available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Mitochondrial dysfunction in mouse trisomy 16 brain

L.L. Bambrick[a,b,c,*], G. Fiskum[a,c]

[a]Department of Anesthesiology, University of Maryland, School of Medicine, USA
[b]Department of Physiology, University of Maryland, School of Medicine, USA
[c]Program in Neuroscience, University of Maryland, School of Medicine, USA

ARTICLE INFO

Article history:
Accepted 17 October 2007
Available online 26 October 2007

Keywords:
Complex I
Pyruvate dehydrogenase
Parkinson's disease
Down syndrome

ABSTRACT

Mitochondrial function in the brain of mouse trisomy 16, an animal model of Down syndrome with accelerated neuron death, was studied in isolated cortex mitochondria. Using an oxygen-sensitive Clarke electrode, a selective 16% decrease in respiration was detected with the Complex I substrates malate and glutamate but not with the Complex II substrate succinate. Western blotting revealed a 20% decrease in the 20 kDa subunit of Complex I in Ts16 brain cortex homogenates with no significant decrease in marker proteins for the other complexes of the electron transport chain. Although no differences in H_2O_2 production or maximal calcium uptake were detected in the Ts16 mitochondria, there was an 18% decrease in pyruvate dehydrogenase levels, a change associated with oxidative stress in ischemia. These results are similar to those found in Parkinson's disease suggesting some neurodegenerative diseases may have mitochondrial pathology as a common step.

1. Introduction

The trisomy 16 (Ts16) mouse has a triplication of genes found on chromosomes 3, 16 and 21 in humans. The region of conserved synteny between human chromosome 21 and mouse chromosome 16 contains 111 orthologous genes pairs preserved in the same order (with one inversion exception) on both chromosomes and includes the Down Syndrome Critical Region (DSCR), which is thought to cover most of the genes responsible for Down Syndrome (DS, human trisomy 21) (Hattori et al., 2000; Mural et al., 2002). The Ts16 mouse is thus a model of aneuploidy that may replicate some of the features of DS. Such abnormalities, including learning defects, altered neuronal migration, smaller brains, and facial, cranial and cardiac malformations have been observed in mice with full or partial triplication of chromosome 16 (see e.g. Epstein, 1986; Haydar et al., 2000; Kleschevnikov et al., 2004; Delabar et al., 2006; Moore, 2006).

Attempts to understand the etiology of these pathologies have involved studies of cultured cells from Ts16 and DS brains. Increased apoptotic neuron death, altered calcium homeostasis and increased reactive oxygen species (ROS) levels and oxidative stress have been reported in DS and Ts16 neurons and astrocytes (Busciglio and Yankner, 1995; Bambrick

* *Corresponding author*. Department of Anesthesiology, University of Maryland, School of Medicine, MSTF Building, Rm 534, 685 W Baltimore St, Baltimore, MD 21201, USA.
E-mail address: lbambric@umaryland.edu (L.L. Bambrick).

Abbreviations: AD, Alzheimer's disease; BDNF, Brain-derived neurotrophic factor; FCCP, Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; CsA, Cyclosporine A; cytc, cytochrome c; DS, Down syndrome; DSCR, Down syndrome critical region; E, Embryonic day; ETC, Electron transport chain; Glu, Glutamate; Mal, Malate; MPT, Mitochondrial permeability transition; NADH, Nicotinamide adenine dinucleotide; PD, Parkinson's disease; PDH, Pyruvate dehydrogenase; ROS, Reactive oxygen species; RCR, Respiratory control ratio; Rot, Rotenone; Suc, Succinate; SOD, Superoxide dismutase; Ts, Trisomy

0006-8993/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2007.10.045

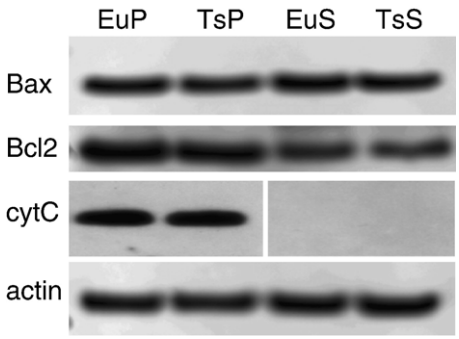

Fig. 1 – Distribution of mitochondrial proteins between the mitochondrial (pellet, lanes 1 and 2) and cytoplasmic (soluble, lanes 3 and 4) fractions for euploid and Ts16 brains. Similar results were found in 5 separate experiments. Densitometry showed no differences between protein amount or distribution between euploid and Ts16 samples.

et al., 1997; Bambrick and Krueger, 1999; Schuchmann et al., 1998; Schuchmann and Heinemann, 2000). A mitochondrial defect has been suggested as the basis of increased ROS, decreased mitochondrial membrane potential and changes in NADH observed in Ts16 mitochondria (see e.g. Schuchmann et al., 1998). We have now directly analyzed mitochondrial function in Ts16 by isolating brain mitochondria and measuring mitochondrial respiration and levels of relevant mitochondrial proteins. We find that Ts16 brain mitochondria have a selective defect in Complex I-mediated respiration, similar to that observed in Parkinson's Disease (PD) (Parker and Swerdlow, 1998; Shults et al., 2004), with decreased levels of the 20 kDa subunit of Complex I protein (ND6) and the E1α subunit of the pyruvate dehydrogenase (PDH) complex: a mitochondrial matrix protein also affected in ischemia and neurodegenerative disorders including Wernicke–Korsakoff syndrome (reviewed in Martin et al., 2005).

