Increased phosphoglycerate kinase in the brains of patients with Down’s syndrome but not with Alzheimer’s disease

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ABSTRACT

Impaired glucose metabolism in Down’s syndrome (DS) has been well-documented in vivo, although information on the underlying biochemical defect is limited and no biochemical studies on glucose handling enzymes have been carried out in the brain. Through gene hunting in fetal DS brain we found an overexpressed sequence homologous to the phosphoglycerate kinase (PGK) gene. This finding was studied further by investigating the activity levels of this key enzyme of carbohydrate metabolism in the brains of patients with DS. PGK activity was determined in five brain regions of nine patients with DS, nine patients with Alzheimer’s disease and 14 controls. PGK activity was significantly elevated in the frontal, occipital and temporal lobe and in the cerebellum of patients with DS. PGK activity in corresponding brain regions of patients with Alzheimer’s disease was comparable with controls. We conclude that our findings complement previously published data on impaired brain glucose metabolism in DS evaluated by positron emission tomography in clinical studies. Furthermore, we show that in DS, impaired glucose metabolism, represented by increased PGK activity, is a specific finding rather than a secondary phenomenon simply due to neurodegeneration or atrophy. These observations are also supported by data from subtractive hybridization, showing overexpressed PGK in DS brains at the transcriptional level early in life.

INTRODUCTION

Down’s syndrome (DS) is the most common genetic cause of dementia and although the trisomic state can be directly associated with the phenotype, the underlying mechanisms are far from being understood. Nearly all subjects with DS over the age of 40 years show neuropathological and neurochemical abnormalities on post-mortem brain examination that are indistinguishable from those seen in Alzheimer’s disease (AD) [1,2]. A series of impaired metabolic functions in DS has been reported [3] including deteriorated glucose metabolism. Specifically, as early as 1971 Hsia et al. [4] reported increased glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in DS; neither enzyme is encoded on chromosome 21.

Anneren et al. [5] have shown that phosphofructokinase (PFK, liver type) activity is elevated in fibroblasts

Key words: Alzheimer’s disease, brain, Down’s syndrome, glucose metabolism, phosphoglycerate kinase, subtractive hybridization.

Abbreviations: AD, Alzheimer’s disease; DS, Down’s syndrome; PET, positron emission tomography; PFK, phosphofructokinase; PGK, phosphoglycerate kinase.

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aneuploid for chromosome 21. As this isoenzyme is encoded on chromosome 21q22.3 [6,7], the authors suggested a gene dose effect to explain the PFK increase. The increase of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase described by Hsia et al. could therefore be seen as compensatory/adaptive to the elevated PFK. Glucose metabolism has been evaluated in the brains of DS patients in vivo using positron emission tomography (PET) with $[^1{\text{H}}]$fluorodeoxyglucose, a well-established method for the measurement of regional cerebral glucose metabolism rates in humans. The DS patients showed lower than normal rates of absolute glucose metabolism in association areas of temporal and parietal neocortices [8], resembling a pattern seen in AD [9]. Dani et al. [10] showed in a longitudinal study that older subjects with DS without cognitive impairment maintain regional cerebral glucose metabolism within the normal range for many years, although repeated measurements showed a progressive decline of glucose metabolism over time and after the onset of dementia.

In a recent publication Pietrini et al. [11] demonstrated by PET that, in patients with DS at risk of AD before dementia, abnormalities in cerebral glucose metabolism appeared during stimulation in cortical regions typically affected in AD. Their data indicate that a stress paradigm can detect metabolic abnormalities in the preclinical stages of AD despite normal cerebral metabolism at rest.

By gene hunting in DS using the principle of subtractive hybridization [12], we detected a sequence with strong homology to phosphoglycerate kinase (PGK), a key enzyme of carbohydrate metabolism, which was significantly up-regulated in the temporal lobe of fetal DS brain when compared with normal fetal brain. We therefore decided to study PGK activity levels in several regions of post-mortem brains of adult patients with DS, AD and controls. The studies were performed in aged patients as no neurodegenerative control could have been recruited for fetal brain of DS and the majority of aged patients with DS show AD disease pathology in their brain [1,2] thus allowing the comparison.

**METHODS**

**Subtractive hybridization [12]**

Fetal brain samples of two fetuses with DS and two age- and sex-matched controls, all in the 23rd week of gestation, were obtained from the Brain Bank of the Institute of Psychiatry (Denmark Hill, London, U.K.). Hippocampus (gyrus parahippocampalis) was taken into liquid nitrogen and ground for the isolation of mRNA.

