Reduction of Calcineurin Enzymatic Activity in Alzheimer's Disease: Correlation with Neuropathologic Changes

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Abstract. Neurofibrillary tangles (NFT), neuritic plaques, and dystrophic neurites are the classic neuropathologic hallmarks of Alzheimer's disease (AD), all of which contain to varying degrees normally and/or hyperphosphorylated forms of the microtubule-associated protein tau. Protein phosphatase 2B (calcineurin) dephosphorylates tau isolated from AD brains to control levels in vitro as well as regulates tau phosphorylation and function in vivo. It has been hypothesized that the changes in tau phosphorylation observed in AD may be due to increases in kinase activity and/or decreases in phosphatase activity. In order to investigate the latter possibility, we examined calcineurin enzyme activity using the substrate para-nitrophenylphosphate (pNPP) in postmortem brain samples from individuals with moderate to severe AD (n=8) and age-matched controls (n=7). The stimulation of calcineurin activity by manganese chloride (1 mM) was reduced by 60% (p<0.01) in whole-cell homogenates prepared from AD temporal cortex (Brodmann area 38). On the other hand, in P2 membrane fractions, the stimulation of calcineurin activity by manganese chloride as well as nickel chloride (1 mM) was reduced by 37% (p<0.05) and 79% (p<0.01), respectively. The manganese-stimulated calcineurin activity in the temporal cortex inversely correlated with both the number of NFT (r = -0.60, p<0.02) and neurite/neuropil plaques (r = -0.63, p<0.02) in whole-cell homogenates, but only with NFT (r = -0.61, p<0.02) in P2 membrane fractions. The nickel-stimulated calcineurin activity did not correlate with neuropathology measures in either whole-cell or P2 membrane fractions. In striate/visual cortex (Brodmann area 17), an area relatively unaffected in AD, neither whole-cell nor P2 membrane calcineurin activity were significantly altered. To our knowledge, this is the first report of a reduction in calcineurin phosphatase activity in AD which correlates with the neuropathological features in a region-, subcellular fraction-, and divalent cation-specific manner.

Key Words: Alzheimer's disease; Calcineurin; Neuropathy.

INTRODUCTION

Alzheimer's disease (AD) is characterized symptomatically by a progressive deterioration of cognitive and mnemonic function accompanied by distinct histopathological lesions in discrete brain regions. Senile plaque, neurofibrillary tangle (NFT), and dystrophic neurite densities have been shown, to varying degrees, to be positively correlated with the extent of clinical dementia in AD (1–3). Recent studies suggest neocortical NFT density is strongly correlated with premorbid severity of dementia (3–7). Examination of the ultrastructure of neurofibrillary tangles (NFT) indicate that they are composed of paired helical filaments (PHF) (for review see 8), a primary constituent of which is abnormally phosphorylated forms of the microtubule-associated protein tau (9). Phosphorylation of threonine and serine residues within the tandem repeat domain, the putative tubulin binding site, attenuates tau's ability to bind to microtubules in vitro (10, 11). PHF-tau isolated from AD brain does not promote microtubule polymerization unless pretreated with a phosphatase (12). Therefore, an alteration of tau phosphorylation in vivo may destabilize microtubule structures, compromise axonal transport, and perhaps contribute to the neurodegeneration observed in AD. Given that the change in tau phosphorylation may play a role in NFT formation, as well as the strong relationship of NFT density with cognitive impairment in AD, the processes underlying AD-related changes in tau phosphorylation may also contribute to the clinical manifestation of dementia.

The phosphorylation state of a protein is determined by the net balance between the activities of kinases and phosphatases. The changes in tau phosphorylation observed in AD may be due to an increase in tau kinase activity and/or a diminution of tau phosphatase activity. Several candidate tau kinases have been identified which can phosphorylate some of the sites phosphorylated in AD (13–15). While an increase in the overall phosphorylation level of a protein may be the result of increased kinase activity, a chronic reduction of phosphatase activity could have the same net effect irrespective of kinase activity.

