SUMMARY: Pyruvate dehydrogenase (PDH) activity was measured in platelets from 10 patients with Friedreich’s ataxia, and 10 age-matched healthy control subjects. Both total PDH and active PDH activity were measured. There were no significant differences between the two groups.

INTRODUCTION

Pyruvate dehydrogenase (PDH) is a multienzyme system that can be purified and separated into at least three separate components (Reed, 1974). The three enzymes comprising the PDH are: pyruvate dehydrogenase (E₁) (E.C.4.1.1.1.), a thiamine pyrophosphate (TPP) dependent enzyme, dihydrolipoyl transacetylase (E₂) (E.C.2.3.12) and dihydrolipoyl dehydrogenase (E₃) (E.C.1.6.4.3) also called lipoamide dehydrogenase (LAD) employing flavin-adenine-dinucleotide (FAD) as co-factor and under the control of the nicotinamide-adenine-dinucleotide (NAD-NADH) reaction. They exist in a molecular ratio of 12:1:6 (Linn et al. 1972). E₁ component in PDH complex appears to be present in excess, whereas either E₂ or E₃ are the apparent limiting components of the PDH system (Farrell et al. 1974). The PDH complex in vivo exists in an active and inactive form (Wieland et al. 1971). The conversion from the active to the inactive form is catalyzed by the kinase, pyruvate decarboxylase: ATP transphosphorylase, with the conversion from the inactive to the active form being catalyzed by a magnesium dependent phosphatase: pyruvate decarboxylase phosphate phosphatase.

PDH is situated at the crossroads of several metabolic routes: the generation of ATP through acetyl-coenzyme A (acetyl-CoA) and the Krebs cycle; carboxylation to form oxaloacetate and the starting point of gluconeogenesis; through acetyl-CoA the biosynthesis of fatty acids, prostaglandins, steroid hormones and acetylcholine. Finally, pyruvate can supply the carbon skeleton for de novo synthesis of non essential amino acids. Many hormonal, ionic and metabolic factors regulate PDH complex at various sites.

Severe impairment of this enzyme is joined with serious clinical features and death (Farrell et al. 1974) whereas patients with partial deficiencies of PDH present cerebellar ataxia as the most prominent and sometimes the only neurologic abnormality (Blass et al. 1971; Oka et al. 1976; Falk et al. 1976; Wick et al. 1977; Blass et al. 1976). Reynolds et al. (1976) demonstrated that the reduction of the activity of PDH to less than 30 percent of normal impaired pyruvate oxidation in the brain and that deficiencies too mild to impair oxidation in several other parts of the brain could do so in the anterior cerebellar vermis.

Decreased PDH activity (40 percent of normal) due to impairment of E₃ (Kark et al. 1977) has been described by Blass et al. (1976) in various tissues from patients affected by Friedreich’s ataxia.

Our previous study (Filla et al. 1978) on platelets from patients affected by Friedreich’s ataxia showed only a slight and not significant decrease of PDH (16%) and E₃ (15%). These results suggested that PDH impairment was not the primary genetic deficiency in that disease, but only a regulatory defect. In order to further investigate the nature of this impairment we studied the activation of PDH in platelets.

SUBJECTS, MATERIALS AND METHODS

Subjects

PDH activity in platelets was estimated in 10 patients with typical Friedreich’s ataxia (Group Ia, according to the clinical criteria of Geoffroy
et al. 1976) from different families and 10 age-matched healthy controls. In each experiment samples from one patient and the control were run together.

**Materials**

Sodium (1-C¹⁴) pyruvate (4.8 Ci/mol) was purchased from New England Nuclear Corp. Glycerol, nicotinamide, NAD (grade III yeast), thiamine pyrophosphate chloride, hyamine hydroxide and bovine serum albumine were obtained from Sigma Chemical Corp. Coenzyme A was bought from P-L Biochemicals Inc. Toluene "scintanalyzed grade", methanol "spectraanalyzed" were from Fisher Scientific Co. and liquifluor from New England Nuclear. All other reagents were of analytical grade.

**Methods**

PDH assay was performed using modifications of the method described by Blass et al. (1977). Platelets were isolated as described previously (Filla et al. 1978). To the final pellet was added 1.2 ml of glycerol buffer mixture (2 parts of glycerol and 3 parts of 0.0324 M potassium phosphate buffer, pH 7.4, containing 30 mM nicotinamide, 7 mM Na₂SO₄, 6 mM DL-dithriothreitol). Then the cells were disrupted by homogenization in an ice bath. Protein detection was done according to the Lowry's technique (1951). In 15 x 50 mm plastic tubes, in ice, were mixed 200 μl of potassium phosphate buffer (pH 7.4) for active PDH (PDHₐ) detection, 200 μl of homogenate and 10 μl of 7.5 mM phosphate buffer for total PDH (PDHₜ) detection. The assays were done in triplicate. Blanks were obtained by adding 0.1 ml of ion H₂SO₄ to the reaction mixture. The samples were incubated for 60 minutes at 30°C, before adding 10 μl of a solution containing CoA, NAD, TPP (2 mM each) in 0.0324 M potassium phosphate buffer, pH 7.4, and 20 μl of a solution of 1.8 x 10⁴ cpm sodium (1-C¹⁴) pyruvate in the same buffer. Then the samples were incubated again for 30 min at 37°C and CO₂ was collected after acidification of the mixture and counted as described previously (Filla et al. 1978).

**RESULTS AND DISCUSSION**

The values of PDHₐ and PDHₜ in platelets are shown in Table I. Twenty percent of PDH was usually in the active form. Diet did not appear to alter this ratio thus confirming previous results (Blass et al. 1979). No significant difference was found between patients and controls with Student's paired t test, although the ataxic patients showed slightly lower values of PDHₜ and PDHₐ (respectively 10% and 15% less than controls).

The present results confirm our previous report (Filla et al., 1978) that PDH activity in platelets from patients affected with Friedreich's ataxia is normal. The patients selected for this study were different from the initial group and responded to all the criteria of typical Friedreich's ataxia listed by Geoffroy et al. (1976). We now add that the percentage of activated PDH from the total PDH measured is not different from normal in these patients. This overall situation confirms the results of Stumpf and Parks (1978), but not those of Kark et al. (1980). However, we have not measured the Michaelis-Menten constants for this enzyme. The non significant decrease in PDH activity is probably the result of a regulatory deficit elsewhere in the system.

**TABLE I**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>PDH Active</th>
<th>PDH Total</th>
<th>% Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>10</td>
<td>43.46 ± 7.79</td>
<td>200.57 ± 19.86</td>
<td>21.67</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>50.21 ± 7.93</td>
<td>220.93 ± 27.27</td>
<td>22.73</td>
</tr>
</tbody>
</table>

PDH activity, mean ± S.E., is expressed in pmol/min/mg protein.

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**REFERENCES**


Oka, Y., Matjuda, I., Arashima, S., Anakura, M., Mituyama, T. and Nagamatsu, I. (1976): Citrate treatment...


