

J.P.BLASS,¹ K.-F.R.SHEU,¹ A.J.L.COOPER,² E.H.JUNG¹
and G.E.GIBSON¹

¹Altshul Laboratory for Dementia Research, Cornell University Medical College, Burke Medical Research Institute, 785 Mamaroneck Avenue, White Plains, NY 10605 (USA)

²Department of Biochemistry, Cornell University Medical College, 1300 York Avenue, New York, NY 10021 (USA)

I. INTRODUCTION

Two sets of observations prompted a systematic study of thiamin dependent processes in brain from patients with dementia of the Alzheimer type (DAT). The first was the dramatic reduction of the activity of one of the thiamin pyrophosphate (TPP) dependent enzymes, namely the pyruvate dehydrogenase complex (PDHC), in DAT brain obtained at autopsy [1-3]. The second was the loss of cholinergic cells of the rostral part of the reticular activating formation in the brains of patients with a thiamin-deficiency syndrome, namely the Wernicke-Korsakoff syndrome [4]. Wernicke-Korsakoff syndrome and DAT [5] are clearly distinguishable clinically, neuropsychologically, and pathologically. On the other hand, there are areas of clinical, neuropsychological, and neuropathological overlap [5]. The recognition of these overlaps led to a systematic examination of TPP-dependent enzymes in DAT [5,6].

II. THIAMIN AND TPP-DEPENDENT ENZYMES IN DAT TISSUES

Three TPP-dependent enzymes have been studied in DAT brain and non-neural tissues: the cytoplasmic enzyme transketolase (TK) and two mitochondrial multi-enzyme dehydrogenase complexes, PDHC and the α -ketoglutarate dehydrogenase complex (KGDHC). No studies of the branched chain ketoacid dehydrogenase complex in DAT tissues have been done.

1. **Transketolase** TK activity is reduced in DAT brain obtained at autopsy [6], compared to appropriate non-DAT controls (Table 1). In the three DAT brains examined which exhibited <50% of normal TK activity, immunostaining of the enzymatically active 69 kDa species was also grossly diminished. Antibody-activity titration analysis [6] indicated that the TK in the affected DAT brain is functionally less competent when compared to TK in non-DAT control brain. Conceivably, however, the reduction of TK activity and of immunoreactive 69 kDa TK protein in DAT brain might be a nonspecific result of brain damage, which is now recognized to involve not only the classic areas of "selective vulnerability" but at the molecular level much of the rest of the brain as well [7]. Therefore, TK has also been examined in histologically normal non-neural DAT tissues. In erythrocytes, TK activity and behavior on immunoblots after SDS-PAGE was comparable to controls, but heat inactivation studies suggested the presence of a subtle structural variation in the TK protein [6]. In cultured skin fibroblasts, TK activity in the DAT cells was slightly but statistically significantly lower than in non-DAT controls matched for chronological age of donor, passage and sex (Table 2).

An unresolved issue is the molecular nature of the multiple forms of TK that can be resolved by isoelectric focusing. Studies by Paoletti, Sorbi and coworkers in Florence indicated the presence of characteristic high pI TK variants in cultured fibroblasts from 70%

Table 1: Activity of TPP-Dependent Enzymes in DAT and Non-DAT Autopsy Brain

	KGDHC	PDHC	TK
<i>Frontal Cortex</i>			
Non-DAT	2.25 $\pm$ 0.49(9)	10.6 $\pm$ 0.80(7)	3.83 $\pm$ 0.47(8)
DAT	0.29 $\pm$ 0.44(8)	2.80 $\pm$ 1.20(7)	1.63 $\pm$ 0.33(9)
<i>Occipital Cortex</i>			
Non-DAT	1.42 $\pm$ 0.52(7)	10.7 $\pm$ 1.8(7)	2.28 $\pm$ 0.25(7)
DAT	0.11 $\pm$ 0.07(6)	2.9 $\pm$ 1.2(7)	1.14 $\pm$ 0.43(6)
<i>Caudate Nucleus</i>			
Non-DAT	1.28 $\pm$ 0.73(5)	-	1.55 $\pm$ 0.52(5)
DAT	0.33 $\pm$ 0.26(3)	-	0.72 $\pm$ 0.25(5)

Values represent nmol/min/mg protein, mean $\pm$ SEM. The difference between DAT and Non-DAT was statistically significant for all differences shown ($p < 0.05$ - $p < 0.005$). For original data, and details of experiments, see references 2,5,6.

of the DAT patients they studied [8]. Disruption of the cells in the presence of the protease inhibitor phenylmethylsulfonyl fluoride reduced the immunostaining for several high pI species. This result is consistent either with aberrant protease activity in the DAT cell extracts or with an aberration in the TK protein substrate of the protease. Our studies have confirmed the observations of Paoletti et al, although the discrimination between DAT and non-DAT by isoelectric focusing pattern was not clear cut in the relatively small number of patients studied as yet. Molecular cloning of TK is in progress, in order to compare the DAT and non-DAT gene products and genes.

