Functional Alterations in Alzheimer's Disease: Selective Loss of Mitochondrial-encoded Cytochrome Oxidase mRNA in the Hippocampal Formation

NANCY A. SIMONIAN, M.D. AND BRADLEY T. HYMAN, M.D., PH.D.

Abstract. The activity of cytochrome oxidase (CO), the terminal enzyme of the electron transport chain, has been reported to be decreased in the brains of individuals with Alzheimer's disease (AD). In experimental models, CO activity decreases following functional deafferentation of neural circuits. CO is a holoenzyme composed of 13 nuclear- and mitochondrial-encoded subunits and experimental data indicate that the change in CO activity following deafferentation is controlled primarily by regulation of mitochondrial CO gene expression. It has been proposed that the hippocampal formation is deafferented in AD. We therefore hypothesized that an alteration in mitochondrial CO gene expression might underlie the reduction in CO activity in AD. Using in situ hybridization, we found a selective reduction in mRNA levels for a mitochondrial-encoded subunit, CO II, with preservation of mRNA for a nuclear-encoded subunit, CO IV, in the hippocampal formation of individuals with AD. The reduction in CO II mRNA levels was seen both in regions with neurofibrillary tangles, senile plaques, and neuronal loss and regions relatively spared from these neuropathological changes. These data suggest that the reduction in CO activity in brain regions from individuals with AD may be a result of an alteration in mitochondrial CO gene expression that extends beyond neurons directly affected by structural pathology.

Key Words: Alzheimer's disease; Cytochrome oxidase; In situ hybridization; Mitochondria; Oxidative phosphorylation.

INTRODUCTION

Cytochrome oxidase (CO), or complex IV, is the terminal enzyme of the electron transport chain (1, 2). This enzyme catalyzes the transfer of electrons to generate adenosine triphosphate (ATP) via the coupled process of oxidative phosphorylation. CO activity is high in the dendrites of neurons receiving excitatory input, and low in axons and glia. CO activity is highly regulated and correlates closely with the functional level of neuronal activity (1). For example, in experimental systems, CO activity decreases in primary visual cortex following monoclonal visual deprivation and in the molecular layer of the dentate gyrus following lesions of the perforant pathway (3–5). CO is a holoenzyme composed of 13 subunits in a 1:1 stoichiometry (6). The three largest polypeptide subunits, CO I–III, are encoded in the mitochondrial genome and are responsible for catalytic function. CO subunits IV–XIII are encoded in the nucleus. CO activity is thus dependent on the coordinated expression of both mitochondrial and nuclear genes.

We and others have recently reported a reduction in CO activity in the brains of individuals with Alzheimer's disease (AD) (7–9). The molecular basis for this alteration is unknown. We previously suggested that the decrease in CO activity in brain regions from individuals with AD may be secondary to altered neuronal functional activity from deafferentation of neural circuits (8). Experimental data indicate that mitochondrial and nuclear genes for oxidative phosphorylation enzymes are differentially regulated by decrements in neuronal function. For example, a disproportionate reduction in mRNA for mitochondrial-encoded CO subunits has been reported in the lateral geniculate nucleus of the monkey following monoclonal visual deprivation (10) and in CA1 neurons of the gerbil following transient forebrain ischemia (11). Based on these data, we hypothesized that an alteration in mitochondrial CO gene expression might underlie the decrease in CO activity in AD. Using in situ hybridization, we investigated the levels of a mitochondrial-encoded transcript, CO II, and of a nuclear-encoded transcript, CO IV, in the hippocampal formation of individuals with AD and aged controls.

MATERIALS AND METHODS

Tissue Preparation

Temporal lobes from individuals with and without a clinical (12) and neuropathological (13) diagnosis of AD were obtained from the Alzheimer's Disease Research Center Brain Bank. Tissue was fixed in paraformaldehyde-lysine-metaperoxidase at 4°C for 24–48 hours and then cryoprotected in 15% glycerol in 0.1 M phosphate buffered saline (PBS), pH 7.4. Fifty-micron sections were cut on a freezing slide microtome and individually stored in 15% glycerol in PBS at -80°C until use.

Design and Synthesis of Oligonucleotide Probes

Oligodeoxyribonucleotide probes 45 bases in length for CO II (14) and 48 bases for CO IV (15) were designed using published sequences obtained from Genebank. A search of the Genebank and EMBL databases using the National Center for
Biotechnology Information BLAST network service (16) revealed no significant homology of these probes to known sequences other than intended targets. Probes were synthesized using an Applied Biosystems 392 Synthesizer and purified by electrophoresis through a 12% polyacrylamide gel containing 8 M urea. Probes were 3' end labeled with ^32P-alpha dATP (specific activity > 1,000 Ci/mM, Dupont-NEN, Wilmington, DE) and terminal deoxynucleotidyl transferase using a Dupont-NEN kit (NEP 100).