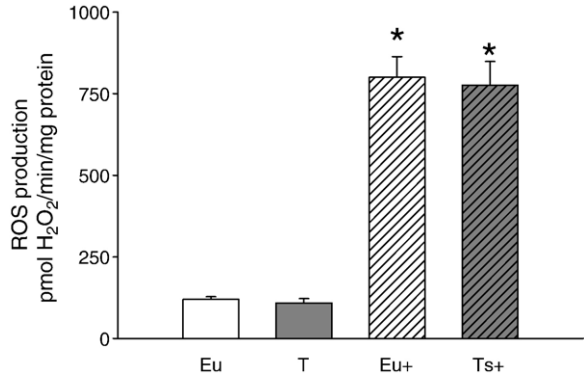

Fig. 2 – ROS production measured by Amplex red in euploid (white) and Ts16 (grey) cortical mitochondrial fractions in the presence of malate (5 mM) and glutamate (5 mM) with ADP (1 mM open bars) or ADP and rotenone (4 μM, stippled bars). Means and S.E.M. for N = 4 euploid and 3 Ts16 experiments. * Significantly different from 0 rotenone, $p < 0.01$.

2. Results

At E15.5, Ts16 brains are slightly smaller than euploid (Haydar et al., 2000), as reflected in the reduced protein yield/brain found for Ts16 (0.8 Ts16/euploid). However, the Ts16 porin/actin ratio was not different (1.0 ± 0.1 of euploid, $n = 6$) suggesting that the mitochondrial content per cell was not changed. Levels of the pro- and anti-apoptotic proteins bax and bcl-2 and of cytochrome c were measured by Western blot. Bax is known to redistribute from the cytoplasm to the mitochondria in response to apoptotic signals. Moreover, release of cytochrome c from mitochondria is associated with initiation of both apoptosis and necrosis. We found no differences in the amounts of these proteins in Ts16 (Fig. 1) nor in their distribution between mitochondrial and cytoplasmic fractions.

Increased oxidative damage has been reported in Ts16 and DS cells in vitro. Since mitochondria are a principle generator of ROS, ROS generation was measured in euploid and Ts16 cortical fractions using the horseradish peroxidase/amplex red

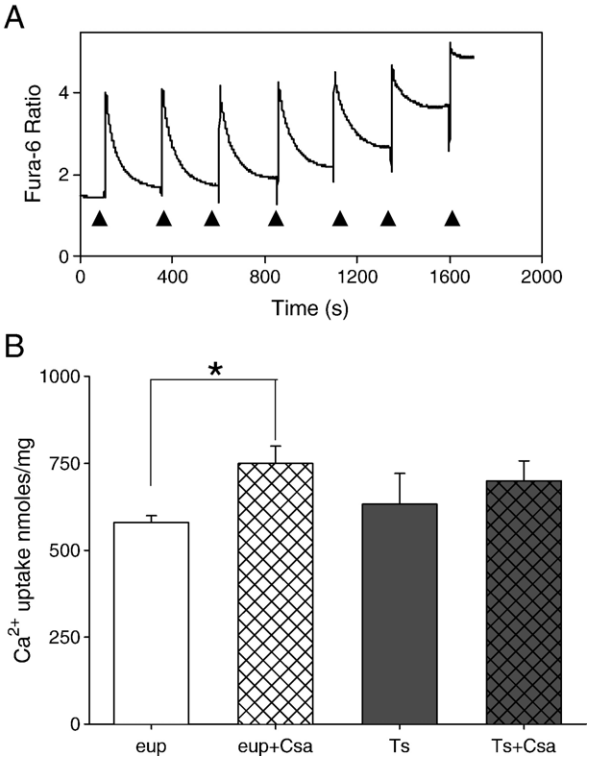

Fig. 3 – Calcium uptake in Euploid and Ts16 cortical mitochondria. A: euploid mitochondria (20 mg) with repeated pulses of calcium (20 nmol/addition, arrow heads) added at 5-min intervals. The solution calcium initially rises and then the mitochondria take up the calcium and solution calcium returns to baseline. The cumulative calcium to the last completely taken up addition is calculated as the calcium capacity and expressed per mg protein of the mitochondrial fraction. B: means and S.E.M. of N = 4–6 experiments for euploid (white) and Ts16 (grey) cortical mitochondrial fractions in the absence (open bars) or presence (hatched bars) of CsA. *Significantly from 0 CsA by paired t-test, $p < 0.05$.

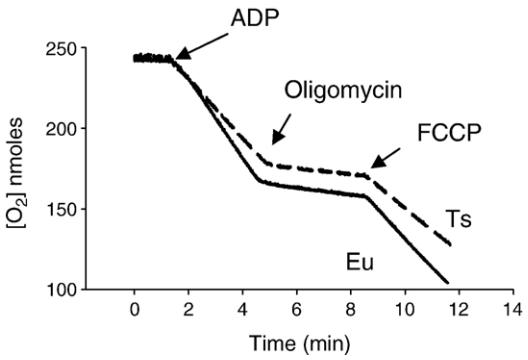

Fig. 4 – Respiration is impaired in Ts16 mitochondria. Euploid (solid line) and Ts16 (dotted line). Oxygen consumption in the presence of malate and glutamate was initiated by the addition of ADP to measure State 3; state 4_o respiration was determined after the addition of oligomycin and uncoupled respiration by the addition of FCCP.

assay (Starkov and Fiskum, 2003). This assay detects both H_2O_2 and superoxide, which is converted to H_2O_2 by the exogenous SOD in the reaction mixture. Fig. 2 shows that no significant difference was found in H_2O_2 production in Ts16. Addition of 4 μM rotenone to inhibit Complex I increased ROS production equally in euploid and Ts16.

The maximum calcium uptake capacity of isolated Ts16 mitochondria was measured as previously described in the absence and presence of the mitochondrial permeability transition (MPT) pore inhibitor cyclosporine A (CsA) (Bambrick et al., 2006). Using fura-6FF to detect solution calcium, repeated additions of calcium were made. Uptake into mitochondria is reflected by the decrease in fluorescence (Fig. 3A). Fig. 3B shows that there was no difference in calcium capacity, the calcium accumulated up to the last completely taken up addition, between mitochondria from Ts16 or euploid brain. Unlike in adult brain or permeabilized cultures of postnatal cerebellar granule cells (Bambrick et al., 2006), CsA slightly enhanced the Ca^{2+} uptake capacity of euploid E15.5 cortex mitochondria under these experimental conditions, although no significant effect of CsA was seen for Ts16.

