An Assay for Dopamine-β-Hydroxylase Activity in Tissues and Serum

Although dopamine-β-hydroxylase (DβH) activity was demonstrated in vivo in various sympathetic innervated tissues, the enzyme activity could not be measured in homogenates obtained from these tissues. Evidence has been presented that endogenous inhibitors interfere with the assays of DβH activity in vitro[1,2].

This paper describes a sensitive and specific enzymatic assay for determination of DβH activity. The enzymatic activity can be determined by this procedure in various tissues as well as in serum. The principle of the assay is outlined in the following reaction scheme.

$$RCH_2CH_2NH_2 + O_2 + XH_2 \xrightarrow{D\beta H} RCHOHCH_2NH_2 + H_2O + X$$

$$RCHOHCH_2NH_2 + C^{14}H_3\text{–S–AM} \xrightarrow{PNMT} RCHOHCH_2NHC^{14}H_3 + SAHCys.$$

XH_2 = ascorbate; X = dehydroascorbate;
$C^{14}H_3$–S–AM = S-adenosylmethionine;
S–AHCys = S-adenosylhomocysteine; R = phenyl

It can be seen from this scheme that the assay depends on 2 enzymatic reactions. In the first reaction tyramine is added to the homogenate and is converted by DβH to octopamine. In the subsequent second reaction the enzymatically formed octopamine is further converted by the added PNMT to N-methyl octopamine. $C^{14}H_3$–S–AM serves as a methyl donor and the amount of C^{14}-N-methyl octopamine is proportional to DβH activity. An account of this procedure was previously presented[3,4].

Determination of dopamine-β-hydroxylase activity in the serum. Serum obtained from humans was diluted 50–100 fold and used for enzyme activity assay. The incubations were carried out in 2 separate steps.

Step 1. To an aliquot of diluted serum (0.05–0.1 ml) 10 μmoles of acetate buffer pH 5.5 and 0.1 μmole of N-ethyl-maleimide (EMI) were added in a volume of 0.2 ml. The mixture was preincubated at room temperature for 10 min and then the following components in a total volume of 0.3 ml were added (in nmoles): fumarate, 10; ascorbate, 6; pargyline, 0.7; tyramine, 0.4; and 1000 units of catalase (Sigma). The mixture was incubated for 20 min at 37 °C.

The endogenous inhibitors of DβH were inactivated with EMI a known sulfhydryl reactive reagent. EMI inhibits PNMT activity and therefore at the beginning of the second step of the reaction DTT was added to react with the excess of this reagent. The addition of copper also inactivates the endogenous inhibitors[4]; however, excessive copper inhibits DβH activity and the concentration of copper in each assay is critical. It seems therefore that the addition of EMI is preferable to the addition of copper.

Step 2. At the end of the incubation 0.1 μmole of dithiothreitol (Cleland's reagent) (DTT) in 0.1 ml was added and after 5 min the following components were added: *tris* buffer pH 7.5, 100 μmoles; $C^{14}H_3$–S–AM, 2 nmoles and 0.1 ml of purified PNMT. (A PNMT preparation purified by chromatography on Sephadex G-100 column was used[5].) The reaction mixture was incubated for 30 min at 37 °C. The incubation was stopped by the addition of borate buffer pH 10.5 (500 μmoles). The C^{14}-N-methylated octopamine was extracted into a mixture of toluene isoamyl alcohol (3:2) as previously described[6]. An aliquot of the organic phase was transferred into a counting vial and the radioactivity was determined. A sample of boiled enzyme preparation served as blank.

Determination of dopamine-β-hydroxylase activity in tissues. The same procedure as described for serum was used for the determination of DβH activity in tissues. Tissues were homogenized in 30 volumes of 0.05 *M tris* buffer pH 6.8, containing 0.1% triton X-100. The homogenate was centrifuged at 27,000 × *g* for 20 min at 0 °C. Aliquots of the supernatant were used for enzyme activity and for protein determination. When the DβH activity in the homogenates of some tissues was too low the enzyme in the supernatant was concentrated by precipitation with 80% $(NH_4)_2SO_4$. The precipitant was collected on millipore filter and dissolved in a minimum volume of 0.05 *M tris* buffer pH 6.8 (20–30 mg of protein per ml). The dissolved protein was dialyzed against

Table I. Dopamine-β-Hydroxylase activity in the serum of control subjects and in patients with neurological disorders

No. of subjects (age range of subjects)	Disorder and treatment	Physical activity	Range of DβH activity[a]
6 (22–46)	Controls	Active	50–90
2 (70–72)	Controls	Inactive	36–45
2 (70–75)	Parkinson's disease, treated with L-Dopa	Inactive	22–28
2 (70–72)	Parkinson's disease, treated with L-Dopa	Inactive	2.8–5.5
2 (70–75)	Parkinson's disease (no treatment)	Inactive	4.9–6.0
1 (70)	Amyotrophic lateral sclerosis, treated with L-Dopa	Inactive	11.0
1 (72)	Amyotrophic lateral sclerosis (no treatment)	Inactive	12.5

[a] The activity is expressed per nmole of formed product/20 min/ml serum.

