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Altered phosphorylation of τ protein in heat-shocked rats and patients with Alzheimer disease

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ABSTRACT **Six hours after heat shocking 2- to 3-month-old male and female Sprague–Dawley rats at 42°C for 15 min, we analyzed τ protein immunoreactivity in SDS extracts of cerebrums and peripheral nerves by using immunoblot analysis and immunohistochemistry with the anti-τ monoclonal antibody Tau-1, which recognizes a phosphate-dependent nonphosphorylated epitope, and with ^{125}I-labeled protein A. In the cerebral extracts, we found altered phosphorylation of τ in heat-shocked females, characterized by a marked reduction in the amount of nonphosphorylated τ, a doubling of the ratio of total (phosphorylated plus nonphosphorylated) τ to nonphosphorylated τ, and the appearance of the slowest moving phosphorylated τ polypeptide (68 kDa). Similar, but milder, changes were observed in male rats. These changes progressively increased in females from 3 to 6 h after heat shocking. In contrast, both phosphorylated τ and nonphosphorylated τ were reduced in peripheral nerves after heat shocking. In immunoblots of SDS extracts from Alzheimer disease-affected brain, the two slowest moving phosphorylated τ polypeptides (62 kDa and 66 kDa, respectively) were detected by Tau-1 after dephosphorylation and by Tau-2 (an anti-τ monoclonal antibody that recognizes a phosphate-independent epitope) without prior dephosphorylation only in regions that contained τ immunoreactivity in histologic preparations. In addition, quantitative immunoblot analysis of cortex and the underlying white matter with Tau-1 and ^{125}I-labeled protein A showed that the amount of phosphorylated τ progressively increased in the Alzheimer disease-affected cerebral cortex, while concurrently a proportionally lesser amount of τ entered the white matter axons. The similar findings for the rat heat-shock model and Alzheimer disease suggest that life stressors may play a role in the etiopathogenesis of Alzheimer disease.**

Despite a plethora of hypotheses and intense investigation, the etiopathogenesis of Alzheimer disease (AD) is still unknown. This laboratory has suggested (1, 2) that the excessive τ protein immunoreactivity seen in histologic preparations of AD-affected cerebrum results from a protective response to a variety of stressful stimuli. τ is a group of three to five thermostable phosphopolypeptides that stabilize and promote bundling of microtubules (3, 4); other possible functions of τ *in vivo* are unknown, although a nucleolar role has been suggested (5). It has been shown (1, 2) that the association of τ with ribosomes in AD perhaps is a primary pathogenetic event, the significance of which remains to be defined.

The heat shock response is a fundamental cellular function that has been conserved in all organisms across the evolutionary scale. After sublethal heat shocking, many changes take place in the cell that culminate in the selective translation of the mRNA for heat shock proteins, which then facilitate recovery from the toxic effects of heat and provide protection against subsequent increases in temperature (6). However, most of the heat shock proteins also are expressed constitutively, albeit in lesser amounts. A few of the adaptations known to lead to the heat shock response include (*i*) activation of the heat shock factor by phosphorylation (7) and its binding to repeating units of 5 base pairs in the regulatory regions (8), (*ii*) shutdown of rRNA synthesis and ribosomal biogenesis (9), and (*iii*) blocking of splicing of precursor mRNAs (10), which allows selective expression of the intronless heat-shock protein genes. Although the mechanism by which the elevated temperature is sensed and transduced is unknown, neuronal phosphoproteins are known to play an obligatory role in mediating signal transduction (11). For example, rapid dephosphorylation of ribosomal protein S6 (12, 13) and phosphorylation of the α subunit of the eukaryotic translation initiation factor 2 (14, 15) and other proteins (16) occur after heat shocking. Some of these processes (14, 15) correlate with the blocking of peptide chain initiation and partial disaggregation of polysomes.

In testing our hypothesis that life stressors play a role in the etiopathogenesis of AD, we found that phosphorylation of τ is similarly altered in AD and in heat-shocked rats. In addition, in AD, enhanced phosphorylation of two τ polypeptides (62 kDa and 66 kDa) render τ immunodetectable in histologic preparations of cerebrum and diminished amounts of τ enter the white matter axon.