Mitochondrial function in Ts16 brain was assessed by measuring respiration in mitochondrial fractions from euploid and Ts16 cortex. Fig. 4 shows representative traces for respiration with the Complex I substrates glutamate/malate. The rate of oxygen consumption in State 3 is slower in Ts16. When State 3 and State 4_o are compared for Malate/glutamate (Complex I) or Succinate/rotenone (Complex II) respiration (Table 1) there is a selective 16% decrease in Complex I State 3 in Ts16. Data are shown as the means of the absolute respiration values and as the means of the Ts16/euploid ratios from each experiment. State 4_o was slightly but not significantly reduced for Ts16 Complex I-mediated respiration and not affected when respiration was supported by Succinate/rotenone. The respiratory control ratio (RCR), a measure of how well-coupled

Table 1 – Complex I-mediated respiration is decreased in Ts16

	State 3		State 4_o	
	Complex I Mal/Glu	Complex II Suc/Rot	Complex I Mal/Glu	Complex II Suc/Rot
Euploid	30.8 ± 2.1	36.8 ± 2.7	4.4 ± 0.9	6.7 ± 0.7
Ts16	25.9 ± 1.6*	37.6 ± 2.2	3.9 ± 0.9	7.0 ± 0.5
Ts16/euploid	0.84 ± 0.02**	1.02 ± 0.04	1.2 ± 0.4	1.1 ± 0.3

Means ± S.E.M., N = 4–7. *Significantly different from euploid by paired t-test, $p < 0.05$, **significantly different from 1, $p < 0.01$.

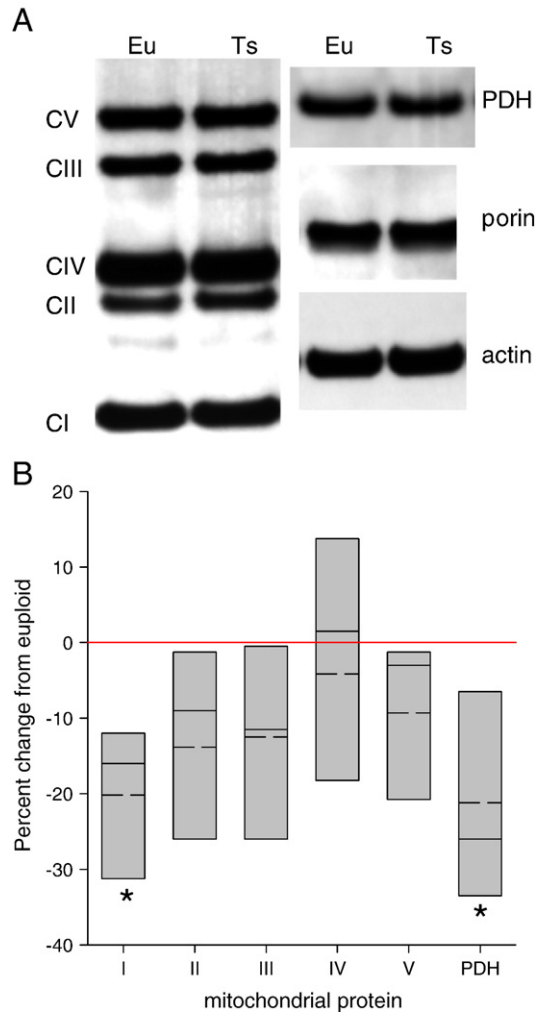

Fig. 5 – A: Euploid and Ts16 cortical homogenates run on 4–12% bis/tris gels transferred to PDF membranes and immunobloted for ETC proteins [Complex I 20 kDa protein: ND6; Complex II 30 kDa protein FeS; Complex III core 2 protein; Complex IV COX I; Complex V α subunit, Mitosciences MS604] or PDH E1α subunit. Gels were stripped and reprobed for porin and actin. B: Box plot of Ts16 mitochondrial protein expression. Densitometry values from Western blots were normalized to porin and expressed as a percent change from the littermate euploid cortex samples for each experiment. Data from N = 4–6 experiments. Boxes are the 5th and 95th percentiles, line is median, dotted line is mean. *Significantly different from euploid by paired t-test of the protein/porin ratios, $p < 0.05$.

oxidative phosphorylation is in the mitochondria, was 7±0.7 for euploid as compared to 6.6±1.3 for Ts16 cortex (with malate/glutamate as substrates). These results are consistent with unaffected respiration through Complex II and suggest that the mitochondria in Ts16 cortex are intact and that respiration is normal from Complex II to Complex IV, but that either the generation of NADH from malate/glutamate or its utilization by Complex I are defective in Ts16.

Changes in levels of mitochondrial proteins could cause the decreased Complex I-mediated respiration reported in Fig. 4 and Table 1. Accordingly, brain homogenates from euploid and Ts16 mice were collected and Western blot analysis performed for selected proteins from each of the 5 electron transport chain (ETC) complexes and the E1α subunit of pyruvate dehydrogenase (PDH). Western blots for the selected ETC complex proteins and PDH were done and then each blot was stripped and reprobed first for porin and second for actin. Fig. 5A shows representative blots of the proteins detected. In Fig. 5B, the Ts16 data are expressed as the average of the percent change from euploid brains for each experiment in box plot format showing the median, mean and 5th/95th percentiles of the distributions. There is a pattern of decreased levels for 5 of the 6 mitochondrial proteins in Ts16 with the exception being Complex IV. In particular, the 20 kDa protein of Complex I and the E1α subunit of PDH are decreased with the "zero" unchanged line outside of the 5–95% confidence limits for the proteins. When the raw Complex I/porin or PDH/porin values are compared for euploid and Ts16 by paired *t*-test, there were significant decreases in levels of the 20 kDa Complex I subunit (20%) and PDH (18%) in Ts16 cortex.