Abnormally hyperphosphorylated tau isolated from AD brains has been shown to be efficiently dephosphorylated by protein phosphatase 2A (PP2A) and calcineurin (PP2B) (16–18). Dephosphorylation of AD tau by these phosphatases restores tau's ability to bind to microtubules and promote tubulin polymerization (12). In the brain, PP2A is expressed in neurites, neuronal perikarya (19, 20), as well as microglia (20). In contrast, the distribution
of calcineurin in the CNS is reported to be entirely neuronal (21, 22). Like other type 2 serine-threonine phosphatases, PP2A and calcineurin are stimulated by divalent cations. While both phosphatases have been reported to be stimulated by Mn²⁺ (23–25), calcineurin is also stimulated by Ni²⁺ (25, 26). Protein phosphatase 2A and calcineurin can be further discriminated based on their sensitivity to selective phosphatase inhibitors. Nanomolar concentrations of okadaic acid or calyculin A selectively block the activity of PP2A (27). Calcineurin activity is inhibited by micromolar concentrations of okadaic acid (28), the antipsychotic trifluoperazine (26), as well as the immunosuppressants cyclosporin A and FK506 (29).

Converging lines of evidence indicate that an impairment of protein phosphatase activities may underlie the changes in tau phosphorylation and function observed in AD. Knock-out mice lacking the gene for the calcineurin catalytic subunit exhibit an accumulation of hyperphosphorylated tau in the hippocampus (30). Incubation of metabolically active slices of human temporal cortex slices with micromolar okadaic acid (which inhibits PP1, PP2A and calcineurin) produced changes in tau phosphorylation similar to PHF tau (28). A recent study of tau phosphorylation in biopsy-derived human temporal cortex found that several phosphorylation sites thought to be normally and/or hyperphosphorylated in AD postmortem tissue were actually phosphorylated in samples from control brain (31). It was hypothesized that these abnormally phosphorylated residues were rapidly dephosphorylated by endogenous phosphatases in the biopsy sample. One interpretation of Matsuo et al (31) is that a reduction in the activity of tau phosphatases in AD may underlie the observed change in tau phosphorylation.

Studies of phosphatases in postmortem brain samples suggest a reduction in the overall activity of phosphatases in AD. Shimohama et al (32) demonstrated a reduction in the activity of low molecular weight acid phosphatase in AD temporal cortex extracts. A study of the activities of PP1, PP2A, calcineurin (PP2B), and PP2C in frontal lobe found a modest reduction in the activities of PP1 and 2A with no change in the activity of calcineurin or PP2C (33). More recently, a 30% reduction in tau phosphatase activity in AD frontal cortex has been reported using tau isolated from AD brain as a substrate (34); however, the specific phosphatase accounting for the reduction was not determined. While one study of calcineurin protein expression in AD found a decrease in calcineurin immunoreactivity in individual tangle-bearing neurons (20), another study found no difference in total calcineurin protein levels measured by Western blotting between AD and control brain samples (22). In order to further investigate the relationship between calcineurin and neuropathological findings in AD, we characterized and quantitated the actual enzymatic activity of calcineurin in AD and control cortical gray matter homogenates and membrane fractions. In addition, we correlated calcineurin activity with neuropathologic findings in tissue sections from the temporal and occipital cortices.

MATERIALS AND METHODS

Materials

Cyclosporin A (Sandimmune®) was purchased from Sandoz (East Hanover, NJ). okadaic acid and calyculin A from Calbiochem (La Jolla, CA). All other materials were purchased from Sigma (St Louis, MO).

