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Cortical Cytochrome Oxidase Activity Is Reduced in Alzheimer's Disease

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Abstract: A defect in energy metabolism may play a role in the pathogenesis of neurodegenerative diseases, such as Alzheimer's disease. In the present study, we examined the activities of the enzymes that catalyze oxidative phosphorylation in frontal, temporal, parietal, and occipital cortex from Alzheimer's disease patients and age-matched controls. Complex I and complex II–III activities showed a small decrease in occipital cortex, but were unaffected in the other cortical areas. The most consistent change was a significant decrease of cytochrome oxidase (complex IV) activity of 25–30% in the four cortical regions examined. These results provide further evidence of a cytochrome oxidase defect in Alzheimer's disease postmortem brain tissue. A deficiency in this key energy-metabolizing enzyme could lead to a reduction in energy stores and thereby contribute to the neurodegenerative process. **Key Words:** Alzheimer's disease—Mitochondria—Cytochrome oxidase—Excitotoxicity—Neurodegenerative disease.
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A substantial body of evidence suggests that a defect in energy metabolism may occur in Alzheimer's disease (AD) (Blass and Gibson, 1991; Blass, 1993). A number of studies have used positron emission tomography to show that a decrease in parietal and temporal glucose metabolism occurs early in the course of AD and may precede cognitive deficits (Duara et al., 1986; Haxby et al., 1986). Further, an increase in inorganic phosphate was found using nuclear magnetic resonance spectroscopy (Brown et al., 1989). Abnormal glucose metabolism in brain biopsies, interpreted as partial uncoupling of mitochondria, has also been observed (Sims et al., 1987). More recently, much interest has focused on cytochrome oxidase, following a report that there was a marked decrease in the activity of this enzyme in platelets of AD patients (Parker et al., 1990). A subsequent report confirmed a 16–26% decrease in cytochrome oxidase activity in the cerebral cortex of AD patients (Kish et al., 1992).

In the present study, we extended this work by measuring all of the enzymes that catalyze oxidative phosphorylation in four representative regions of cerebral cortex from patients with pathologically confirmed AD and age-matched controls. We examined enzyme activities in both partially purified mitochondria and brain homogenates. Our results confirm a relatively selective decrease in cytochrome oxidase (complex IV) activity in AD postmortem cerebral cortex.

MATERIALS AND METHODS

Autopsied brain specimens were obtained from clinically demented patients with histopathological confirmation of AD and from age-matched, sex-matched, postmortem interval-matched control patients with a protracted illness, but without evidence of neuropsychiatric disease or neuropathology. The patient characteristics are shown in Table 1. All brains were frozen at −80°C immediately following autopsy. Subsequently, the following regions were dissected from these brains for examination: Brodmann area 7 in the parietal cortex (19 AD patients, 23 controls); Brodmann areas 8 and 9 in the frontal cortex (19 AD patients, 23 controls); Brodmann areas 20, 21, and 22 in the temporal cortex (21 AD patients, 15 controls); and Brodmann areas 17 and 18 in the occipital cortex (22 AD patients, 22 controls). Crude mitochondrial preparations were then made by applying the P_2 pellet from ~1 g of cortical tissue to a discontinuous Ficoll gradient (Sims and Blass, 1986). Homogenates (1:5, wt/vol) of a subset of the Brodmann area 7 subjects (13 AD patients, 10 controls) and of all the Brodmann areas 20/21/22 subjects were also made.

Enzyme activities were assayed spectrophotometrically in triplicate for each sample by using established procedures with minor modifications for complex I (Hatefi, 1978), complex II–III (Zheng et al., 1989), complex IV (Darley-Usmar et al., 1987), and complex V (Darley-Usmar et al., 1987). Protein concentration was also determined (Bradford, 1976). As the crude mitochondrial and homogenate preparations appeared to have variable amounts of mitochondria, mitochondrial concentration was estimated by measuring the activity of citrate synthase, a mitochondrial matrix enzyme

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Abbreviation used: AD, Alzheimer's disease.