Isolation of mRNA was performed using the Quick Prep Micro mRNA purification kit (Pharmacia Biotech Inc., Uppsala, Sweden). One microgram of mRNA from each (of the two) preparation was quality checked by a cDNA cloning kit (Gibco, Life Technologies, Eggenstein, Germany: cat. 18248-013) using the incorporation of $[^32]{\text{P}}$dATP (Amersham, Bucks, U.K.) with subsequent electrophoresis on 1 % agarose followed by autoradiography. The Reflection film (Dupont, Germany) was exposed to the gel for a period of 2 h at room temperature.

**Construction of the subtractive library**

Ten micrograms each of mRNA from the brains of DS and control subjects were biotinylated by UV irradiation at 360 nm according to the instructions supplied in the subtractor kit (Invitrogen, Leek, Netherlands). One microgram of mRNA from each DS brain sample was subject to reverse transcription (subtractor kit, Invitrogen) and the cDNA pools were hybridized with the corresponding biotinylated mRNAs from controls.

The subtractive hybridization mixture was incubated with streptavidin according to the subtractor kit given above and thus the biotinylated molecules (non-induced biotinylated mRNAs and the hybrid biotinylated mRNAs/cDNAs) formed a complex. The streptavidin complexes were removed by repeated phenol–chloroform extraction and subtracted cDNAs were separated from the aqueous phase by alcohol precipitation (subtractor kit).

The amplification and cloning of subtracted cDNAs, and the primers and vectors used, are given in detail in a previous publication [12]. Recombinant clones were sequenced by K. Granderath, MWG-Biotech (Ebersberg, Germany). Homologies were determined by computer-assisted comparison of data from the gene bank sequence library: fastA@ebi.ac.uk (GBALL, EMBL, Heidelberg, Germany).

Subtractive hybridization was performed cross-wise, i.e. DS sample mRNA was subtracted from control mRNA and vice versa at a ratio of 1:3 (DS mRNA/control mRNA).

**Determination of PGK activity levels**

To study the PGK activity level in the brain, the temporal, frontal, occipital and parietal cortex and the cerebellum of karyotyped patients with DS ($n = 9$; three females and six males; $56.1 \pm 7.1$ years), AD ($n = 9$; six females and three males; $72.3 \pm 7.6$ years) and controls ($n = 14$; seven females and seven males; $61.6 \pm 6.6$ years) were used.

Briefly, post-mortem brain samples were obtained from the MRC London Brain Bank for Neurodegenerative Diseases (Institute of Psychiatry). In all DS brains there was evidence of abundant $\beta$-amyloid plaques and neurofibrillary tangles. The patients with AD fulfilled the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association.
Table 1  Protocol for PGK determination

<table>
<thead>
<tr>
<th></th>
<th>Blank (µl)</th>
<th>System (µl)</th>
<th>Low system (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris–HCl, 1 M at pH 8.0 cont. 5 mM EDTA</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MgCl2, 0.1 M</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>ATP, 0.02 M</td>
<td>400</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>GAPDH, 40 units/ml</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NADH, 2 mM</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Supernatant of brain tissue homogenate</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>H2O</td>
<td>190</td>
<td>90</td>
<td>185</td>
</tr>
<tr>
<td>Incubate at 37 °C for 1 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-phosphoglyceraldehyde</td>
<td>–</td>
<td>100</td>
<td>5</td>
</tr>
</tbody>
</table>

(NINCDS/ADRDA) for probable AD [13]. The histological diagnosis of AD was established and was consistent with the CERAD criteria [14] for a ‘definite’ diagnosis of AD. The controls were brains from individuals with no history of neurological or psychiatric illness. The major cause of death was bronchopneumonia in DS and AD patients and heart disease in controls. The post-mortem interval of brain dissection was 34.1 ± 13.7 h in AD patients, 30.6 ± 17.5 h in DS patients and 34.8 ± 15.0 h in controls. Tissue samples were stored at −70 °C and the freezing chain was not interrupted. DS patients, with very few exceptions, do not survive into their seventies and therefore the oldest DS population available was selected.

The PGK assay was performed according to the principle and procedure described by Beutler [15]. Brain tissue aliquots (about 10 mg each) from DS, AD and control brain regions were homogenized in a Potter homogenizer on ice in 0.1 M Tris/HCl buffer pH 8.0 [16]. The homogenate was spun down at 4000 g in a cooled centrifuge and the supernatant was used for the enzyme determinations.

The principle is that PGK catalyses the phosphorylation of ADP to ATP by 1,3-diphosphoglycerate (1,3-DPG):

\[
1,3\text{-DPG} + \text{ADP} \rightarrow \text{PGK} \rightarrow 
3\text{-phosphoglyceraldehyde} + \text{ATP}
\]

In this assay procedure, the reaction is measured in the reverse direction (from right to left).