Table II. DβH activity in various tissues of male Sprague-Dawley rats

Tissues	cpm × 10^{-2} per g tissue	cpm × 10^{-2} per organ
Adrenals[a]	18,000 ± 2,000	720 ± 80 (per pair)
Heart	3,420 ± 390	1,710 ± 190
Salivary gland	4,050 ± 520	610 ± 30
Brain stem	2,900 ± 260	900 ± 125

The results are the mean ± S.E.M. obtained from tissues of 5 rats.
[a] Aliquots of the whole homogenate (1:100 dilution) were used for enzyme assay.

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0.15 *M* acetate buffer pH 5.5 and the volume adjusted to contain 20 mg of protein per ml. Aliquots of these preparations were used for determination of enzyme activity and of protein.

The results presented in Table I show DβH activity in the serum of various human control subjects and in patients with some neurological disorders. DβH activity varies within a certain range in the serum of the control subjects. Preliminary results indicate that the DβH activity depends on the age and physical activity of the human subjects. The DβH activity in the serum of some patients with Parkinson's disease and with amyotrophic lateral sclerosis was lower as compared with the activity in the serum of the controls. However, these patients have been physically inactive for a long period of time and the low DβH activity in the serum may be due to the physical inactivity and may not necessarily be associated with the disease. Furthermore, the prolonged treatment of some patients with L-Dopa may affect the DβH activity. Studies are now in progress to determine the factors which are responsible for the low DβH activity in the serum of these patients.

The presence of DβH activity in human serum was also assayed with immunochemical techniques. The addition of purified rabbit anti-DβH antiserum[7] to human serum resulted in an inhibition of DβH activity. This antiserum exhibited a specific effect on DβH activity and had no effect on PNMT activity. DβH activity was also measured in the serum of various species. In rats, mice and monkeys the DβH activity was much lower than in humans. In C-1300 mice bearing neuroblastoma tumors the DβH activity in the serum was significantly higher than in the corresponding serum of control mice.

The results presented in Table II show the DβH activity in various tissues of rats. The adrenal gland shows the highest DβH activity, while in the heart and salivary glands the DβH activity is much lower. DβH activity was determined in various regions of the brain, but at present reproducible results were obtained only in the brain stem. It is noteworthy that DβH was also localized with immunofluorescence techniques in the cell bodies in the medulla oblongata and the pons[7,8]. Thus, the presence of DβH in the brain stem was confirmed with 2 different procedures. Studies on the effects of stress and of psychoactive drugs on DβH activity in serum and in tissues are now in progress.

Zusammenfassung. Eine isotopische Methode zur Bestimmung der DβH-Aktivität wird beschrieben. Die Methode wurde verwendet zur Bestimmung der DβH-Aktivität im menschlichen Serum und in verschiedenen Organen der Ratte. Das sympatische Nervensystem des Menschens kann mit dieser Methode weiter erforscht werden.

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The Effect of Pyruvate on Cyanide-Inhibited Respiration in Intact Ascites Tumor Cells

Cyanide is known to inhibit mitochondrial electron transport by combining with cytochrome oxidase[1]. The cyanide inhibition is considered not readily reversible. Indeed, even the previously accepted reversibility by physical methods of this inhibition[1-5] has been later questioned on the basis of results obtained by spectrophotometric techniques[6].

The combination of cyanide with different substances, such as fructose, acetaldehyde, pyruvic and oxalacetic acids, results in the formation of cyanhydrins which can mask the expected poisoning effect[7-10]. In this respect the only reports in biological systems were those relative to cyanide inhibition in long-term experiments. Acetaldehyde and pyruvic acid, employed as substrates for bacteria[8] and kidney slices[9], respectively, were added from the beginning of the experiments together with the inhibitor. In this way, because of the early formation of cyanhydrins, the inhibitory effect of cyanide was obviously not feasible.

In this communication we report how the effect of cyanide on the endogenous respiration of ascites tumor cells may be readily and almost completely removed by the further addition of pyruvate even when the binding of cytochrome oxidase to the inhibitor is already established. It has therefore been of interest to investigate under these conditions the real efficiency of the mitochondrial respiratory chain in these cells. The results obtained show that the possibility exists of blocking and releasing quickly the phosphorylating electron flow through the whole respiratory chain.

Material and methods. ELD (Ehrlich-Lettré hyperdiploid) ascites tumor cells were grown in albino Swiss mice by weekly transfer of 0.2 ml of ascites fluid. The cells were harvested 7–8 days after the inoculation, washed in an isotonic phosphate-buffered medium and resuspended in the same medium for the experiments. Rat liver mitochondria were prepared by the method of CHANCE and HAGIHARA[11]. Oxygen uptake was measured

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