Decrease of the 3,4-Dihydroxyphenylalanine (DOPA) Decarboxylase Activities in Human Erythrocytes and Mouse Tissues after Administration of DOPA

(Parkinson's disease/brain/liver/kidney/catecholamines)

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ABSTRACT  Human erythrocytes have been found to contain appreciable amounts of DOPA decarboxylase (EC 4.1.1.26) activity. The enzyme activity in erythrocytes from patients with Parkinson's disease who were treated with DOPA was significantly lower than that of untreated patients and of normal individuals. Administration of the drug to mice led to a marked decrease of DOPA decarboxylase in liver and kidney, but not of the brain enzyme. The findings thus indicate that administration of DOPA leads to a decrease in peripheral DOPA decarboxylase, an effect that is expected to be of benefit in DOPA therapy of patients with Parkinson's disease. Peripheral DOPA decarboxylase concentration also decreases in mice after short periods of fasting; the findings suggest that the peripheral enzyme activities may be affected by various nutritional and perhaps hormonal influences, which may be partially responsible for the observed fluctuations in the motor abilities of Parkinsonian patients receiving constant doses of the drug. Study of DOPA decarboxylase activity in erythrocytes may be useful in following changes in patients receiving DOPA therapy and may also be of general interest and value in investigations of catecholamine metabolism in man.

The medically-significant finding that the administration of L-DOPA results in striking improvement in the condition of many patients with Parkinson's disease developed from basic biochemical research that revealed, in part, that the DOPA produced by hydroxylation of tyrosine is a precursor of dopamine and other catecholamines (1). There is substantial evidence that dopamine is an inhibitory neurotransmitter in the nigrostriatal pathway, and that there is a deficiency of dopamine in the striatum of patients with Parkinson's disease. Although the mode of action of DOPA in these patients is not yet fully explained, the weight of evidence favors the view that the therapeutic effect of the amino acid is closely associated with its conversion to dopamine, which is catalyzed by brain DOPA decarboxylase. Although DOPA readily crosses the blood-brain barrier (2), dopamine and other catecholamines do not readily penetrate the barrier (3). DOPA decarboxylase (3,4-dihydroxy-L-phenylalanine carboxylase; EC 4.1.1.26) is widely distributed in animal tissues (4), and has been found in human liver, kidney, heart, lung, and brain (5, and manuscript in preparation). Relatively large oral doses of DOPA (1-10 g/day) are required for the effective therapy of Parkinson's disease. Presumably, most of the administered amino acid is decarboxylated peripherally, while only a small fraction enters the brain and undergoes decarboxylation to yield striatal dopamine. It thus seems probable that the rate and extent of decarboxylation of DOPA by peripheral tissues is of considerable significance in relation to therapy with this amino acid. In the present work, we have studied the effect of DOPA administration on the DOPA decarboxylase activity of several tissues in the mouse. We have found, as documented here, that administration of the amino acid leads to decreased liver and kidney DOPA decarboxylase activity, but does not affect brain DOPA decarboxylase significantly. In an extension of this work to man, we have discovered that human erythrocytes exhibit substantial amounts of DOPA decarboxylase activity. In analogy with the results of the studies on mouse tissues, we found that the DOPA decarboxylase in erythrocytes is markedly decreased in Parkinsonian patients receiving therapy with DOPA.

EXPERIMENTAL

Treatment of mice with DOPA

Male mice (strain AH/J; 4 weeks old), average weight, 19 ± 2 g were used. L-DOPA (1.5 mg/g of body weight) was administered subcutaneously as a suspension in 0.9% sodium chloride containing 0.1% Tween-80 daily for 7 or 15 days. The effect of fasting was observed by withdrawing food (but not water) for 15 or 24 hr. The mice were decapitated and their tissues were quickly removed, chilled to 0 °C, and then homogenized with 5 volumes (w/v) of cold 0.05 M potassium phosphate buffer (pH 7.2) containing 1 mM EDTA, 10 μM pyridoxal 5'-phosphate, and 5 mM 2-mercaptoethanol (referred to below as “phosphate buffer”) in a Potter-Elvehjem homogenizer equipped with a Teflon pestle.