Oligonucleotide probes:

CO II—atatgaggaactaaggatagtgggcccagga-
      tattgcaggggtttct;
CO IV—ttcttttctagctccatgtgacaggtcata-
      gccctggatggggttccac

In situ Hybridization

Free-floating sections were mounted on poly-L-lysine-coated slides (Sigma, St. Louis, MO). Slides were fixed in 4% paraformaldehyde in PBS, pH 7.4, for 10 minutes (min), washed in three changes of PBS, pH 7.4, acetylated in 0.1 M triethanolamine, pH 8.0, with 0.25% acetic anhydride, rinsed in PBS and dehydrated through graded ethanol solutions. Hybridization was performed in 50% formamide, 0.3 M NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA, 10% dextran sulfate, 1 X Denhardt’s solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.2 mg/ml bovine serum albumin, Sigma), and 100 mM dithiothreitol. Radiolabeled DNA probes were added to a concentration of about 30,000 DPM per ml of hybridization solution, and sections were incubated in 80 ml of this solution under glass coverslips overnight at 37°C. Following hybridization, the slides were washed sequentially in 2X SSC (20 min at room temperature [RT]), 1X SSC (10 min, RT), 0.5X SSC (10 min, RT), 0.5X SSC (40 min, 60°C), 0.5X SSC (10 min, RT), rinsed briefly in 70% ethanol, air dried, and exposed to film (βmax, Amersham, Arlington Heights, IL) for 1 to 14 days.

The specificity of labeling was examined by pretreating sections with Ribonuclease A (Sigma, 50 mg/ml in 100 mM Tris, 25 mM EDTA, pH 8.0, 37°C for 30 min), or including a 100-fold excess of unlabeled DNA probe in the hybridization solution. In all cases, these treatments abolished hybridization signal. In addition, no signal was seen with a complementary sense probe to CO II.

Analysis of Autoradiograms

Film autoradiograms were analyzed using a computer-assisted image analysis system (MCID, Imaging Research, St. Catherine's, Ontario, Canada). The instrument was calibrated using a Kodak Optical Density Standard (Eastman Kodak, Rochester, NY). Anatomical borders were identified by direct inspection of the images. The mean optical density in each region was measured. Optical density in each region was compared in AD and aged control brains using an unpaired t-test. In order to correct for multiple chance effects, p values adjusted for multiple tests were computed using the bootstrap resampling method.

RESULTS

CO II

In situ hybridization for CO II was performed in the hippocampal formation from eight control individuals (mean age 71 years, range 49–94; mean postmortem interval [PMI] 12 hours) and ten individuals with AD (mean age 75 years, range 52–95; mean PMI 12 hours). CO II mRNA was present in the cell layer of the dentate gyrus (DG), CA4, CA3 and CA1 (Fig. 1A, B). In addition, CO II mRNA was present diffusely throughout the molecular layer of the DG. There was no difference in the pattern of distribution of CO II mRNA in AD.

There was a large reduction in the optical density of CO II mRNA in all subfields of the hippocampal formation in AD (Figs. 1B, 2). The mean optical density in the DG was 0.374 in controls and 0.196 in AD (t = −2.82; p < 0.01); in the inner molecular layer of the DG, 0.334 in controls and 0.155 in AD (t = −2.89; p < 0.01); in the outer molecular layer of the DG, 0.287 in controls and 0.141 in AD (t = −2.76; p < 0.01); in CA4, 0.281 in controls and 0.119 in AD (t = −3.23; p < 0.005); in CA3, 0.369 in controls and 0.194 in AD (t = −3.29; p < 0.005); and in CA1, 0.292 in controls and 0.155 in AD (t = −2.88; p < 0.01). There was no correlation between age or PMI and optical density.

CO IV

In situ hybridization for CO IV was performed in the hippocampal formation from seven aged control individuals (mean age 72 years, range 49–94; mean PMI 12 hours) and eight individuals with AD (mean age 74 years, range 52–95; mean PMI 13 hours). CO IV mRNA was present in the DG, CA4, CA3 and CA1 (Fig. 1C, D). Unlike CO II mRNA, CO IV mRNA was not present in the molecular layer of the DG. There was no difference in the anatomic pattern of distribution of CO IV mRNA in individuals with AD. There was no significant difference in the optical density of CO IV mRNA in any hippocampal region in AD (Figs. 1D, 3).