TABLE 1. *Patient characteristics*

	Age	Sex	Postmortem interval (h)
Brodmann area 7			
AD (19)	73.5 ± 3.0	10 F, 9 M	10.7 ± 1.5
Control (23)	75.9 ± 2.7	10 F, 13 M	9.4 ± 1.3
Brodmann areas 8/9			
AD (19)	75.3 ± 2.6	12 F, 7 M	8.7 ± 1.2
Control (23)	70.1 ± 3.5	9 F, 14 M	11.8 ± 1.3
Brodmann areas 17/18			
AD (22)	73.2 ± 2.0	14 F, 8 M	9.2 ± 1.1
Control (22)	72.8 ± 3.6	8 F, 14 M	12.4 ± 1.3
Brodmann areas 20/21/22			
AD (21)	74.3 ± 2.2	13 F, 8 M	11.7 ± 1.3
Control (15)	73.9 ± 3.0	6 F, 9 M	12.3 ± 1.6

(Shepherd and Garland, 1969). The activities of the oxidative phosphorylation enzymes were then "corrected" by dividing by citrate synthase activity. The protein assay reagent was purchased from Bio-Rad (Richmond, CA, U.S.A.). Ubiquinone-1 was kindly donated by Eisai Chemical Co. (Tokyo, Japan). All other reagents were obtained from Sigma (St. Louis, MO, U.S.A.).

The data were analyzed by unpaired t test analysis (two-tailed). Additionally, regression analysis was used to examine possible correlations of enzyme activities with age and postmortem interval in both AD and control patients. A $p < 0.05$ was considered significant. The data are expressed as the means ± SEM.

RESULTS

The number of samples in the different cortical regions varied due to tissue availability. There were no significant differences between the AD and control groups for age, sex, or postmortem interval in any of the regions examined. Furthermore, no significant differences in age, sex, or postmortem interval existed between the AD and control samples in the subset used for homogenates in area 7. There were no significant correlations of enzyme activities with either age or postmortem interval in any of the four cortical regions examined.

Using unpaired t test analysis, no significant difference was found between controls and AD patients in complex I activity in mitochondrial preparations of Brodmann areas 7, 8/9, or 20/21/22 (Tables 2–4). Complex I activity in homogenates of Brodmann areas 7 and 20/21/22 also did not exhibit significant differences. Interestingly, assays of mitochondrial preparations of Brodmann areas 17/18 revealed a significant decrease of 46% in complex I activity when AD patients were compared with controls ($p < 0.003$; Table 5). Complex II–III activity was measured in Brodmann areas 8/9, 20/21/22, and 17/18. Although no significant changes were noted in mitochondrial preparations of areas 8/9 and 20/21/22, a significant decrease of 21% was noted in complex II–III activity in mitochondrial preparations of areas 17/18 of AD brains ($p < 0.02$), and a decrease of 30% in activity was found in homogenates of areas 20/21/22 ($p < 0.05$). Complex IV activity in mitochondrial preparations from AD brains exhibited decreases of 30% in area 7 ($p < 0.001$), 26% in areas 8/9 ($p < 0.02$), 25% in areas 20/21/22 ($p < 0.01$), and 29% in areas 17/18 ($p < 0.001$). Homogenate preparations of areas 20/21/22 also revealed a significant decrease of 27% in complex IV activity ($p < 0.01$) in AD brains relative to controls. Complex V activity in mitochondrial preparations and homogenates from areas 7 and 20/21/22 exhibited no significant differences between AD and control brains. Significant increases of 27% in areas 8/9 ($p < 0.03$) and 29% in areas 17/18 ($p < 0.01$) were observed in mitochondrial preparations from AD brains.