Brain Specimens

Brain samples were obtained from the Loyola University Brain Bank (Maywood, IL). All brain samples were collected at autopsy, dissected into specific Brodmann regions, snap-frozen at −60°C in isopentane, and stored at −80°C until use. Adjacent sections were fixed in 10% buffered formalin and stained using a modified Bielschowsky method. The diagnosis of AD was determined using the CERAD criteria based on isocortical neuritic plaque density (35). In order to quantitate the neuropathological lesions, silver-stained brain sections were examined under high power with the number of NFT, total amyloid plaques, and neuritic/core plaques counted and expressed as the number per high power field (200×).

Samples used in the present study were taken from temporal cortex (Brodmann area 38) and striate cortex (Brodmann area 17). Control and AD samples were matched with respect to age and postmortem interval. Brain tissue from seven controls (mean age ± S.D., 73 ± 8 years; mean postmortem interval, 14 ± 3 hours) were from individuals without a history of neuropsychological or cognitive impairment and with neuropathological findings consistent with age. Eight AD patients (mean age ± S.D., 79 ± 8 years; mean postmortem interval, 14 ± 2 hours) were included in the study. Linear regression analysis of both postmortem interval (range of 6 to 24 hours) and age (range: 53 to 85 years) with phosphatase activity found no evidence of a relationship between these two variables (data not shown).

Sample Preparation: Whole Cell Homogenate and P2 Membrane Fractions

Samples of frozen cortex were fractured, trimmed of white matter, weighed (~300 mg), and homogenized (100 mg/ml) in buffer A (20 mM HEPES, pH 7.4, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 0.5 mM EDTA, 0.5 mM EGTA, 20 µg/ml leupeptin) using a Polytron homogenizer (setting 6, twice for 15 s). An aliquot of the homogenate was removed for phosphatase assay and constituted the whole cell homogenate (cytosol + membrane) preparation. The remaining homogenate was centrifuged at 49,000 g for 10 minutes at 4°C. The resulting supernatant was discarded, the pellet was resuspended in Buffer A (10 volumes/weight), homogenized, and spun again at 49,000 g for 10 minutes at 4°C. The supernatant from this spin was discarded and the resulting pellet resuspended in 10 volumes of buffer A, kept on ice, and constituted.
Fig. 1. (A) Nickel chloride (1 mM) stimulates the activity of endogenous phosphatases in brain homogenates. A representative curve from control whole cell homogenate is depicted. Nickel-stimulation of phosphatase activity is completely blocked by the addition of 0.5 μM cyclosporin A (CsA). Based on the sensitivity of the nickel-stimulated phosphatase to CsA, we defined the nickel-stimulated phosphatase activity as calcineurin (protein phosphatase 2B). Similar results were obtained with P2 membrane fractions (data not shown). (B) Manganese chloride (1 mM) stimulates phosphatase activity in brain homogenates. A representative curve from control whole cell homogenate is depicted. Manganese-stimulated phosphatase activity is only partially inhibited by 0.5 μM CsA. The cyclosporin A-sensitive component of the manganese-stimulated phosphatase activity was defined as calcineurin (protein phosphatase 2B), while CsA-resistant phosphatase activity was defined as non-PP2B phosphatase.

The membrane fraction (crude P2 fraction) for subsequent phosphatase assays. Protein concentration was determined using a BioRad protein assay kit based on the Bradford assay (36).

Phosphatase Assay

Phosphatase activity was assayed using the chromogenic substrate para-nitrophenylphosphate (pNPP). Previous studies have shown that this compound is a substrate for purified calcineurin (24, 25). Phosphatase catalyzed formation of p-nitrophenol from pNPP was monitored by measuring changes in absorbance at 405 nm using a Shimadzu UV160U spectrophotometer. For whole cell homogenate assays, 25 μl of brain homogenate was added to a polystyrene cuvette containing buffer B (20 mM HEPES, pH 7.4) in the presence or absence of divalent cations (1 mM NiCl₂ or MnCl₂), or cyclosporin A (0.5 μM). Cyclosporin A was diluted with methanol (2:1). At the concentration of cyclosporin A used in the study (0.5 μM), methanol was 0.8% of total reaction volume and did not influence phosphatase activities. Assays of P2 membrane fractions were identical except that 50 μl of the tissue suspension was used. Final reaction volume was 1 ml. Aliquots of brain homogenates were preincubated for 10 minutes with or without divalent cations and/or phosphatase inhibitors. The reaction was started by the addition of the substrate pNPP (20 mM).

