS.[a] ASSAYS WERE DONE AS INDICATED IN THE TEXT AND IN THE LEGEND TO FIG. 1 ON 100-μl ALIQUOTS OF EACH SAMPLE.

Additives	C.B.	J.H.	P.T. (cpm/ml × 10^{-3})	J.Z.
A None	10.68	7.00	11.95	4.61
B $CuSO_4$ (13 nmoles)	43.08	24.83	53.98	20.49
C A + H_2O (1:8)	12.81	9.10	22.58	6.21
C^1[b]	102.48	72.80	180.64	49.68
D C + NEM[c] (26 μmoles)	13.13	9.75	22.66	6.07
E C + DBH (1.3 μg)	37.27	32.72	47.77	30.43
Summary			**Percent recovery**	
$\frac{A}{C^1}$	10	10	7	9
$\frac{B - A}{C^1}$	32	24	23	32
$\frac{C^1B}{C^1}$	58	66	70	59
$\frac{E - C}{DBH}$	100	96	102	99

[a] One international unit is equivalent to 1 μmole/min/liter of plasma at 37° (18). Under our conditions 1 unit = 90 cpm; 1.3 μg DBH = 2460 cpm.

[b] Calculated from C by correcting for dilution.

[c] NEM is the abbreviation for *N*-ethylmaleimide.

not maleimide, now inhibits the reaction (Fig. 1C and Table I). These data, coupled with the fact that DBH added to diluted plasma is completely recovered (Table 1), suggest that the reaction no longer is inhibited by endogenously derived compounds. 2. There are two types of inhibitors in undiluted plasma. Under the conditions used by us, optimal concentrations of copper reduces the inhibition by only about 30% (Fig. 1A and Table I). This copper-sensitive component is also counteracted by *N*-ethylmaleimide, or it can be removed by dialysis (Fig. 1A). The remaining portion of the inhibition, amounting to about 60–70% of the total activity, appears to be due to a factor(s) in blood which interferes with the assay. The effects of both types can be alleviated by dilution.

In Table I, a summary of the data obtained from four individuals is presented. Among these subjects, DBH activity varied by about 3-fold; but the inhibitory effects were constant. This suggests that data for undiluted plasma can be corrected for the effects of inhibitors, providing that the assay mixture did not contain copper (7) and/or that the plasma had not been dialyzed.

The nature of the copper-insensitive, undialyzable inhibitor of plasma DBH has not as yet been identified. However, the data (Fig. 1) suggest interference with the assay procedure, and since the inhibition occurs within the first 5–10 min of reaction, the agent is probably an enzyme. Two copper-containing possibilities readily come to mind, superoxide dismutase and ceruloplasmin. Under conditions which are not likely to occur in blood, dismutase has been shown to stimulate, not inhibit DBH (11). On the other hand, ceruloplasmin is optimally active under the conditions of this experiment, occurs in concentrations of from 20–40 mg/100 ml in serum, and has been reported to oxidize ascorbate (12). In our hands, ceruloplasmin (1–8 U, specific activity 16 U/mg, Sigma Chemical) inhibits bovine DBH-catalyzed reactions by 25–72%. Whatever the inhibitors turn out to be, they are apparently different from those present in other tissues (7).

Recently several sensitive procedures and techniques have been used to analyze DBH in human blood serum (18, 19). The method outlined above can be used on from 10–20 μl of undiluted plasma, depending upon the DBH content of the latter. The sensitivity can be increased by 2-fold (5–10 μl) by increasing the incubation time to 60 min. The noteworthy advantage in the present method is the lack of interference by inhibitors or by inactivators thereof.

Conclusion. There are two types of endogenous inhibitors of DBH in human plasma. One is sensitive to copper, or *N*-ethylmaleimide, and is dialyzable. The other is not sensitive to copper, maleimide, or dialysis. Both types can be counteracted by diluting the plasma, thus eliminating the need for inactivators during the assay of plasma.

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