The formation of 1,3-DPG is then measured through the glyceraldehyde phosphate dehydrogenase (GAPD) reaction:

\[
3\text{-phosphoglyceraldehyde} + \text{NAD}^+ + \text{Pi} \rightarrow 
\text{GAPD} \rightarrow \text{NADH} + \text{H}^+ + 1,3\text{-DPG}
\]

in the reverse (right to left) direction. The oxidation of NADH is measured at 340 nm according to the procedure described in Table 1.

A blank assay was carried out to be certain that the GAPD used was free of PGK activity. β-Mercaptoethanol–EDTA was substituted for brain tissue homogenate in both the blank and system mixtures and the absorbance of the brain-tissue-free system was measured against that of the blank for 45 min without preincubation. One mole of NADH is oxidized for each mole of 3-phosphoglyceraldehyde converted into 1,3-DPG which forms the basis for the calculations that were carried out as given in the reference and expressed as units/mg of protein [15].

Protein determination was carried out according to the method of Bradford [17].

Statistical calculations

For comparison of groups ANOVA with subsequent Kruskal Wallis and Mann–Whitney’s U-test was carried out. A value of \( P < 0.05 \) was considered significant.

RESULTS

Subtractive hybridization

The corresponding up-regulated sequence found by subtractive hybridization is given in Figure 1 and reveals 100% homology with PGK (human phosphoglycerate kinase, EMBL accession number AC V00572).

Enzyme determinations

Results are presented in Figure 2. The enzyme distribution in control brain showed that the highest PGK activities were in the temporal lobe > occipital > frontal lobe > parietal lobe > cerebellum. PGK activity in the DS patients was significantly higher in the frontal, temporal and occipital lobe and in the cerebellum.
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Figure 1 Alignment of the nucleic acid sequence found by subtractive hybridization (our clone 109 u) with the sequence obtained from EMBL Heidelberg (HSPGK)

When 1258 bp of our truncated sequence were aligned to the HSPGK sequence obtained from EMBL with 1348 bp, 100% homology could be revealed at the nucleic acid level. Capitals indicate the open reading frame, small letters the 3' or 5' untranslated regions.

compared with the control brain regions. PGK activity in the brain regions of patients with AD was not significantly different from controls.

DISCUSSION

Gene hunting using subtractive hybridization revealed that in fetal DS brain at the 23rd week of gestation a sequence homologous to PGK was overexpressed by about threefold. This intriguing finding, which may reflect impaired glucose metabolism in DS at a very early stage, prompted us to investigate PGK activity in five individual brain regions of aged DS patients with trisomy 21.

PGK activity levels were clearly elevated in four of five brain regions examined in patients with DS (Figure 2), which should be seen in context with deteriorated brain glucose metabolism in patients with DS as described above.

In order to show whether impaired glucose metabolism is a hallmark of DS per se or simply reflects metabolic deterioration or the general mechanism of neurodegenerative disease, we have included PGK activity determinations in the corresponding regions of brain specimen with AD, showing neuropathological changes identical to those found in our patients with DS.

A series of reports have described changes in glucose metabolism in AD [18]. Most reports have demonstrated a reduced cerebral metabolic rate of glucose using PET [19–21] and the decreased metabolic rate was either explained by decreased hexokinase activity [22], atrophy [23] or diminished glucose transport and phosphorylation [24]. Salmon et al. [25] have addressed the problem of cortical tissue loss and cerebral glucose metabolism and concluded from their data that atrophy is not sufficient to explain cortical glucose hypometabolism which may reflect reduced synaptic connectivity.

Our results from PGK activity studies indicate that dysregulated brain carbohydrate metabolism represented by increased PGK activity may be a specific trait of DS pathobiology. Although Anneren et al. [5] did not show increased PFK activity in brain one could suggest that our findings of increased PGK could be a consequence of or adaptation to increased PFK as the concerted
action of glycolytic enzymes can be expected even at altered levels. We cannot explain differences of PGK activity in the individual areas but we are currently studying PFK and other glucose handling enzymes in order to further elucidate the biochemical mechanisms. The demonstration of increased PGK may be a piece in the puzzle of the complex derangement of brain glucose metabolism in DS, which is far from being solved. Our study confirms impairment of glucose metabolism in the brains of patients with DS and demonstrates that brain glucose metabolism represented by increased PGK is a finding specific to DS rather than to AD. Data from subtractive hybridization suggest that this derangement may be found early in human life. We are now proceeding to investigate more brain samples with a different severity of expression of AD pathology and more glucose handling enzymes to expand on the observation reported here in order to show whether PGK activity is associated with dementia or with DS per se.

ACKNOWLEDGMENT

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