Phosphatase activity was assayed at 25°C in a darkened room (to prevent photolysis of pNPP) for 20 minutes with absorbance readings taken at two minute intervals. The first absorbance reading was taken after a two minute lag time to allow for substrate equilibration. Under these conditions, the change in absorbance due to phosphatase activity was linear with respect to time (mean r² value > 0.99) and tissue concentration (data not shown). A standard curve was constructed using known concentrations of the dephosphorylation product p-nitrophenol and these values were used to convert changes in absorbance to nanomoles of p-nitrophenol formed. Specific enzyme activity was quantitated by dividing the nanomoles of phosphate released over twenty minutes by the protein concentration to give final activity units expressed as nanomoles p-nitrophenol formed per minute per mg protein. Experimental conditions employed in the study included basal activity (absence of divalent cations), nickel (1 mM NiCl₂), manganese (1 mM MnCl₂), and manganese + cyclosporin A (activity in presence of 1 mM MnCl₂ and 0.5 μM cyclosporin A). For a given brain sample, all experimental conditions were assayed simultaneously in a staggered fashion (15s intervals) to minimize time-dependent changes in extract phosphatase activity due to proteolytic degradation and/or activation of phosphatases.

Statistical Analysis

Computer-assisted data analysis utilized Statview software (v1.03, Abacus Concepts, Berkely, CA). Group comparisons were made using a paired t-test (two-tailed) to determine whether the addition of ions and/or inhibitors had a significant effect on phosphatase activity. Comparisons between control and AD groups utilized an unpaired t-test (two-tailed) to compare mean phosphatase activity under the different experimental conditions. Linear regressions were performed using neuroanatomical findings and phosphatase activity as covariates. Results were considered significant if p values were less than 0.05 on any of the above tests.

RESULTS

Characterization of Phosphatase Activity in Human Brain Extracts

The identification of specific calcineurin activity in brain homogenates is supported by a number of experimental findings. In controls, the addition of nickel chloride or manganese chloride (reported activators of calcineurin) produced a 134% and 316% increase in brain
whole-cell homogenate phosphatase activity over basal activity (p<0.01, two-tailed paired t-test) (Fig. 1A, B). Second, both the nickel- and manganese-stimulated phosphatases were inhibited by nanomolar concentrations of cyclosporin A, a specific inhibitor of calcineurin. The nickel-stimulated phosphatase was completely blocked by 0.5 μM cyclosporin A (p<0.01, two-tailed t-test) (Fig. 1A), suggesting that all of the nickel-stimulated phosphatase activity present in the homogenate is attributable to calcineurin. The addition of cyclosporin A (0.5 μM) significantly reduced manganese-stimulated phosphatase activity (p<0.01, two-tailed paired t-test) to approximately 50% of total manganese-stimulated phosphatase activity (Fig. 1B). In preliminary experiments, we also observed a similar inhibition of nickel and manganese-stimulated phosphatase activity by trifluoperazine, another inhibitor of calcineurin (data not shown). Although pNPP is reported to be a substrate for PP2A (37), both nickel and manganese-stimulated phosphatase activities were totally insensitive to 10 mM calyculin A and 10 mM okadaic acid, both of which selectively inhibit PP1 and PP2A (data not shown). Collectively, these observations suggest the presence of active calcineurin (PP2B) in both whole cell homogenate and P2 membrane preparations. Based on these findings, we defined calcineurin activity as the nickel-stimulated phosphatase activity over basal activity, as well as the component of the manganese-stimulated phosphatase activity that is sensitive to cyclosporin (0.5 μM) (Fig. 1A, B).