3. Discussion

Analysis of mitochondrial respiratory protein immunoreactivity and bioenergetic function, revealed that the Ts16 mitochondria showed selective defects in Complex I-mediated respiration with decreased levels of the 20 kDa Complex I subunit and of pyruvate dehydrogenase (PDH).

Mitochondrial content (expressed as the porin/actin ratio in the homogenate), levels of critical mitochondrial proteins such as cytochrome *c* and the electron transport chain (ETC) proteins and respiration are grossly normal in Ts16 cortex. These results are consistent with Ts16 neurons being viable at E15.5, with normal numbers of mitochondria: consistent with the report that at E15.5, the Ts16 cortex is still growing (Haydar et al., 2000); a catastrophic failure of mitochondrial function would not support the cell proliferation and migration occurring at this time. The same study reported a small increase in cortical cell apoptosis at E15.5; we did not see any corresponding increase in bax or decrease in bcl-2 expression. Either these pro- and anti-apoptotic proteins are not involved in the Ts16 cell death or the absolute numbers of cells dying in Ts16 cortex were too small for a change in bcl-2-family proteins to be detected in cortical homogenates.

We found no difference in maximal calcium uptake in Ts16 mitochondria (Fig. 4) using glutamate and malate as substrates. Although calcium dysregulation is a consistently observed feature of Ts16 with increased cytoplasmic and endoplasmic reticulum store calcium (see e.g. Bambrick et al., 1997; Schuchmann et al., 1998) and an impaired calcium buffering capacity (Cardenas et al., 1999), the present results suggest that the small decrease in Complex-I respiration we measure (16%, Table 1) might only lead to a slight slowing of mitochondrial calcium uptake and not affect capacity. Such a change in mitochondrial calcium uptake kinetics could, however, affect the shape of calcium transients in neurons (see e.g. Malli et al., 2003). Euploid mitochondria showed an increase in calcium capacity upon addition of CsA. Neuronal mitochondria from adult brain are not sensitive to CsA — the embryonic sensitivity likely reflects a developmental gradient in cyclophilin D (the CsA receptor) expression (Eliseev et al., 2007). The lack of detectable effect of CsA in Ts16 may be a consequence of the increased variability in Ts16 — it is noticeable that an increased variability in cytoplasmic and endoplasmic reticulum calcium is also seen among individual cells in Ts16 cultures (see e.g. Bambrick et al., 1997).

We detected a small but significant decrease in Complex I-mediated respiration together with a decrease in mitochondrial levels of PDH subunit E1α and of the 20 kDa subunit of Complex I in Ts16 mitochondria. PDH levels and activity have been reported to be decreased after cerebral ischemia/reperfusion (Zaidan et al., 1998; Bogaert et al., 2000; Martin et al., 2005; Vereczki et al., 2006) and levels of Complex I proteins or Complex I activity have been reported to be decreased in PD (Keeney et al., 2006). Levels of both the 20 kDa subunit protein and PDH are known to be sensitive to oxidative damage with oxidation leading to increased degradation and decreased protein levels. It is possible that inhibition of Complex I could lead to an increased generation of free radicals, depending on the site of inhibition within this macromolecular assembly. However, Fig. 3 shows that no significant difference was found in H_2O_2 production in Ts16. This is most consistent with the defect in Complex I respiration occurring prior to the NADH dehydrogenase site or with the major sites of free radical production in the absence of inhibitors being outside of the electron transport chain, e.g. at the ketoglutarate dehydrogenase complex (Starkov et al., 2004). However, because respiration is decreased, the rate of generation of H_2O_2/nmol oxygen consumed is slightly increased in Ts16. It is not clear whether there is a primary defect in Complex I respiration leading to a slight increase in free radical generation which damages the sensitive 20 kDa protein and PDH or whether there is an increased generation of free radicals from some other cause which damages PDH and the 20 kDa protein leading, secondarily, to decreased Complex I-mediated respiration.

Mitochondrial function in Ts16 has been previously studied in cultured hippocampal neurons, where a decreased reduction of pyridine nucleotides after glutamate stimulation, lower levels of glutathione and rescue of accelerated Ts16 neuron death by antioxidants (Schuchmann and Heinemann 2000; Schuchmann et al., 1998) have been interpreted to be the consequence of an underlying mitochondrial defect. In the Ts1Cje mouse, a partial trisomy model, cultured neurons and astrocytes have increased ROS levels and astrocytes have a decrease in mitochondrial membrane potential (Shukkur et al., 2006). Similarly, cultured neurons from human trisomy 21 brains show evidence of increased ROS and free radical damage (Busciglio and Yankner, 1995) while cultured human Ts21 astrocytes have a decreased mitochondrial membrane potential

(Busciglio et al., 2002). One limitation of previous studies is that the increased ROS and decreased mitochondrial membrane potential could be secondary to the increased apoptosis in Ts16 and DS neurons. The present results showing a significant defect in mitochondrial respiration in the absence of increased ROS generation and at a time in vivo when there is not a significant increase in Ts16 neuron death suggest that mitochondrial dysfunction may be an early event in the pathology of Ts16.