Determination of the DOPA decarboxylase of human erythrocytes

Freshly obtained peripheral venous blood (10 ml) was centrifuged at 8000 × g for 15 min at 4°C. The packed cells were suspended in phosphate buffer and the volume was brought to 10 ml. Preliminary studies showed that little or no decarboxylation occurred with plasma or with plasma that contained platelets and leukocytes. Fresh erythrocyte suspensions gave less than one-third of the activity found with frozen and thawed or sonicated cell suspensions, indicating that enzyme activity is intracellular and is released by cell lysis. Therefore, the cell suspensions were assayed after sonication (for 3 min) with a Bronson ultrasonic sonifier.
Determination of DOPA decarboxylase activity

The assays were performed in standard Warburg flasks. The side-arm contained 0.2 ml of 2 N HSO₄, and the center well was supplied with 0.1 ml of 1 N KOH. The main compartment contained either 0.05 ml of tissue homogenate and 0.55 ml of phosphate buffer, or 0.9 ml of sonicated human erythrocyte suspension. The reactions were initiated by the addition of 0.1 ml of 0.01 M L-DOPA containing [1-¹⁴C]DL-DOPA (total radioactivity, 2 x 10⁶ cpm). ([¹⁴C]DL-DOPA was obtained from CalBiochem.) The flask was stoppered and incubated at 37°C for 15 min. The reaction was terminated by tipping the H₂SO₄ flask and the flask was then placed on a reciprocating shaker for 90 min. The KOH solution containing the trapped ¹⁴CO₂ was then mixed with 10 ml of liquid scintillation medium and the radioactivity was determined in a liquid scintillation counter. Controls containing heat-inactivated sonicates and extracts were performed.

A unit of enzyme activity is defined as the amount of enzyme that catalyzes the decarboxylation of 1 nmol of L-DOPA in 15 min under the conditions described above. Activity is expressed as units/g of tissue or as units/100 ml of fresh blood. The protein concentration of the erythrocyte sonicates was determined by the method of Lowry et al. (6); the values for the various blood samples analyzed were very similar. None of the patients studied exhibited evidence of anemia.

RESULTS

As indicated in Table 1, liver and kidney exhibit considerably more DOPA decarboxylase activity than does brain. Administration of the amino acid led to a decrease of 40-45% of DOPA decarboxylase activity in the liver and kidney, while the activity of the brain was much less affected. Withdrawal of DOPA (Expt. 4) led to restoration of control levels of decarboxylase. That the observed decrease in DOPA decarboxylase activity was only apparent, and was due to dilution of the [¹⁴C]DOPA used in the assay by the administered amino acid, was excluded by studies in which the tissue homogenates were dialyzed against phosphate buffer for 18 hr at 4°C before assay; no change in the relative enzymatic activities of the tissues of controls and treated animals was observed.

It is notable that short periods of fasting also led to substantial decreases in DOPA decarboxylase in the liver and kidney, but not in the brain (Expts. 5 and 6). The mice that were treated with DOPA were observed to consume normal amounts of food and to gain weight normally during the experimental period. It is thus unlikely that the effects of DOPA and of fasting on liver and kidney decarboxylase are mediated by the same mechanism.

To gain some insight into the metabolic fate of DOPA in patients being treated with this amino acid, we investigated the possibility that human erythrocytes might contain DOPA decarboxylase. It was found that this enzyme is indeed present in human erythrocytes; thus, studies on eight apparently normal individuals revealed an average DOPA decarboxylase activity of 1420 ± 70 units/100 ml. As noted in Table 2, very similar values were found in the erythrocytes of four patients with Parkinson's disease, who had never been treated with DOPA. In contrast, the activities of erythrocytes from patients with Parkinson's disease who had taken DOPA for 1-2 years were significantly lower than both the patient group and the group of normal individuals. The possibility that the low activities found for the treated patients were a consequence of dilution of [¹⁴C]DOPA by administered unlabeled DOPA was excluded by assays performed after dialysis, as described above for the mouse-tissue experiments.