Calcineurin Activity in AD and Control Gray Matter Homogenates

Data from whole cell homogenate and crude P2 membrane preparations from temporal (Brodmann area 38) and striate cortices (Brodmann area 17) are presented in Figures 2 and 3. In temporal cortex whole cell homogenates, total manganese-stimulated phosphatase activity was decreased in AD by 35% (p<0.01, two-tailed t-test) This reduction in total manganese-stimulated phosphatase activity was due to a 60% decrease in calcineurin (cyclosporin A-sensitive) phosphatase activity (p<0.002, two-tailed t-test), while no changes in non-PP2B (cyclosporin A-insensitive) manganese-stimulated phosphatase activity were observed (Fig. 2A). In addition, there was no change in nickel-stimulated calcineurin activity in whole cell homogenates.

In P2 membrane fractions prepared from temporal cortex, both nickel-(PP2B) and total manganese-(PP2B and non-PP2B) stimulated phosphatase activity were reduced in AD by 80% and 37%, respectively (p<0.005 and p<0.02 respectively) (Fig. 2B). Although manganese-dependent calcineurin activity was not significantly reduced, it appeared that the reduction in total manganese-stimulated phosphatase activity was due to a cyclosporin A-sensitive (PP2B) component. In contrast, in striate cortex whole cell and P2 membrane fractions, divalent cation-stimulated calcineurin activity was not significantly affected in AD preparations compared to controls (Fig. 3). Basal (cation-independent) phosphatase activities were not changed in either cellular fraction or brain region in AD samples (data not shown).

Regression Analysis of Calcineurin Activity and Neuropathologic Findings

Linear regression analyses of control and AD cases were performed between neuropathological variables (counts of NFT, total amyloid plaques, and neuritic/core amyloid plaques) and phosphatase activities. In the temporal cortex whole cell homogenates, both NFT and neuritic/core plaque densities were inversely correlated with manganese-stimulated calcineurin activity (r = -0.62, p<0.02 for NFT and r = -0.61, p<0.02 for neuritic/core plaques) (Table 1). In the P2 membrane fraction, the manganese-stimulated calcineurin was inversely correlated with NFT density only (r = -0.61, p<0.02). No significant correlations were observed between nickel-stimulated calcineurin activity and neuropathological findings in either fraction in the temporal lobe. Neither nickel nor manganese-stimulated calcineurin activity was correlated with neuropathological findings in the striate cortex. Although there were no differences in absolute
activity was reduced in temporal cortex, an area exhibiting extensive neurodegenerative changes in AD, while no significant differences in absolute phosphatase activity were observed in striate cortex. Furthermore, there appeared to be a difference in the sensitivity of calcineurin to ion stimulation in different subcellular fractions in AD. That is, manganese-stimulated activity was reduced in temporal cortex whole cell and P2 membrane fractions, whereas a reduction in nickel-stimulated calcineurin activity was present only in the P2 membrane fraction. Although total manganese-stimulated phosphatase activity was reduced in AD temporal cortex P2 fractions and appeared to be largely due to a reduction in calcineurin activity, the group difference did not reach statistical significance.

A previous study examining calcineurin activity in AD brains failed to find any changes relative to controls (33). Although the mean postmortem interval in the present study was greater than that of the Gong et al study (33), we observed no relationship between postmortem interval and phosphatase activity. The discrepancy between Gong et al (33) and the present study may be due to differences in methodology and experimental design. Gong et al (33) examined calcineurin phosphatase activity in the cytosolic component of superior frontal cortex homogenates using 32P-labeled phosphorylase kinase, with calcium and nickel as calcineurin activators, and trifluoperazine as a calcineurin inhibitor. In the present study we examined calcineurin activity in different brain regions (temporal and occipital cortices), in different subcellular fractions (homogenate vs membrane), with a different substrate (pNPP), as well as different calcineurin activators (manganese as well as nickel) and inhibitor (cyclosporin A). By using a high concentration of substrate (pNPP) we were able to measure the Vmax activity of calcineurin. This is more problematic with phospho-protein substrates. Thus, one must be cautious in making

**DISCUSSION**

In the present study, regional differences in the activity of calcineurin (PP2B) were observed in AD. Calcineurin activities of phosphatases in area 17, total manganesestimulated phosphatase activity was inversely correlated with neuritic/core plaque density \( r = -0.63, p < 0.02 \) in whole cell homogenates prepared from striate cortex. The paucity of NFT in area 17 precluded meaningful regression analysis on NFT and calcineurin activity.