Besides the sequence of genes from the human DSCR, mouse chromosome 16 contains major stretches of genes encoded on human chromosomes 3 and 16 with minor contributions of sequences encoded on human chromosomes 8, 12 and 22 (Mural et al., 2002). Relating a specific defect to triplication of a specific gene has proven difficult. Given that triplication results in only a 1.5-fold gene dosage effect, there may not be such a one-to-one relation and, instead, trisomy likely leads to disease due to an accumulation of minor defects that can even affect the expression of genes outside the triplicated chromosome. Indeed, one molecular difference that has been detected inTs16 is an over-expression of the truncated form of the neurotrophin receptor trkB, encoded on mouse chromosome 3 (Tessarollo et al., 1993), which leads to a decreased sensitivity to brain-derived neurotrophic factor (BDNF) (Dorsey et al., 2002). The improved neuron survival in Ts16 mice with a knockdown of truncated trkB expression (Dorsey et al., 2006) shows that the altered expression of this neurotrophin receptor contributes to the Ts16 phenotype. The present results show that there is a selective, mitochondrial-intrinsic defect in Ts16, which exists either in parallel to or as a consequence of the decreased BDNF signaling in these brains.

Is the magnitude of the observed changes in Complex I-mediated respiration and 20 kDa subunit and PDH levels sufficient to cause increased death in Ts16 neurons? In a fly model of oxidative stress and mitochondrial dysfunction (Walker et al., 2006) a selective defect in subunit b of succinate dehydrogenase (Complex II) was associated with a 56% decrease in Complex II mediated respiration, a 25% increase in ROS generation and a reduction in lifespan to ¼ wildtype. In a recent study of PD (Keeney et al., 2006), isolated brain mitochondrial proteins showed a 30% reduction in the rate of complex I catalytic activity and small 11–34% changes in Complex I, but not Complex II–V, proteins, while 20% or smaller decreases in Complex I catalytic activity have been found in mitochondria isolated from peripheral tissue in PD. For PDH, studies have found that both activity and expression in brain drop rapidly after cardiac arrest and resuscitation due to oxidative damage to the protein (Bogaert et al., 2000; Martin et al., 2005). In the dog hippocampus there is a 40% decrease in PDH abundance by Western blot 24 h after cardiac arrest/resuscitation which is correlated with extensive brain damage (including, e.g. a 50% reduction in neuronal cell numbers in the CA1 region, Vereczki et al., 2006). Therefore the 18% decrease in PDH reported here for Ts16 brain might represent considerable damage and could be consistent with the accelerated death reported in cultured Ts16 neurons (Bambrick and Krueger, 1999). Clearly the magnitude of the changes reported here for Ts16 is in the same range as those found in PD and after ischemia, raising the possibility that distinct neurological disorders may have common mitochondrial dysfunctions as part of their pathophysiologies.

4. Experimental procedures

4.1. *Mouse breeding and identification of Ts16 fetuses*

Male double Robertsonian mice (Jackson Laboratory, Bar Harbor, ME) were mated overnight with female C57BL/6J mice. The next day was designated as day 0.5 of gestation (E 0.5). Pregnant female mice were killed by cervical dislocation at E 15.5 and the fetuses removed, chilled on ice and decapitated. Normal and Ts16 fetuses were easily distinguishable (Bekker et al., 2006) using criteria previously validated by karyotyping and by fluorescence in situ hybridization (Bambrick et al., 1995; Strovel et al., 1999). Between 3 and 6 brains were pooled in each experiment. Our average Ts16 fetus/litter frequency was one. Some litters had no Ts16 fetuses and were not used in this study. The average number of litters/experiment was four. Because brain development at this age is so rapid (see e.g. Haydar et al., 2000), euploid and Ts16 littermate pairs were used in all experiments to minimize the effects of an up to 16 hr differences in gestational age between litters. Animal care and all procedures were performed in accordance with National Institute of Health guidelines and under the supervision of the institutional Animal Care and Use Committee.

4.2. *Preparation of mitochondrial fractions and of homogenates*

Embryonic brains were dissected out of the skulls and the cerebral hemispheres were freed of meninges, transferred to ice-cold homogenization buffer [in mM mannitol 225; sucrose 75, HEPES 5, EGTA 1; pH 7.4 with KOH] at a concentration of 200 μl/brain and dissociated in a glass homogenizer. After centrifugation at 5 min at 1000×*g*, the supernatant was recovered and centrifuged again at 10,000×*g*. The resulting pellet was resuspended in KCl buffer [in mM: KCl 125; K_2PO_4 2; $MgCl_2$ 1; HEPES 20; pH 7] at 20 μl/brain and this crude mitochondrial fraction was kept on ice until used for respiration, calcium uptake or H_2O_2 experiments. All respiration and calcium uptake experiments were performed within 2 h of sample isolation. At this age there are very few synapses in the mouse brain (Demarque et al., 2002) so the synaptosomal contribution is minimal. Aliquots of the homogenate (after the first centrifugation), of the crude mitochondrial fraction (the pellet after the second centrifugation) or the cytoplasmic fraction (the supernatant after the second centrifugation) were frozen at −20 °C for use in Western blotting. Protein was assayed by Biorad.

4.3. *Respiration*

Oxygen consumption was measured with a Clarke-type electrode from Strathkelvin at 37 °C. Rates of respiration are expressed as nmol O_2/min/mg protein. State 3 respiration through Complex I was determined in the presence of 5 mM glutamate, 5 mM malate, 1 mM ADP in KCl buffer. State 3 respiration through Complex II was determined by replacing the malate/glutamate with 5 mM succinate and 4 μM rotenone. State 4_o respiration was measured in the presence of 0.5 mg/ml oligomycin, an inhibitor of the ATP synthase. While the State 4_o respiration determined in the presence of oligomycin is not equivalent to the classical State 4 rate obtained after a small

bolus of ADP is almost completely converted to ATP, the use of oligomycin eliminates the contribution of ATP cycling via hydrolysis by contaminating ATPases and resynthesis by the mitochondrial ATP synthase to State 4 respiration and is therefore a more specific indicator of mitochondrial proton cycling limited by passive proton leak across the inner mitochondrial membrane. The respiratory control ratio was calculated as State 3/State4_o (Kristian et al., 2006). Uncoupled respiration was measured with malate/glutamate/ADP and 1 μM Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP).