DISCUSSION

We previously reported a decrease in CO activity in the hippocampal formation from brains of individuals with AD (8). We now report a reduction in mRNA for the mitochondrial-encoded CO II with preservation of mRNA for the nuclear-encoded CO IV. Decreased levels of CO II mRNA were found in the dentate granule cell layer, CA4, CA3 and CA1. This anatomic distribution of CO II mRNA loss paralleled the sites of CO activity loss previously reported (8). In general, though, there was a greater reduction in the optical density of CO II mRNA, approximately 50% in each area, than in enzyme activity which ranged from 20–32% (8). A large decrease in CO II mRNA levels was also seen in the molecular layer of the DG, presumably reflecting loss of dendritic transcripts (17). The observation that the degree of CO II mRNA loss was similar in an area that has neuronal loss and atrophy (CA1) and in areas spared (CA3 and CA4) suggests that functional status, rather than neuronal number, is the primary determinant of CO II mRNA signal. The comparable reduction in CO activity (8) and CO II mRNA levels in CA1 and in CA3 and CA4 may be re-
lated to a smaller change in the density of neurons in CA1 because of atrophy.

Our results are consistent with those of Chandrasekaran et al. (18) who found a 50% decrease in mRNA levels for two other mitochondrial-encoded CO subunits, CO I and III, with preservation of transcripts for lactate dehydrogenase, beta actin and 12S rRNA in the mid temporal gyrus in AD. Preliminary data from our laboratory suggest that another nuclear-encoded CO transcript, CO VIII, is also preserved in AD. Taken together, these results suggest that the reduction in CO activity in AD may be related to an alteration in CO mitochondrial gene expression.

Mitochondrial-encoded CO subunit transcripts have previously been reported to be sensitive to decrements in neuronal function. For example, following monocular deprivation of primates, Hevner et al. (10) examined the lateral geniculate nucleus and found a 49% decrease in mRNA levels for a mitochondrial-encoded CO subunit, CO I, but only an 18% decrease in levels for a nuclear-encoded CO subunit, CO IV. These results suggest that alterations in CO activity following deafferentation may be primarily controlled by the regulation of mitochondrial genes. Likewise, in CA1 neurons following transient forebrain ischemia in the gerbil, Abe et al. (11) found an early and progressive reduction in the mitochondrial-encoded CO I mRNA levels with preservation of activity of a nuclear-encoded oxidative phosphorylation chain enzyme, succinate dehydrogenase. Taken together, these results suggest that mitochondrial-encoded CO mRNA are sensitive to alterations in neuronal activity from both deafferentation and pathologic processes that involve excess glutamatergic stimulation and depleted energy stores.

We have previously shown that neurofibrillary tangles (NFT) selectively affect specific lamina and subfields in the entorhinal cortex, hippocampus and higher order association areas, and have suggested that this neuroanatomic pattern of neural destruction produces a disconnection syndrome (8, 19, 20). This principle is most
clearly illustrated in the hippocampal formation, where neurons in layer II of the entorhinal cortex, the origin of the major afferent to the hippocampus, the perforant pathway, are selectively destroyed. The terminal zone of the perforant pathway, the molecular layer of the DG, loses synaptophysin immunoreactivity (21), glutamate content (22), and undergoes neuroplasticity-mediated remodeling (23, 24).

In our earlier study, we demonstrated that CO activity is reduced in the terminal zone of the perforant pathway. We hypothesized that the reduction in CO activity was related to deafferentation from NFT at the origin of the perforant pathway. A direct relationship between the severity of NFT in the entorhinal cortex and CO activity, however, was not possible because the number of NFT in the entorhinal cortex reaches a peak very early in the course of the disease (20). In addition to the molecular layer of the DG, we found reductions in CO activity in downstream projection areas, CA4, CA3, and CA1, as well (8). While NFT and neuronal loss are extensive in CA1, CA4 and CA3 are relatively spared (20, 25), suggesting that alterations in neuronal metabolic activity in AD occur both in regions with and without structural pathology.

We have now expanded this observation to CO gene expression. We found a large reduction in mitochondrial-encoded mRNA for CO II both in hippocampal subfields with and relatively spared from NFT, senile plaques, and neuronal loss. This result is consistent with our hypo-

ACKNOWLEDGMENTS

We thank the Massachusetts Alzheimer’s Disease Research Center Brain Bank (E.T. Hedley-Whyte, director), Dr. Joseph Locascio for statistical advice and Larry Cherkas for photography assistance.

REFERENCES

4. Wong-Riley MTT. Changes in the visual system of monocularly reared or enucleated cats demonstrable with cytochrome oxidase histochemistry. Brain Res 1979;171:11–28
10. Hevner RF, Wong-Riley MTT. Mitochondrial and nuclear gene ex-


Received March 3, 1994
Revision received April 21, 1994
Accepted April 21, 1994