DISCUSSION

In the present study, we found a significant decrease of cytochrome oxidase activity of 25–30% in all four

TABLE 2. *Oxidative phosphorylation enzyme activities in Brodmann area 7*

		Mitochondria uncorrected	Homogenates uncorrected	Mitochondria corrected by citrate synthase	Homogenates corrected by citrate synthase
Complex I	AD	21.2 ± 4.5	20.8 ± 4.6	0.068 ± 0.013	0.022 ± 0.004
	Control	26.9 ± 2.9	23.4 ± 3.8	0.093 ± 0.010	0.025 ± 0.004
Complex IV	AD	240.8 ± 84.8	168.8 ± 13.6	0.569 ± 0.036[a]	0.188 ± 0.015
	Control	285.7 ± 25.1	191.4 ± 26.8	0.809 ± 0.051	0.233 ± 0.024
Complex V	AD	521.2 ± 98.6	1,401.4 ± 100.0	1.36 ± 0.157	1.55 ± 0.109
	Control	398.3 ± 37.1	1,315.2 ± 141.1	1.45 ± 0.141	1.40 ± 0.135
Citrate synthase	AD	399.3 ± 108.3			
	Control	298.9 ± 28.9			

Data are means ± SEM in nmol/min/mg of protein. For mitochondrial preparations, n is 19 (AD) and 23 (control); for homogenates, n is 13 (AD) and 10 (control).

[a] $p < 0.001$.

TABLE 3. *Oxidative phosphorylation enzyme activities in Brodmann areas 8/9*

		Mitochondria uncorrected	Mitochondria corrected by citrate synthase
Complex I	AD	17.3 ± 2.2	0.057 ± 0.006
	Control	16.7 ± 1.6	0.056 ± 0.004
Complex II–III	AD	57.2 ± 12.3	0.168 ± 0.013
	Control	53.6 ± 5.2	0.180 ± 0.015
Complex IV	AD	192.8 ± 21.6	0.615 ± 0.063[a]
	Control	246.5 ± 23.0	0.826 ± 0.059
Complex V	AD	532.2 ± 106.9[a]	1.52 ± 0.09[a]
	Control	324.5 ± 27.3	1.20 ± 0.11
Citrate synthase	AD	358.4 ± 64.0	
	Control	292.6 ± 12.9	

Data are means ± SEM in nmol/min/mg of protein. n is 19 for AD and 23 for control.
[a] $p < 0.05$.

cortical regions examined. Our results concur with those of Kish et al. (1992), who found that cytochrome oxidase activity was reduced by 26% in frontal cortex and 17% in temporal cortex. In their study, smaller decreases in parietal and occipital cortex did not reach significance, and there was no decrease in activity in either the putamen or hippocampus.

We have extended their findings by examining the other enzymes that catalyze oxidative phosphorylation. Although there was a small decrease in complex I and II–III activities in occipital cortex, the activities of these enzymes were not altered significantly in other cortical regions. Of interest, complex V activity was increased in both the frontal and occipital cortices, which could represent a compensation for reduced cytochrome oxidase activity. Although some of our patients were receiving low doses of neuroleptic medications, these have been reported to have effects on complex I activity, but not on complex IV activity (Burkhardt et al., 1993). No significant changes in citrate synthase activity were observed. We used the activity of this enzyme as a biochemical marker for mitochondria and corrected oxidative phosphorylation enzyme activities by dividing enzyme activity by citrate synthase activity. This correction should compensate for variable mitochondrial enrichment and variable amounts of mitochondria in the samples. In general, this correction resulted in less variance in the data, and in some brain regions significant decreases were detected with corrected values, but not uncorrected values.

We examined the oxidative phosphorylation enzyme activities in both partially purified mitochondria and tissue homogenates. The partially purified mitochondria have the advantage of being less affected by cell loss; however, if some mitochondria are damaged, they may not survive the procedures employed in the isolation of mitochondria. Partially purified mitochondrial preparations may also preferentially reflect nonsynaptic mitochondria (Clark and Nicklas, 1970). Although we obtained similar results with both crude mitochondria and tissue homogenates, the crude mitochondria showed only about a twofold increase in enzyme activities. This indicates that they had significant contamination, which is probably due to both postmortem factors and freezing/thawing prior to their preparation. Using highly purified brain mitochondria from nonfrozen postmortem tissue, Parker et al. (1994) recently found ~50% decrease in cytochrome oxidase activity, with much smaller decreases in the activities of the other electron transport enzymes.