4.4. *Peroxide production*

Hydrogen peroxide production was measured fluorometrically in KCl buffer with 0.5 mg BSA, 0.25 mM EGTA, 5 U/ml horseradish peroxidase, 40 U/ml Cu,Zn superoxide dismutase and 1 μM amplex red (Starkov and Fiskum, 2003). The generation of H_2O_2 in the mitochondrial suspension was measured as an increase in fluorescence measured at 585 nm with 550 nm excitation. The dye response was calibrated by the addition of a known amount of hydrogen peroxide solution.

4.5. *Calcium uptake*

Calcium uptake capacity was measured in the mitochondrial fraction in 125 mM KCl, 20 mM HEPES, 2 mM KH_2PO_4, 4 mM $MgCl_2$, 3 mM ATP, 5 mM malate, 5 mM glutamate, pH 7.0, 1 mM EGTA with 250 nM fura-6F (pentapotassium salt) in a Hitachi F-2500 spectrophotometer, 37 °C, excitation at 340/380. Repeated pulses of calcium (20 nmol/addition) were added at 5-min intervals. The solution calcium initially rises and then falls due to mitochondrial uptake. "Calcium capacity", the total amount of calcium accumulated through the last addition, is expressed per mg protein of the mitochondrial fraction (Bambrick et al., 2006).

4.6. *Western blotting*

Homogenate, mitochondrial and cytoplasmic fractions from E15.5 euploid and Ts16 mouse brain pairs were separated by SDS-polyacrylamide gel electrophoresis using MES SDS buffer on 4–12% bis tris gels (NuPAGE, Invitrogen), transferred to PVDF membranes (NuPAGE Transfer buffer, Invitrogen) and probed for cytochrome *c* (1/5000, PharMingen), bax (1/1000, Upstate) or bcl-2 (C-2, 1/5000, Santa Cruz) and then stripped using Restore Western Blot Stripping Buffer (Pierce) and reprobed for actin (1/5000, Sigma). In experiments assaying levels of ETC complex proteins or PDH, the blots were probed with the rodent OXPHOS cocktail from Mitosciences (see Fig. 5A), or PDH (1/1000, Molecular Probes) stripped and reprobed for porin (1/5000, Calbiochem) then stripped and probed a third time for actin. The presence of the primary antibody was detected using horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary (1/5000, Vector) in Tris-buffered saline with Tween-20. Following incubation with ECL Plus (Amersham, Piscataway, NJ, USA) bands were exposed to CL-X Posure Film (Pierce). The film was developed, band density was analyzed with a 2000R Image station (Kodak) and normalized to either porin or actin. Each experiment consisted of one lane containing the homogenate from euploid cortices and the adjacent lane containing the homogenate prepared from that day's littermate Ts16 cortices. So that for any experiment the euploid and Ts16 samples were treated identically.

4.7. *Materials*

All cell culture supplies were from Invitrogen and chemical reagents from Sigma unless otherwise noted.

4.8. *Statistics*

Three to six Ts16 and euploid brains from an average of four litters were pooled in each experiment. *N* is the number of separate experiments. The significance of differences in respiration, ROS generation and calcium uptake was determined by paired *t*-test. For Western blots, the Ts16 data are reported as percent change from euploid (change score). Where the 0% change level was outside the 5–95% percentile of the Ts16 distribution, the raw protein/porin values for euploid and Ts16 samples were compared by paired *t*-test. Paired *t*-tests were used to compare littermate samples and thus control for differences in average gestational age of the fetuses or any differences in maternal environment from experiment to experiment.

Acknowledgments

This work was supported by DAMD 17-03-1-0745 to LLB. We would like to thank Ms. Weili Dai for her expert technical assistance and Dr. Bruce Krueger for reading this manuscript.

REFERENCES

Bambrick, L.L., Krueger, B.K., 1999. Neuronal apoptosis in mouse trisomy 16: mediation by caspases. J. Neurochem. 72, 1769–1772.

Bambrick, L.L., Yarowsky, P.J., Krueger, B.K., 1995. Glutamate as a hippocampal neuron survival factor: an inherited defect in the trisomy 16 mouse. Proc. Natl. Acad. Sci. U. S. A. 92, 9692–9696.

Bambrick, L.L., Golovina, V.A., Blaustein, M.P., Yarowsky, P.J., Krueger, B.K., 1997. Abnormal calcium homeostasis in astrocytes from the trisomy 16 mouse. Glia 19, 352–358.

Bambrick, L., Chandrasekaran, K., Mehrabian, Z., Wright, C., Krueger, B.K., Fiskum, G., 2006. Cyclosporin A increases mitochondrial calcium uptake capacity in cortical astrocytes but not cerebellar granule neurons. J. Bioenerg. Biomembranes 38 (1), 43–47 (Feb).

Bekker, M.N., Twisk, J.W., Bartelings, M.M., Gittenberger-de Groot, A.C., van Vugt, J.M., 2006. Temporal relationship between increased nuchal translucency and enlarged jugular lymphatic sac. Obstet. Gynecol. 108, 846–853.

Bogaert, Y.E., Sheu, K.F., Hof, P.R., Brown, A.M., Blass, J.P., Rosenthal, R.E., Fiskum, G., 2000. Neuronal subclass-selective loss of pyruvate dehydrogenase immunoreactivity following canine cardiac arrest and resuscitation. Exp. Neurol. 161, 115–126.

Busciglio, J., Yankner, B.A., 1995. Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. Nature 378, 776–779.