**TABLE 1**

<table>
<thead>
<tr>
<th>Assay conditions</th>
<th>Whole cell homogenates</th>
<th>P2 membrane fractions</th>
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<tbody>
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<td></td>
<td>NFT</td>
<td>Neuritic/ core plaques</td>
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<tr>
<td>Nickel-stimulated phosphatase (activity)</td>
<td>n.s.</td>
<td>n.s.</td>
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<tr>
<td>Cyclosporin-sensitive Manganese-stimulated phosphatase activity (PP2B)</td>
<td>( r = -0.61, p &lt; 0.02 )</td>
<td>( r = -0.63, p &lt; 0.02 )</td>
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<tr>
<td>Cyclosporin A-resistant Manganese-stimulated phosphatase activity (non-PP2B)</td>
<td>n.s.</td>
<td>n.s.</td>
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</table>

n.s. = not significant.
REDUCTION OF CALCINEURIN ACTIVITY IN ALZHEIMER'S DISEASE

929

direct comparisons between Gong et al (33) and the present study. It is interesting to note that in the previous study (33) the absolute value of calcineurin activity was by far the lowest of all phosphatases measured. This, despite the fact that calcineurin is a major protein phosphatase in the brain, constituting at least 1% of total brain protein (38). The extremely low calcineurin activity in Gong et al (33) may be explained by a recent study demonstrating a rapid inactivation of calcineurin in crude brain extracts by millimolar concentrations of calcium (39).

More recently, Gong et al (34) demonstrated a 30% reduction in a Mn³⁺/Ca²⁺-stimulated phosphatase activity in AD frontal cortex extract using abnormally phosphorylated AD tau as a substrate. Although the identity of the affected phosphatase(s) in the above study was not determined, their data are in close agreement with our finding of a 35% reduction in total manganese-stimulated phosphatase activity in AD temporal cortical tissue homogenate. Interestingly, although calcineurin is reported to be a calcium-dependent enzyme, we failed to detect a stimulation of phosphatase activity by calcium in tissue homogenate. This is consistent with several other reports showing a lack of stimulation of purified calcineurin by calcium using pNPP as a substrate (24, 25).

At least two phenomena may underlie the reduction in specific calcineurin activity detected in AD brain homogenates in the present study. Calcineurin appears to be exclusively expressed in neurons in the central nervous system (21, 40). Given that a decrease in cortical neuron density is a pathological feature of AD (41, 42), the reduction of calcineurin phosphatase activity in the temporal cortex may in part reflect a loss of calcineurin protein secondary to neuronal loss. However, this explanation cannot entirely account for the reduction in calcineurin phosphatase activity since there are differences in calcineurin stimulation by divergent cations in different cellular fractions. In addition, studies of overall calcineurin protein expression in tissue sections (20, 22) and measured by Western blots (22) indicate that calcineurin protein levels are not reduced in AD. These data suggest the reduction of calcineurin activity observed in the present study may represent an intrinsic defect in enzyme activity and/or the presence of a yet unidentified endogenous calcineurin inhibitor, such as a ligand for CNS immunophilins. The finding of an inverse correlation between NFT and manganese-stimulated calcineurin activity is consistent with a recent report of a reduction of calcineurin in AD tissue (20). Thus, a combination of both neuronal loss and changes in intrinsic enzyme activity, particularly a difference in the sensitivity of calcineurin to stimulation by divalent cations, may explain the present findings.