A number of previous studies have implicated mitochondrial abnormalities in the pathogenesis of AD. Electron microscopic studies showed mitochondria with increased matrix densities and intercrystal paracrystalline inclusions (Saraiva et al., 1985). Further-

TABLE 4. *Oxidative phosphorylation enzyme activities in Brodmann areas 20/21/22*

		Mitochondria uncorrected	Homogenates uncorrected	Mitochondria corrected by citrate synthase	Homogenates corrected by citrate synthase
Complex I	AD	10.92 ± 2.26	5.05 ± 0.74	0.040 ± 0.007	0.028 ± 0.004
	Control	7.71 ± 1.04	5.11 ± 0.55	0.034 ± 0.004	0.032 ± 0.005
Complex II–III	AD	38.46 ± 5.06	19.89 ± 2.25[a]	0.164 ± 0.023	0.116 ± 0.017[a]
	Control	48.64 ± 4.41	27.02 ± 2.00	0.225 ± 0.019	0.165 ± 0.017
Complex IV	AD	152.0 ± 11.6	67.39 ± 4.56[a]	0.600 ± 0.048[b]	0.377 ± 0.032[b]
	Control	178.6 ± 13.1	85.86 ± 6.26	0.795 ± 0.053	0.514 ± 0.040
Complex V	AD	454.5 ± 71.6	345.3 ± 19.8	1.64 ± 0.12	1.90 ± 0.096
	Control	380.5 ± 23.5	340.1 ± 19.8	1.70 ± 0.09	2.00 ± 0.091
Citrate synthase	AD	283.8 ± 37.7	188.1 ± 8.0		
	Control	228.7 ± 18.7	172.2 ± 8.8		

Data are means ± SEM in nmol/min/mg of protein. n is 21 for AD and 15 for control.
[a] $p < 0.05$; [b] $p < 0.01$.

TABLE 5. *Oxidative phosphorylation enzyme activities in Brodmann areas 17/18*

		Mitochondria uncorrected	Mitochondria corrected by citrate synthase
Complex I	AD	3.58 ± 0.64[a]	0.013 ± 0.002[a]
	Control	6.62 ± 0.73	0.024 ± 0.002
Complex II–III	AD	50.56 ± 4.59[b]	0.200 ± 0.015[b]
	Control	71.60 ± 4.51	0.253 ± 0.014
Complex IV	AD	126.3 ± 7.6[c]	0.508 ± 0.029[c]
	Control	203.3 ± 14.3	0.712 ± 0.041
Complex V	AD	281.7 ± 18.0	1.16 ± 0.084[a]
	Control	251.8 ± 15.1	0.90 ± 0.056
Citrate synthase	AD	253.1 ± 11.8	
	Control	289.0 ± 15.1	

Data are means ± SEM in nmol/min/mg of protein. n is 22 for both AD and control.
[a] $p < 0.01$; [b] $p < 0.05$; [c] $p < 0.001$.

more, we recently reported several mutations in mitochondrial DNA that are found with increased frequency in patients with AD or Parkinson's disease and that may serve as risk factors for the diseases (Shoffner et al., 1993). The activities of several other mitochondrial enzymes have been reported to be reduced in AD postmortem brain tissue, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase (Sheu et al., 1985; Gibson et al., 1988; Butterworth and Besnard, 1990; Mastrogiacomo et al., 1993). The latter enzyme was also reduced in fibroblasts of familial AD patients with chromosome 14 defects (Sheu et al., 1994).