Busciglio, J., Pelsman, A., Wong, C., Pigino, G., Yuan, M., Mori, H., Yankner, B.A., 2002. Altered metabolism of the amyloid β

precursor protein is associated with mitochondrial dysfunction in Down's syndrome. Neuron 33, 677–688.
Cardenas, A.M., Rodriguez, M.P., Cortes, M.P., Alvarez, R.M., Wei, W., Rapoport, S.I., Shimahara, T., Caviedes, R., Caviedes, P., 1999. Calcium signals in cell lines derived from the cerebral cortex of normal and trisomy 16 mice. NeuroReport 10, 363–369.
Delabar, J.M., Aflalo-Rattenbac, R., Creau, N., 2006. Developmental defects in trisomy 21 and mouse models. Sci. World J. 6, 1945–1964.
Demarque, M., Represa, A., Becq, H., Khalilov, I., Ben-Ari, Y., Aniksztejn, L., 2002. Paracrine intercellular communication by a Ca2+- and SNARE-independent release of GABA and glutamate prior to synapse formation. Neuron 36, 1051–1061.
Dorsey, S.G., Bambrick, L.L., Balice-Gordon, R.J., Krueger, B.K., 2002. Failure of brain-derived neurotrophic factor-dependent neuron survival in mouse trisomy 16. J. Neurosci. 22, 2571–2578.
Dorsey, S.G., Renn, C.L., Carim-Todd, L., Barrick, C.A., Bambrick, L., Krueger, B.K., Ward, C.W., Tessarollo, L., 2006. In vivo restoration of physiological levels of truncated TrkB.T1 receptor rescues neuronal cell death in a trisomic mouse model. Neuron 51, 21–28.
Eliseev, R.A., Filippov, G., Velos, J., Vanwinkle, B., Goldman, A., Rosier, R.N., Gunter, T.E., 2007. Role of cyclophilin D in the resistance of brain mitochondria to the permeability transition. Neurobiol. Aging 28, 1532–1542.
Epstein, C.J., 1986. Developmental genetics. Experientia 42, 1117–1128.
Hattori, M., Fujiyama, A., Taylor, T.D., Watanabe, H., Yada, T., Park, H.-.S., Toyoda, A., Ishii, K., Totoki, Y., Choi, D.-.K., Soeda, E., Ohki, M., Takagi, T., Sakaki, Y., Taudien, S., Blechschmidt, K., Polley, A., Menzel, U., Delabar, J., Kumpf, K., Lehmann, R., Patterson, D., Reichwald, K., Rump, A., Schillhabel, M., Schudy, A., Zimmermann, W., Rosenthal, A., Kudoh, J., Shibuya, K., Kawasaki, K., Asakawa, S., Shintani, A., Sasaki, T., Nagamine, K., Mitsuyama, S., Antonarakis, S.E., Minoshima, S., Shimizu, N., Nordsiek, G., Hornischer, K., Brandt, P., Scharfe, M., Schon, O., Desario, A., Reichelt, J., Kauer, G., Blocker, H., Ramser, J., Beck, A., Klages, S., Hennig, S., Riesselmann, L., Dagand, E., Wehrmeyer, S., Borzym, K., Gardiner, K., Nizetic, D., Francis, F., Lehrach, H., Reinhardt, R., Yaspo, M.-L., 2000. The DNA sequence of human chromosome 21. Nature 405, 311–319.
Haydar, T.F., Nowakowski, R.S., Yarowsky, P.J., Krueger, B.K., 2000. Role of founder cell deficit and delayed neuronogenesis in microencephaly of the trisomy 16 mouse. J. Neurosci. 20, 4156–4164.
Keeney, P.M., Xie, J., Capaldi, R.A., Bennett Jr., J.P., Keeney, P.M., Xie, J., Capaldi, R.A., Bennett Jr., J.P., 2006. Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. J. Neurosci. 26, 5256–5264.
Kleschevnikov, A.M., Belichenko, P.V., Villar, A.J., Epstein, C.J., Malenka, R.C., Mobley, W.C., 2004. Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. J. Neurosci. 24, 8153–8160.
Kristian, T., Hopkins, I.B., McKenna, M.C., Fiskum, G., 2006. Isolation of mitochondria with high respiratory control from primary cultures of neurons and astrocytes using nitrogen cavitation. J. Neurosci. Methods 152, 136–143.
Malli, R., Frieden, M., Osibow, K., Zoratti, C., Mayer, M., Demaurex, N., Graier, W.F., 2003. Sustained Ca2+ transfer across mitochondria is Essential for mitochondrial Ca2+ buffering, sore-operated Ca2+ entry, and Ca2+ store refilling. J. Biol. Chem. 278, 44769–44779.
Martin, E., Rosenthal, R.E., Fiskum, G., 2005. Pyruvate dehydrogenase complex: metabolic link to ischemic brain injury and target of oxidative stress. J. Neurosci. Res. 79, 240–247.
Moore, C.S., 2006. Postnatal lethality and cardiac anomalies in the Ts65Dn Down syndrome mouse model. Mamm. Genome 17, 1005–1012.
Mural, R.J., Adams, M.D., Myers, E.W., Smith, H.O., Miklos, G.L., Wides, R., Halpern, A., Li, P.W., Sutton, G.G., Nadeau, J., Salzberg, S.L., Holt, R.A., Kodira, C.D., Lu, F., Chen, L., Deng, Z., Evangelista, C.C., Gan, W., Heiman, T.J., Li, J., Li, Z., Merkulov, G.V., Milshina, N.V., Naik, A.K., Qi, R., Shue, B.C., Wang, A., Wang, J., Wang, X., Yan, X., Ye, J., Yooseph, S., Zhao, Q., Zheng, L., Zhu, S.C., Biddick, K., Bolanos, R., Delcher, A.L., Dew, I.M., Fasulo, D., Flanigan, M.J., Huson, D.H., Kravitz, S.A., Miller, J.R., Mobarry, C.M., Reinert, K., Remington, K.A., Zhang, Q., Zheng, X.H., Nusskern, D.R., Lai, Z., Lei, Y., Zhong, W., Yao, A., Guan, P., Ji, R.R., Gu, Z., Wang, Z.Y., Zhong, F., Xiao, C., Chiang, C.C., Yandell, M., Wortman, J.R., Amanatides, P.G., Hladun, S.L., Pratts, E.C., Johnson, J.E., Dodson, K.L., Woodford, K.J., Evans, C.A., Gropman, B., Rusch, D.B., Venter, E., Wang, M., Smith, T.J., Houck, J.T., Tompkins, D.E., Haynes, C., Jacob, D., Chin, S.H., Allen, D.R., Dahlke, C.E., Sanders, R., Li, K., Liu, X., Levitsky, A.A., Majoros, W.H., Chen, Q., Xia, A.C., Lopez, J.R., Donnelly, M.T., Newman, M.H., Glodek, A., Kraft, C.L., Nodell, M., Ali, F., An, H.J., Baldwin-Pitts, D., Beeson, K.Y., Cai, S., Carnes, M., Carver, A., Caulk, P.M., Center, A., Chen, Y.H., Cheng, M.L., Coyne, M.D., Crowder, M., Danaher, S., Davenport, L.B., Desilets, R., Dietz, S.M., Doup, L., Dullaghan, P., Ferriera, S., Fosler, C.R., Gire, H.C., Gluecksmann, A., Gocayne, J.D., Gray, J., Hart, B., Haynes, J., Hoover, J., Howland, T., Ibegwam, C., Jalali, M., Johns, D., Kline, L., Ma, D.S., MacCawley, S., Magoon, A., Mann, F., May, D., McIntosh, T.C., Mehta, S., Moy, L., Moy, M.C., Murphy, B.J., Murphy, S.D., Nelson, K.A., Nuri, Z., Parker, K.A., Prudhomme, A.C., Puri, V.N., Qureshi, H., Raley, J.C., Reardon, M.S., Regier, M.A., Rogers, Y.H., Romblad, D.L., Schutz, J., Scott, J.L., Scott, R., Sitter, C.D., Smallwood, M., Sprague, A.C., Stewart, E., Strong, R.V., Suh, E., Sylvester, K., Thomas, R., Tint, N.N., Tsonis, C., Wang, G., Wang, G., Williams, M.S., Williams, S.M., Windsor, S.M., Wolfe, K., Wu, M.M., Zaveri, J., Chaturvedi, K., Gabrielian, A.E., Ke, Z., Sun, J., Subramanian, G., Venter, J.C., Pfannkoch, C.M., Barnstead, M., Stephenson, L.D., 2002. A comparison of whole-genome shotgun-derived mouse chromosome 16 and the human genome. Science 296, 1661–1671.
Parker Jr., W.D., Swerdlow, R.H., 1998. Mitochondrial dysfunction in idiopathic Parkinson disease. Am. J. Hum. Genet. 62, 758–762.
Schuchmann, S., Heinemann, U., 2000. Increased mitochondrial superoxide generation in neurons from trisomy 16 mice: a model of Down's syndrome. Free Radic. Biol. Med. 28, 235–250.
Schuchmann, S., Muller, W., Heinemann, U., 1998. Altered Ca^{2+} signaling and mitochondrial deficiencies in hippocampal neurons of trisomy 16 mice: a model of Down's syndrome. J. Neurosci. 18, 7216–7231.
Shukkur, E.A., Shimohata, A., Akagi, T., Yu, W., Yamaguchi, M., Murayama, M., Chui, D., Takeuchi, T., Amano, K., Subramhanya, K.H., Hashikawa, T., Sago, H., Epstein, C.J., Takashima, A., Yamakawa, K., 2006. Mitochondrial dysfunction and tau hyperphosphorylation in Ts1Cje, a mouse model for Down syndrome. Hum. Mol. Genet. 15, 2752–2762.
Shults, C.W., Flint Beal, M., Song, Fontaine, D., 2004. Pilot trial of high dosages of coenzyme Q10 in patients with Parkinson's disease. Exp. Neurol. 188, 491–494.
Starkov, A.A., Fiskum, G., 2003. Regulation of brain mitochondrial H_2O_2 production by membrane potential and NAD(P)H redox state. J. Neurochem. 86, 1101–1107.
Starkov, A.A., Fiskum, G., Chinopoulos, C., Lorenzo, B.J., Browne, S.E., Patel, M.S., Beal, M.F., 2004. Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. J. Neurosci. 24, 7779–7788.

