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Inhibition of Dopamine-β-Hydroxylase by Hydralazine[1] (37796)

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(Introduced by G. Brecher)

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The drug *l*-hydrazinophthalazine hydrochloride (hydralazine, Apresoline) has been used for the past 20 years as an antihypertensive agent (1). However, little is known at the present time about the precise mechanism or mechanisms of its anti-hypertensive action.

Bydgeman and Atjärne (2, 3) reported that dihydralazine markedly depleted catecholamine in the heart and adrenal of rats. This finding aroused considerable interest, since catecholamine might be an important factor in hypertension (4). Coyle and Axelrod (5) found that an increase in dopamine-β-hydroxylase (DBH) activity during the maturation of brain tissue parallels the increase in the level of endogenous norepinephrine. One might, therefore, speculate that hydralazine-induced catecholamine depletion may be secondary to the depression of DBH, which is required for norepinephrine biosynthesis. Consequently, we studied the effect of hydralazine on the partially purified DBH from bovine adrenal glands. Our data indicate that hydralazine inhibits DBH activity. This inhibition is shown to result from the chelation by the drug of cupric ions required for the inactivation of the DBH endogenous inhibitors.

Materials and Methods. [*side chain*-1, 2-^{3}H]Tyramine hydrochloride (sp act 5.5 Ci/mmole) was purchased from Amersham/Searle Corporation. Catalase was obtained from Boehringer Mannheim Corporation. Pargyline (Eutonyl) was a product of Abbott Laboratories (Chicago, IL). Tyramine HCl was obtained from Sigma Corporation (St. Louis, MO).

Protein assay. Protein determinations were carried out by the method of Lowry *et al.* (6).

Purification of DBH. Partially purified DBH was obtained from bovine adrenal glands. The fresh bovine adrenal glands were obtained from Allen Meat Company, San Francisco, through the courtesy of Dr. Roy. The purification procedure was that of Molinoff *et al.* (7). Separation of phenylethanolamine-*N*-methyltransferase fraction from DBH was done on a Sephadex G-200 column. The fractions which contained highest DBH activity were pooled and used for the present studies. The DBH fraction prepared by this procedure catalyzed the formation of 53 nmoles octopamine from tyramine substrate per mg protein in 30 min at 37° as determined by the method of Molinoff *et al.* (7).

DBH assay. The assay of DBH activity was carried out by a simple and rapid procedure developed by us recently. Details are to be reported in a subsequent communication from this laboratory. The principle of the assay was based on the conversion by DBH of [*side chain*-1,2-^{3}H] tyramine to the β-hydroxylated amine, which simultaneously involved a release of tritium and the formation of tritiated water in the reaction mixture. The quantity of tritiated water formed was used as an index for DBH activity. The low blank radioactivity (ranging from

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100 to 300 cpm) indicated that the tritium–hydrogen exchange during the reaction was negligible.

The standard incubation mixture contained tyramine substrate in 1.0 *M* Tris buffer, pH 5.5, (500 μg cold + 5 × 10^6 cpm hot per ml), 200 μl; catalase (1.5 mg/ml), 100 μl; ascorbic acid (5.6 mmole/liter), 200 μl; sodium fumarate (22.0 mmole/liter), 200 μl; pargyline (20 mmole/liter), 50 μl; $CuSO_4$ (1.44 mmole/liter), 25 μl; enzyme, 50–400 μg; and H_2O. The total volume of the incubation was 1.1 ml. Water was replaced by different concentrations of hydralazine in some incubation tubes. The reaction mixture was incubated at 37° for 30 min. The reaction was then stopped by adding 0.1 ml of 50% trichloroacetic acid (TCA) solution. The tritiated water was removed from the reaction mixture by vacuum distillation. A measured quantity of aliquot was mixed with 10 ml of aqueous scintillation fluid and counted. A sample of boiled enzyme preparation was used as a blank.

Results and Discussion. It is well documented that the full activity of DBH is variably reduced in different tissue by endogenous inhibitors that have not been characterized completely, but can be neutralized by binding with cupric ions (8, 9). Therefore, it is of importance to quantify the concentration of endogenous inhibitors present in each preparation and to establish the optimal copper concentration necessary to inactivate them before any given experiment is carried out. As indicated in Fig. 1, the narrow range of optimal copper concentration required to inactivate the endogenous inhibitors of a given batch of enzyme preparation emphasizes the importance of this quantitation. The optimal copper concentration required for our enzyme preparation was estimated to be 3.6 × 10^{-5} *M*.

