QUEBEC COOPERATIVE STUDY
OF FRIEDREICH'S ATAXIA

Cardiac Malic Enzyme in Friedreich's Disease

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ABSTRACT: We measured the activity of cytosolic and of mitochondrial malic enzyme in the hearts from 4 patients with Friedreich's disease and from two non-ataxic control subjects. There was a wide variability in the results and the slight overall decreases in both enzyme activities were not considered to be statistically significant. From these and other results, we conclude that deficient mitochondrial malic enzyme activity is not a constant or primary feature of Friedreich's disease.

RÉSUMÉ: Nous avons mesuré l'activité des enzymes maliques cytosoliques et mitochondriales dans le coeur de quatre patients souffrant de la maladie de Friedreich et celui de deux témoins non-ataxiques. Quoiqu'il y ait une grande variabilité dans les résultats, la diminution générale d'activité des deux enzymes n'est pas statistiquement significative. Nous concluons qu'un déficit en enzyme malique mitochondriale n'est pas un défaut constant ou primaire dans la maladie de Friedreich.

Can. J. Neurol. Sci. 1984; 11:643-645

Previous studies from this group and others have concluded that many of the symptoms of Friedreich's disease are secondary to a deficit in the mitochondrial control of energy production (Barbeau, 1980, 1982). Defects have been postulated at the level of the pyruvate dehydrogenase complex, of lipoamide dehydrogenase, of pyruvate carboxylase, and of mitochondrial membranes. Despite much work, none of these modifications can be considered primary to the disease. It was therefore of great interest when Stumpf et al. (1981, 1982) reported to have found severely depressed activity of mitochondrial malic enzyme (L-malate:$NADP^+$ oxidoreductase, decarboxylating; E.C.1.1.1.40) in cultured fibroblasts from 10 patients with Friedreich's disease. This defect was also claimed to be partially present in obligate heterozygotes (Stumpf et al., 1983) and to explain the cardiomyopathy of Friedreich (Stumpf, 1982). In Friedreich muscle, activity of the mitochondrial $NADP^+$-linked enzyme was also found to be significantly decreased in another study from Italy (Bottachi and Di Donato, 1983).

The pathology of Friedreich's disease involves the dorsal root ganglia (Lamarche et al., 1982), the posterior columns (Hughes et al., 1968), the cerebellar vermis and brain stem (Oppenheimer, 1979), and the heart (Lamarche et al., 1980). If a mitochondrial malic enzyme deficiency is the cause of the disease, it would be reasonable to expect expression of the defect in the main target tissues. We report the results of our exploration of this aspect of the problem in the heart.

SUBJECTS AND METHODS

Hearts were obtained at autopsy (and kept frozen) from 3 patients (2 males, 1 female) with the typical signs and symptoms

of Friedreich's disease as defined by Geoffroy et al. (1976) and from one patient with the slow progression form of the disease (female) (Barbeau et al., 1984). The pathology and histories of the cases were previously described (Lamarche et al., 1980). The brains from two of these patients were studied independently by Stumpf and his collaborators. Ages at death ranged from 18 to 38 and duration of the ataxic illness from 11 to 31 years. A severe symmetrical hypertrophic cardiomyopathy was present in all cases (Lamarche et al., 1980). Control hearts were obtained from the pathology department of the Hôtel-Dieu Hospital in Montreal. One was from a man age 55 who died from intestinal neoplasia and the other from a man age 39 who died accidentally. These hearts were normal to gross pathology. Delay between death and autopsy did not exceed 14 hours in all cases.

Malic enzyme activity was estimated in cytosol and mitochondrial fractions from the hearts according to the protocole recommended by Bottachi and Di Donato (1983) as modified by Melançon et al. (1984) in this issue (for details see that paper).

RESULTS

As can be seen in Table 1, "total" malic enzyme activity is slightly (- 33%) lower in the ataxic group. This is due equally to a decrease in mitochondrial (- 25%) and cytosolic activity (-34%). There is a wide variability in the results which makes them non significant statistically. However, when ataxic values are compared to their respective mean control values for mitochondrial activity, two Friedreich cases have higher activities (+ 24%, + 45%) and two, lower activities (- 83%, - 52%). Only one of the latter is within the range of the deficit reported by Stumpf and his collaborators (1982) i.e. circa 90% decrease.

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Table 1: Cardiac Malic Enzyme Activity in Ataxia

		CONTROL			ATAXIC	
PAIRS	Cytosol	Mitoch.	Total	Cytosol	Mitoch.	Total
$C_1 \times A_1$	3.13	0.81	3.94	2.19	0.19	2.38
$C_1 \times A_2$	2.04	1.48	3.52	2.67	0.54	3.21
$C_2 \times A_3$	3.82	0.71	4.53	1.96	0.72	2.68
$C_2 \times A_4$	3.92	0.46	4.38	1.70	1.05	2.75

nM/min/mg Prot.