Strovel, J., Stamberg, J., Yarowsky, P.J., 1999. Interphase FISH for rapid identification of a down syndrome animal model. Cytogenet. Cell Genet. 86, 285–287.
Tessarollo, L., Tsoulfas, P., Martin-Zanca, D., Gilbert, D.J., Jenkins, N.A., Copeland, N.G., Parada, L.F., 1993. trkC, a receptor for neurotrophin-3, is widely expressed in the developing nervous system and in non-neuronal tissues. Development 118, 463–475.
Vereczki, V., Martin, E., Rosenthal, R.E., Hof, P.R., Hoffman, G.E., Fiskum, G., 2006. Normoxic resuscitation after cardiac arrest protects against hippocampal oxidative stress, metabolic dysfunction, and neuronal death. J. Cereb. Blood Flow Metab. 26, 821–835.
Walker, D.W., Hajek, P., Muffat, J., Knoepfle, D., Cornelison, S., Attardi, G., Benzer, S., 2006. Hypersensitivity to oxygen and shortened lifespan in a Drosophila mitochondrial complex II mutant. Proc. Natl. Acad. Sci. U. S. A. 103, 16382–16387.
Zaidan, E., Sheu, K.F., Sims, N.R., 1998. The pyruvate dehydrogenase complex is partially inactivated during early recirculation following short-term forebrain ischemia in rats. J. Neurochem. 70, 233–241.