DISCUSSION

The present studies indicate that administration of DOPA leads to a decrease in peripheral DOPA decarboxylase, an effect that would be expected to be beneficial in the treatment of Parkinson's disease. Thus, it has been reported that the administration of inhibitors of peripheral DOPA decarboxylase potentiates the desirable therapeutic effects of the amino acid (7). The mechanism by which administered DOPA produces a decrease in DOPA decarboxylase requires

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**Table 1. Effect of DOPA administration and of fasting on the DOPA decarboxylase activity of mouse tissues**

<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Treatment†</th>
<th>No. of animals</th>
<th>Enzyme activity, units*/g ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None (controls)</td>
<td>8</td>
<td>Brain: 1,300 ± 240, 10,600 ± 1,830, 7,710 ± 1,560</td>
</tr>
<tr>
<td>2</td>
<td>DOPA (7 days)</td>
<td>6</td>
<td>Liver: 1,020 ± 30, 6,310 ± 770, 4,980 ± 550</td>
</tr>
<tr>
<td>3</td>
<td>DOPA (15 days)</td>
<td>4</td>
<td>Kidney: 1,050 ± 110, 6,090 ± 720, 4,860 ± 290</td>
</tr>
<tr>
<td>4</td>
<td>DOPA (7 days); then no treatment (3 days)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Fasted (15 hr)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Fasted (24 hr)</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* Nanomoles of CO₂ produced in 15 min at 37°C (see text).
† Details are given in the text.

**Table 2. DOPA decarboxylase activity in human erythrocytes**

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Age of patients (years ± SE)</th>
<th>Enzyme activity (units*/100 ml blood ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>8</td>
<td>63 ± 3</td>
</tr>
<tr>
<td>Patients with Parkinson's disease not treated with DOPA</td>
<td>4</td>
<td>1240 ± 100</td>
</tr>
<tr>
<td>Patients with Parkinson's disease receiving DOPA therapy</td>
<td>10</td>
<td>800 ± 50</td>
</tr>
</tbody>
</table>

* Nanomoles of CO₂ produced in 15 min at 37°C (see text).
additional study. DOPA is known to form a tetrahydroisoquinoline derivative with pyridoxal 5'-phosphate, and the formation of this complex has been found to inhibit DOPA decarboxylase (8). It will be of interest to learn whether the inhibition observed in the present studies is due to such complex formation. However, other types of inhibition must also be considered, and it is also possible that the synthesis of DOPA decarboxylase is repressed by DOPA or one or more of its metabolites. If the lowered enzyme activity of erythrocytes from Parkinsonian patients treated with DOPA is associated with decreased cellular content of the enzyme, it would seem that repression of enzyme synthesis must have taken place during the developmental stages of the cells, since the mature erythrocyte cannot synthesize protein. It might, therefore, be expected that erythrocyte DOPA decarboxylase activity in patients beginning DOPA therapy would diminish at a rate related to that at which the erythrocyte population turns over. This possibility, which is capable of experimental test, is currently being examined.

The susceptibility of liver and kidney DOPA decarboxylase (in contrast to the brain enzyme) to depression by DOPA and by brief periods of fasting suggests that the peripheral DOPA decarboxylases may be subject to alteration by various nutritional and hormonal influences. Such influences may be of significance in relation to the effectiveness of treatment of Parkinson’s disease by large oral doses of DOPA, since the amount of dopamine formed in the brain is apparently affected by peripheral utilization of the amino acid. Treatment of Parkinsonian patients with DOPA is sometimes complicated by fluctuations in motor abilities that are unrelated to the dosage of the amino acid. Changes in peripheral DOPA decarboxylase activities might be responsible in part for such effects.

To our knowledge, DOPA decarboxylase has not previously been demonstrated in human erythrocytes. Although dopamine has been found in erythrocytes (9), the functions of these and other catecholamines in erythrocytes remain to be elucidated. The presence of DOPA decarboxylase in erythrocytes also raises the question as to whether the erythrocyte enzyme may play a significant role in the normal metabolism of DOPA. The ready accessibility of human blood seems to offer an approach that may well be of practical value in following therapy with DOPA and may also be of general interest and significance in studies of catecholamine metabolism in man.

**Ed. Note:** Similar findings for the liver and brain enzymes are independently reported in this issue [Proc. Nat. Acad. Sci. USA, 68, 2117–2120 (1971)] by Dairman et al.

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