MATERIAL AND METHODS

Heat Shocking. Age-matched 2- to 3-month-old male and female Sprague–Dawley rats were lightly anesthetized with pentobarbital [20 mg/kg (body weight)] and heat-shocked by placing them in an incubator no longer used for human newborns containing ambient air heated to 42°C. The rectal temperature of the rats was monitored continuously until it reached 42°C; this procedure lasted 45–60 min. The rats were then transferred to a 37°C environment and maintained for 15 min. At the end of this period the rectal temperature rose to 42.3–42.8°C. The rats were then transferred to room temperature. Control rats were kept at room temperature and were not anesthetized.

Preparation and Immunoblot Analysis of Rat SDS Extracts. Six hours after heat shocking, the heat-shocked and control rats were sacrificed under pentobarbital anesthesia (35 mg/kg), almost always in pairs, by intracardiac perfusion with 100 mM Tris·HCl, pH 7.6/6 mM EGTA at 4°C until blood-free fluid began exiting from the right atrium. To study whether the changes resulting from heat shocking were progressive, four additional rats were sacrificed either at 3 h or 6 h after heat shocking. By working on ice, the brain (excluding the brain stem and cerebellum), sciatic nerves, brachial plexi, and spinal nerve roots (peripheral nerves) were removed rapidly and homogenized in a buffer containing 62.5 mM

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Abbreviation: AD, Alzheimer disease.

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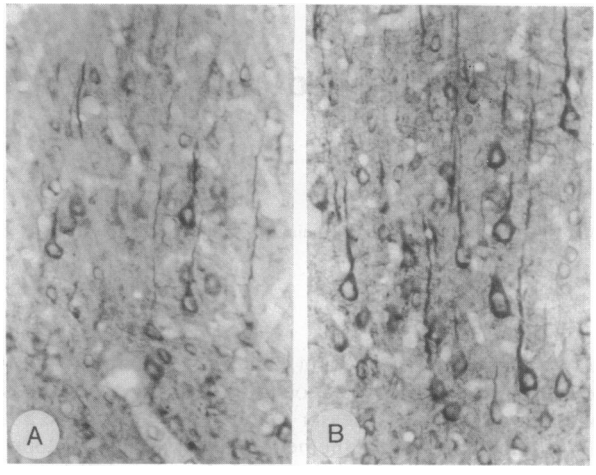

FIG. 1. More intense staining is present in heat-shocked than in control neurons of female rats. Six hours after heat shocking, rats were perfused with 4% paraformaldehyde/0.25% glutaraldehyde, tissue samples were fixed further in 4% paraformaldehyde at 4°C overnight, and Vibratome sections 20 μm thick were stained with Tau-1 after pretreating with alkaline phosphatase using the peroxidase-antiperoxidase technique (19, 21). Both sections were taken from the frontoparietal motor cortex. (*A*) Control rat. (*B*) Heat-shocked rat. Note the somatodendritic staining. Heat-shocked nuclei appear to be larger than the control. (*A* and *B*, ×180.)