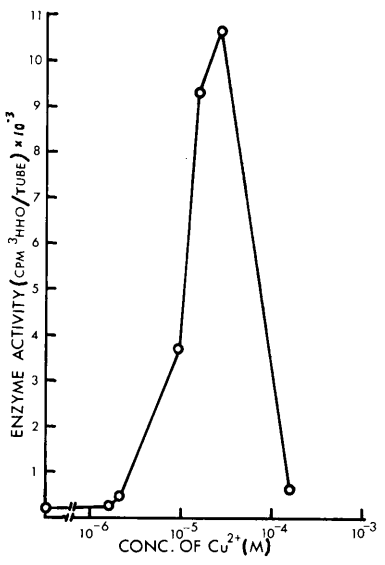

FIG. 1. Effect of cupric ions on partially purified bovine adrenal dopamine-β-hydroxylase. Enzyme protein concentration used for the assay was 400 μg. Each point represents the mean value of two separate assays.

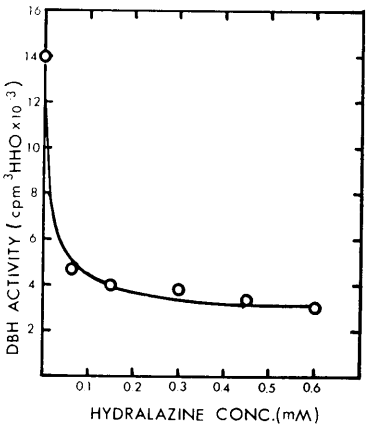

FIG. 2. The effect of hydralazine on dopamine-β-hydroxylase activity *in vitro*. The complete incubation mixtures are described in the text. The enzyme concentration used was 400 μg per tube.

As shown in Fig. 2, when partially purified DBH was incubated with various concentrations of hydralazine along with appropriate cofactors, inhibition of enzyme activity occurred. Figure 3 shows that, in the absence of hydralazine, DBH activity rises linearly with the increase of enzyme protein concentration used. However, in the presence of hydralazine (0.1–0.2 m*M*), the enzyme activity was reduced to below 10% of the control.

The Lineweaver–Burk plot was examined in the absence and presence of hydralazine. As indicated in Fig. 4, the two lines shared a common intercept on the abcissa. This result suggests that the inhibition of DBH activity was noncompetitive in nature. Therefore, the

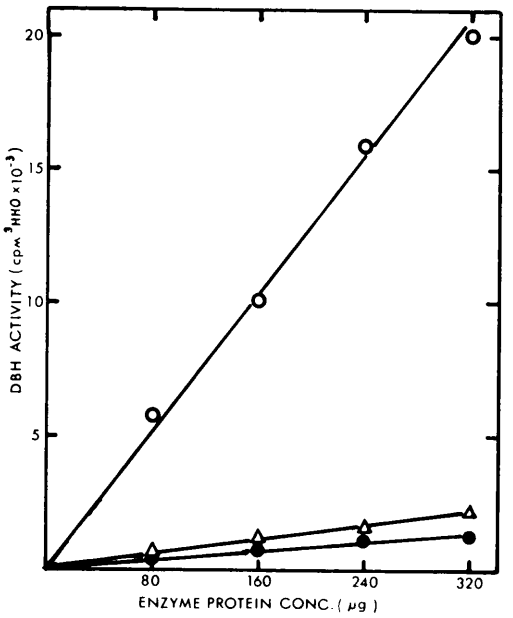

FIG. 3. Effect of protein concentration on dopamine-β-hydroxylase activity in the presence or absence of hydralazine. (○—○) no hydralazine; (△—△) 0.1 m*M* hydralazine added; (●—●) 0.2 m*M* hydralazine added.

drug does not seem to interfere with the substrate binding site of the enzyme.

It was of interest to ascertain whether the drug and DBH are competing for an essential constituent directly involved in the enzymic action. Therefore, a double reciprocal plot of the initial velocity against enzyme protein concentration in the absence and presence of the drug was constructed. As shown in Fig. 5, all three lines shared a common intercept on the abcissa, indicating that the drug and DBH do not compete for an active component required for the enzymic catalysis.

Bhatnagar *et al.* (10) reported that the hydroxylation of peptidyl proline residues in protocollagen was markedly inhibited by hydralazine. They showed that the inhibition resulted from the formation of a chelating complex between the drug and the ferrous ions required for the optimal activity of protocollagen proline hydroxylase. This, together with several other reports (11, 12), suggests that hydralazine is a potentially good chelating agent. This prompted us to investigate the nature of hydralazine-induced inhibition of DBH activity further with special reference to the chelating ability of the drug. As shown in Fig. 6, after DBH was incubated with hydralazine for 10 min, various concentrations of cupric ions were then added back to the drug-inhibited system. The reversal of the enzyme inhibition was evident. These data strongly suggest the formation of a chelating complex between cupric ions and the drug which is responsible for the inhibition of DBH activity.