2. PDHC Reduction of PDHC activity in DAT brain was first reported by workers in Newcastle in 1980 [1] and has been repeatedly confirmed [2,3,9]. The reduction is greater than 70% (Table 1). Immunochemical studies are consistent with the presence of a reduced amount of normal PDHC proteins [2]. PDHC appears to be relatively concentrated in the cholinergic neurons which are characteristically lost in DAT [10]. The reduction in PDHC in DAT brain may therefore be primarily the result of the brain damage [11]. In accord with this interpretation, the activity of PDHC appears to be normal in cultured DAT fibroblasts (Table 2). In platelets from 17 patients with DAT, PDHC activity averaged $105 \pm 9\%$ of the activity for simultaneously studied controls. These observations, however, do not exclude the possibility of subtle alterations of the PDHC proteins, including the regulatory PDH kinase and phosphatase.

The TPP-binding E1p component of PDHC has been cloned [12,13]. In humans, the gene for the TPP-binding α E1p component is located on the X chromosome, apparently in the pseudo-autosomal region [13]. Comparisons of the E1p genes in DAT and non-DAT have not yet been done.

3. KGDHC The activity of KGDHC is dramatically reduced in DAT brain (Table 1), by more than 80% in every region examined [5]. Although TK and PDHC appear to be remarkably stable agonally and post mortem, KGDHC is not. The KGDHC measurements were done on samples for which DAT and non-DAT were matched as closely as possible for agonal state and post-mortem time, as well as for age and sex.

KGDHC activity is also reduced in DAT fibroblasts (Table 2). Results were consistent for patients from each of 4 families with DAT, including the large Canadian-American kindred. In that kindred, fibroblasts from 3 affected subjects showed KGDHC activities comparable to other DAT patients, while cultures from the 3 escapees studied had

Table 2: Activity of TPP-Dependent Enzymes in DAT Cultured Fibroblasts

	KGDHC	PDHC	TK
Non-DAT	2.61 $\pm$ 0.14(8)	0.87 $\pm$ 0.29(8)	14.4 $\pm$ 0.8
DAT	1.47 $\pm$ 0.10(7)**	1.19 $\pm$ 0.24(6)	12.2 $\pm$ 0.6*

Values are nmol/min/mg protein, mean $\pm$ SEM. **, $p < 0.005$; *, $p < 0.05$, by 2-tailed t-test. See reference 5 for original data on TK.

activities comparable to non-DAT controls. These results were obtained with cells grown under meticulously controlled conditions [14], in which variability was lower than in previous studies [5].

Preliminary studies suggest the presence of immunochemical differences of the TPP-binding E1k component of KGDHC between DAT and non-DAT cultured fibroblasts, on immunoblots after SDS-PAGE. The E2k component has not been studied in detail for lack of suitable antibodies. The E3k component appeared normal. Further immunoblotting studies are in progress, including examination of the effects of different protease inhibitors on the patterns. Molecular cloning of E1k and E2k is in progress, preparative for the test for a genetic basis for the KGDHC abnormality in DAT. E3 has been cloned by others [15].

4. Thiamin and Derivatives Butterworth and coworkers [9] recently reported in an abstract the existence of a deficiency of TPP in DAT brain as well. Previous studies documented normal levels of total thiamine in DAT blood [16]. These data raise the possibility of an aberration in DAT in the transport of thiamin or in its pyrophosphorylation to TPP. Complete definition of the role of thiamin in DAT will require study of these processes as well.

III. PATHOGENESIS

An impairment of oxidative metabolism or thiamin deficiency is known to lead to specific neurologic changes. It is plausible that an abnormality in TPP-dependent processes in DAT and specifically of KGDHC has a significant role in the pathogenetic mechanisms responsible for the disease. This issue has been explored extensively elsewhere [5,17,18].

IV. TREATMENT

A cross-over trial of 3 grams a day of thiamin hydrochloride in 11 patients showed a statistically but not clinically significant slowing of deterioration compared to treatment with the niacin placebo [19]. Each arm, placebo and control, was three months. Subsequently, a one year trial using a less sensitive but more robust parallel-group design in 15 patients showed no significant difference; if anything, the patients on thiamin deteriorated more rapidly [20]. Whether or not even larger doses of thiamin or treatment with lipid-soluble derivatives might be more effective is not yet known.

V. CONCLUSION

Abnormalities exist in TPP-dependent processes in DAT brain and other tissues, notably in KGDHC. The molecular basis and functional importance of these abnormalities is not yet known. Further studies of thiamin-dependent mechanisms in DAT seem justified, both scientifically and because of the possibility of adding another approach to the developing armamentarium of treatments for this devastating and common disease.

SUMMARY

Because of clinical and neuropathological overlap between the characteristics of dementia of the Alzheimer type (DAT) and of a human thiamin deficiency syndrome (Wernicke-Korsakoff syndrome), thiamin pyrophosphate (TPP) dependent processes have been studied in DAT brain and other tissues. The activities of 3 TPP-dependent enzymes are reduced in DAT brain: transketolase (TK), the pyruvate dehydrogenase complex (PDHC), and the α -ketoglutarate dehydrogenase complex (KGDHC). Quantitatively, the most marked reductions are in KGDHC (to less than 20% of normal). In cultured skin fibroblasts, KGDHC activity is reduced to 50-60% of normal, TK activity to 80-90% of normal, and PDHC is normal. Structural and molecular studies of the DAT and non-DAT enzymes are in process. A lesion of KGDHC may be related to the pathogenesis of DAT. Treatment with large doses of thiamin has not been beneficial, but the data are not totally negative. Further studies of thiamin-dependent mechanisms in DAT seem justified.

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