The inverse correlation between manganese-stimulated calcineurin activity and NFT density in the temporal lobe appears to be due primarily to a change in membrane-associated calcineurin since correlation coefficients were similar in whole cell and P2 membranes. In contrast to the association of neurofibrillary pathology with P2 membrane manganese-stimulated calcineurin activity, correlations between manganese-stimulated calcineurin activity and neuritic/core plaques were present only in whole cell homogenates. By inference, this would indicate that a cytosolic calcineurin deficit correlates with neuritic/core plaques. However, subtraction of P2 membrane calcineurin activity from whole cell homogenate calcineurin activity indicates that phosphatase activities in the different fractions are not simply additive, since negative activity values were obtained. In particular, 4 of the 8 AD cases demonstrated greater manganese-stimulated phosphatase activity in the P2 membranes than the whole cell homogenates, whereas this occurred only in a single control who exhibited the greatest neuropathologic changes of any of the controls. One explanation of this nonadditivity of calcineurin activity is the presence of endogenous calcineurin inhibitory factors in the cytosolic fraction which may then be washed away during membrane preparation. The finding that total manganese-stimulated phosphatase activity correlated with neuritic/core plaques in striate cortex despite no change in mean absolute phosphatase activities suggests that changes in phosphatase activity may be an early event in the development of AD neuropathology.

In addition to dephosphorylating serine and threonine residues, calcineurin also possesses intrinsic phosphotyrosine phosphatase activity (24, 43). The finding of a reduction in calcineurin activity using pNPP (structurally analogous to phosphotyrosine) implicates a role for calcineurin in alterations in tyrosine phosphorylation systems documented in AD. While studies of tyrosine kinase activities in AD brain have demonstrated a reduction in enzymatic activity (44, 45), an increase in phosphotyrosine immunoreactivity of several proteins has been reported (44, 46, 47). In particular, senile plaques in the brains of AD patients are stained by antibodies against phosphotyrosine (47). Further, tangle-bearing neurons contain elevated levels of phosphotyrosine (46). Thus, a loss of calcineurin phosphotyrosine activity may in part explain the increase in tyrosine phosphorylation in AD.

Calcineurin activity was detected in the P2 membrane fractions in the present study. Although calcineurin activity has been reported to be associated with T-cell plasma membranes (48) and placental membranes (43), to our knowledge, characterization of calcineurin activity in human brain membrane fractions has not been previously reported. Nickel- and manganese-stimulated membrane-associated phosphatase activity was 50 to 70% of control whole cell homogenate activity. Recent studies demonstrate that calcineurin associates with membranes via an

anchoring protein (49) and is targeted to membrane structures, such as postsynaptic densities (40). Whether or not cytosolic calcineurin undergoes translocation to the membrane (or vice versa) is unknown. The presence of calcineurin activity in P2 membrane fractions is in agreement with functional studies demonstrating both presynaptic and postsynaptic modulation of neuronal signal transduction by calcineurin (50–52).

In conclusion, we observed significant changes in divergent cation-stimulated calcineurin activity in postmortem AD brain specimens using pNPP as a substrate. Furthermore, the changes in calcineurin activity were inversely correlated with NFT and neuritic/core plaque density. To our knowledge, this is the first report of a reduction in AD brain calcineurin phosphatase activity. Since calcineurin is subject to regulation by a diverse array of activators (divalent cations, calmodulin) and inhibitors (immunosuppressant-immunophilin complexes, trifluoperazine, autoinhibitory domain), the AD-specific changes in sensitivity to any of these activators or inhibitors may provide clues as to the mechanism of reduced calcineurin activity. Future studies will examine specific calcineurin activity towards different substrates, particularly protein substrates (e.g. PHF-tau).

REFERENCES

REDUCTION OF CALCINEURIN ACTIVITY IN ALZHEIMER'S DISEASE


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