It was previously reported that Dopa decarboxylase was also inhibited by hydralazine (13). It will be interesting to study the effect of this drug on tyrosine hydroxylase along with DBH *in vivo*. Furthermore, the correlation between these enzyme activities and the catecholamine contents in different organs will be of significance. These ideas are currently under investigation in our laboratory.

Summary. The effect of the antihypertensive drug, hydralazine (*l*-hydrazinophthalazine) on the partially purified dopamine-

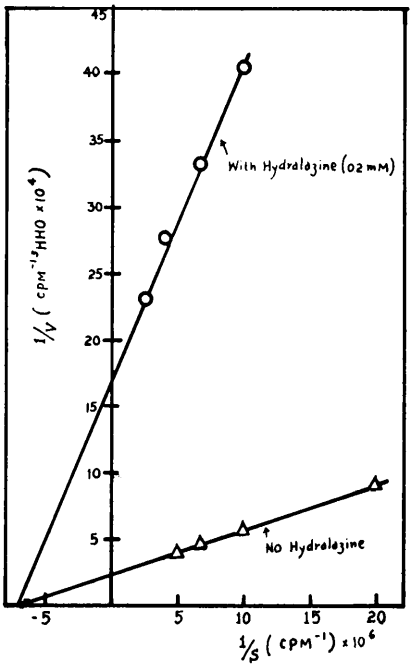

FIG. 4. Lineweaver–Burk plot of bovine adrenal dopamine-β-hydroxylase activity in the presence or absence of hydralazine.

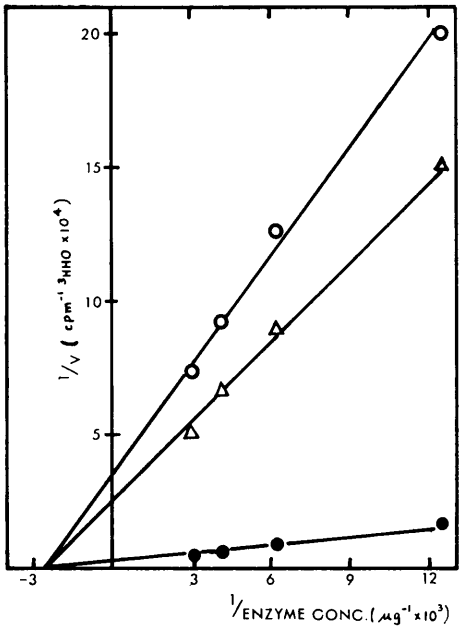

FIG. 5. Double reciprocal plot of the initial velocity against enzyme protein concentration in the presence or absence of hydralazine. (●—●) no hydralazine; (△—△) 0.1 m*M* hydralazine added; (○—○) 0.2 m*M* hydralazine added.

β-hydroxylase from bovine adrenal gland was investigated. The enzyme preparation so prepared was shown to be activated by the appropriate concentration of Cu^{2+} known to inactivate endogenous inhibitors. When the drug was added to this enzyme preparation along with the appropriate components, the inhibition of the hydroxylase enzyme activity could be demonstrated. The inhibition was shown to be noncompetitive, suggesting that the drug did not interfere with the substrate-binding site of the enzyme. The double reciprocal plot of the initial velocity against enzyme concentration shared a common intercept on the abcissa, indicating that DBH activity was inhibited by the drug through a mechanism other than competition for an essential constituent directly involved in the optimum activity of the enzyme. The drug-induced inhibition of the enzyme activity was reversed by adding various concentrations of Cu^{2+} to the system. These data suggest that the drug inhibits DBH activity by chelating Cu^{2+} which is essential for the inactivation of the endogenous inhibitors present in the enzyme preparation used in these experiments.

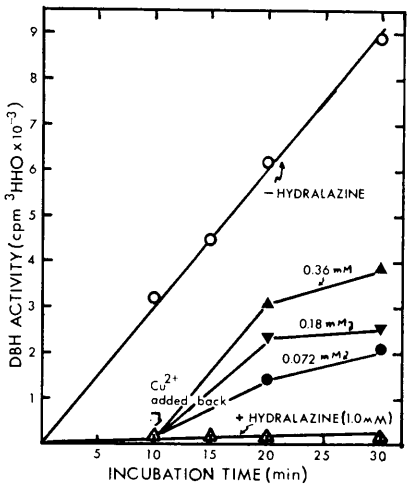

FIG. 6. Time course of the hydralazine inhibition of dopamine-β-hydroxylase and the reversal of the inhibited enzyme activity by cupric ions.

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