Cytochrome oxidase is of particular interest, because it is tightly coupled to ATP production by the electron transport chain and it has been used as a marker of neuronal activity (Wong-Riley, 1989; Hevner and Wong-Riley, 1993). The findings of Parker et al. (1990) of a 68% decrease in cytochrome oxidase activity in AD platelets are, therefore, of great interest. Using simplified methods for purifying platelet mitochondria, a subsequent study did not replicate the finding (Van Zuylen et al., 1992); however, it has been confirmed in a further study of Parker et al. (1994*b*). Reduced activity in platelets would suggest a systemic abnormality that could not be attributed to neuronal loss. The recent studies of Chandrasekaran et al. (1992*a*) showed that cytochrome oxidase subunits I, II, and III are preferentially expressed in primate association cortex. Within the entorhinal cortex, cytochrome oxidase subunit II mRNA and cytochrome oxidase activity are expressed preferentially in layer II and VI neurons, which are prone to neurofibrillary tangle formation in AD (Hyman et al., 1990; Chandrasekaran et al., 1992*b*; Hevner and Wong-Riley, 1992). Therefore, a reduction in cytochrome oxidase activity may contribute to selective neuronal vulnerability in AD. There is a twofold decrease in cytochrome oxidase subunit I and III mRNA expression in the midtemporal gyrus in AD, with no change in mRNA for actin or lactate dehydrogenase (Chandrasekaran et al., 1993). Alberts et al. (1992) found a gene homologous to cytochrome oxidase subunit III that was overexpressed in AD, which raised the possibility of a compensatory increase in expression of this gene. Cytochrome oxidase activity is reduced in the perforant pathway terminal zone in AD; however, this finding could be a consequence of either neuronal loss or dysfunction (Simonian and Hyman, 1993).

The significance of the present results is unclear. Cytochrome oxidase activity has been reported to reflect neuronal activity (Wong-Riley, 1989; Hevner and Wong-Riley, 1993). The decrease in enzyme activity could be a result of decreased enzyme synthesis due to reduced energy requirements in AD brains. Arguing against this, however, is the observation that levels of cytochrome aa_3, a component of cytochrome oxidase, are normal in AD brains (Parker et al., 1994*a*). This suggests that cytochrome oxidase is present in normal concentrations, but is catalytically abnormal. The decrease in cytochrome oxidase activity is unlikely to be merely a consequence of neuronal degeneration, because it would be expected that other oxidative phosphorylation enzymes would be equally affected. A decrease in cytochrome oxidase activity could be a consequence of lipid abnormalities, such as decreased cardiolipin content, or a consequence of oxidative stress. Both complex I and complex IV are susceptible to injury from free radicals and exhibit age-related decreases in their activities (Zhang et al., 1990; Bowling et al., 1993). Complex I activity was largely preserved in the present study, except in occipital cortex, which is the most metabolically active of all cortical regions. It is, therefore, possible that oxidative damage could contribute to the changes observed in occipital cortex.

A decrease in cytochrome oxidase activity could contribute to the pathogenesis of AD in several ways. Head trauma has been reported to be a risk factor for AD in several studies (Blass, 1993), and experimental concussive brain injury is followed by a decrease in cortical cytochrome oxidase activity for up to 10 days after the insult (Hovda et al., 1991). Inhibition of cytochrome oxidase with sodium azide leads to a marked increase in production of potentially amyloidogenic fragments in cultured neurons (Gabuzda et al., 1994). Moreover, a reduction in ATP can activate specific kinases, which then phosphorylate the τ protein and lead to neurofibrillary tangle formation (Roder and Ingram, 1991; Roder et al., 1993). A defect in energy production may also result in an increase in vulnerability of neurons to excitatory amino acids, leading to neuronal degeneration by a slow excitotoxic process (Beal, 1992). Lastly, a defect in cytochrome oxidase activity may contribute to free radical generation. A decrease in cytochrome oxidase will lead to reduction

of more proximal components of the electron transport chain, including ubiquinone. This can lead to the generation of superoxide and other free radicals, which then damage adjacent DNA, proteins, and lipids. Consistent with this possibility, we found a threefold increase in oxidative damage to mitochondrial DNA in AD postmortem brain tissue (Mecocci et al., 1994). Additional consequences of increased free radical formation may be aggregation and deposition of amyloid, as well as a contribution to the formation of neurofibrillary tangles (Dyrks et al., 1992, 1993; Troncoso et al., 1993). Studies of inhibitors of cytochrome oxidase in animals show cognitive deficits (Bennett et al., 1992), raising the possibility that cytochrome oxidase deficiency could contribute to the behavioral manifestations of AD.

The present results provide further confirmation that there is a deficiency in cytochrome oxidase activity in AD. It is as yet unclear whether this is a primary or secondary consequence of the disease process. Nevertheless, these findings provide further evidence that a defect in energy metabolism may contribute to the pathogenesis of AD.

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