C: CONTROL;   A: ATAXIC

Figure 2 — Position of malic enzyme in the general scheme of mitochondrial and cytosolic metabolisms.
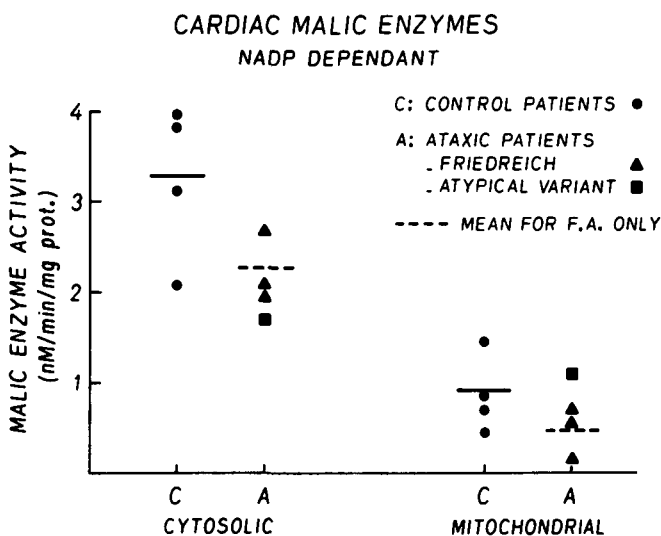

Figure 1 — Cardiac malic enzyme activity in 4 cases of Friedreich's disease (including one slightly atypical) and 2 non-ataxic controls. The activities are expressed in nM/min/mg protein.

These results are better illustrated in Fig. 1 , where the wide variability is apparent. One clear pattern emerges from our results: an inverse relationship between cytosolic and mitochondrial values. The lower the cytosolic activity of malic enzyme, the higher the mitochondrial activity. This suggests an exchange between compartments and perhaps a defective mitochondrial membrane barrier, as proposed by Barbeau (1980).

DISCUSSION

Malic enzyme (Fig. 2) is an inducible enzyme whose function is still not clearly understood. It has been known to exist in mammalian tissue in at least two forms: soluble (cytosolic) and mitochondrial. In the human, the cytosolic enzyme is found in all tissues except red cells and serum. It is even present in fibroblasts. The mitochondrial enzyme is seen in brain, kidney, heart muscle, liver and cultured fibroblasts. Like the cytosolic enzyme, mitochondrial malic enzyme is a tetramer. It can show marked polymorphism in human tissue. Absolute activity of the various forms of malic enzyme vary greatly from tissue to tissue: in adrenal medulla and white adipose tissue malic enzyme is located exclusively within the extra-mitochondrial compart-

ment. In liver, 95% of the activity is extra-mitochondrial and only 5% intra-mitochondrial. In heart, 30% of the enzyme activity is usually extra-mitochondrial and 70% intra-mitochondrial (Brdiczka and Pette, 1971).

Although there is a considerable variability in our present results, it is obvious that a major defect in mitochondrial malic enzyme activity in this target tissue is not a constant feature of our cases, even when differences in age with the controls are taken into account. Only one of the hearts had markedly decreased activity of the mitochondrial malic enzyme in the range reported by Stumpf et al. (1982). There are no distinguishing clinical features between the 4 cases that we can detect, except a longer duration of the illness in the atypical variant. All are clearly "classical" cases of Friedreich.

It is recognized that the available number of studied ataxic hearts is low, but it is unlikely that added cases would markedly alter the findings. Our results in heart tissue are supported by the findings of Stumpf (personal communication) in the brain of two of these patients: only one had low activity of the mitochondrial enzyme, while the other had normal activity.

When the present findings are taken in conjunction with our results in cultured fibroblasts (see Melançon et al., this issue) which were essentially normal, one must conclude that, unless unsuspected methodological differences were present, mitochondrial malic enzyme deficiency is not a constant marker of Friedreich's disease as we define it (Geoffroy et al., 1976). The variability of results would tend to favour a manifestation secondary to the primary defect, perhaps as a result of a membrane defect (Barbeau, 1980). This would explain why it is possible, in some cases, to find very low activity of the enzyme, and in others essentially normal values. We have no good reasons to propose to explain the implied discrepancies with the findings of Stumpf et al. (1982, 1983). Our conclusions of a probable secondary defect are nevertheless closer to those of Bottachi and Di Donato (1983).

In view of the proposed role of polyunsaturated fatty acids in the pathophysiology of Friedreich's symptoms (Barbeau, 1980, 1982), it may be pertinent to investigate the diet and therapy of the patients during the months preceeding death before determining malic enzyme activity. In this respect, two of our ataxic patients had been taking Lecithin (6 to 12 grams daily) for some months (see Barbeau, 1978; Melançon et al., 1982). Their

mitochondrial malic enzyme activity in the heart was $+24\%$ and -52% of their respective controls.

Finally another facet of the problem could be considered. It is known that the mitochondrial malic enzyme, but not the cytosolic enzyme, is activated by succinate and aspartate (Frenkel and CoboFrenkel, 1973). The activity of this enzyme may thus increase rapidly under conditions which would favour accumulation of intermediates of the Krebs cycle. Conversely the activity of mitochondrial malic enzyme decreases in the face of a deficiency in Krebs cycle intermediates. In this respect it is worthwhile to remember that the concentrations of aspartic acid were found to be decreased in the brain of Friedreich patients (Huxtable et al., 1979).

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