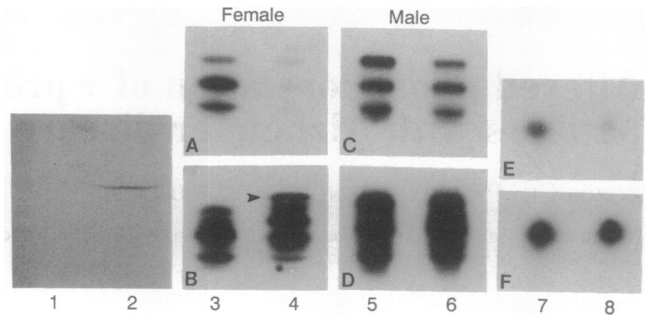

FIG. 2. Six hours after heat shocking, the stress-induced 72-kDa protein is expressed in heat-shocked but not in control rats; the immunostaining intensity of nonphosphorylated τ is much more reduced in SDS cerebral extracts from heat-shocked female rats than in those from male rats, and both the nonphosphorylated τ and total τ are reduced in heat-shocked peripheral nerves. Lanes: 1 and 2, control and heat-shocked samples, respectively, stained with C92F2A-5 (22) using the peroxidase-antiperoxidase technique; 3–8, autoradiographs stained with Tau-1 and ^{125}I-labeled protein A without (*A*, *C*, and *E*) and with (*B*, *D*, and *F*) alkaline phosphatase pretreatment; 3, 5, and 7, control; 4, 6, and 8, heat-shocked. Note the marked decrease in immunostaining intensity of nonphosphorylated τ in *A*, lane 4, and the upward mobility shift, the changes in banding, and the additional 68-kDa τ polypeptide (arrowhead) in *B*, lane 4. Also note that in peripheral nerves only a 115-kDa polypeptide is present (lanes 7 and 8).

Tris·HCl (pH 6.8), 2% (wt/vol) SDS, 5% (vol/vol) 2-mercaptoethanol, and 10% (vol/vol) glycerol by using a Dounce tissue grinder in a boiling water bath for 5 min. The peripheral nerves were pooled, frozen in liquid nitrogen, and crushed before homogenization. After mixing briefly at room temperature, the homogenates were placed in a boiling water bath for another 10 min and then centrifuged for 3 h at 37,500 rpm in a Sorvall rotor AH-650 at 21°C. The compact layer of lipids that formed at the top of the supernatants was discarded and the protein content was assayed in samples of the supernatants by using a modification of the method of Lowry *et al.* (17), that is, removing SDS by precipitating with 10 vol of 10% (vol/vol) perchloric acid/1% phosphotungstic acid for 1 h on ice, centrifuging, and redissolving the pellet with Lowry reagent C (5). One hundred micrograms and 300 μg of protein of cerebral and peripheral nerve extracts, respectively, from control and heat-shocked rats were loaded in each alternate lane of 5–12.5% linear gradient polyacrylamide gels and electrophoresed in electrode buffer containing 0.1% SDS at a constant current of 30 mA. The proteins then were transferred to nitrocellulose membranes and immunostained with or without prior dephosphorylation with type VII-L alkaline phosphatase (65 μg/ml) from bovine intestinal mucosa (Sigma) in 100 mM Tris·HCl (pH 8.0) containing 1 mM phenylmethylsulfonyl fluoride, pepstatin A (10 μg/ml), and leupeptin (10 μg/ml) for 2.5 h at 32°C (18). Immunoblot analysis was performed as described (19) by incubating the nitrocellulose membranes successively with the monoclonal antibody Tau-1 (19), an IgG2a protein that recognizes a phosphate-dependent epitope (diluted 1:2500; provided by L. I. Binder, University of Alabama, Birmingham), and ^{125}I-labeled protein A (type NEX-146L, NEN). Autoradiography then was performed to visualize the τ region on the nitrocellulose membrane, the τ region was excised, and radioactivity was measured in a γ counter. In a few experiments, the anti-τ monoclonal antibody T46 (20) (provided by J. Q. Trojanowski, University of Pennsylvania, Philadelphia), which recognizes a phosphate-independent epitope, also was used. Rabbit anti-mouse IgG was used as a bridge between T46 and ^{125}I-labeled protein A. The peroxidase-antiperoxidase technique (21) also was used to prepare many immunoblots and to verify the heat shock response by using the monoclonal antibody C92F2A-5 against the 72-kDa stress-induced heat-shocked protein (22) provided by W. J. Welch (University of California, San Francisco).

Immunohistochemistry. The peroxidase-antiperoxidase technique was used to immunostain paraffin-embedded and Vibratome sections at the light and electron microscopic levels as described (19, 21).

Correlative Analysis of Immunohistochemical and Immunoblot Data in AD. Paraffin-embedded and/or Vibratome sections of formalin-fixed human brain tissue obtained at autopsy from 20 control individuals without neurologic disease and 27 AD patients were immunostained. Fresh frozen tissue was procured from 3 control individuals and 5 AD patients with a mean postmortem interval of 5 h. Immunoblots of SDS extracts of cerebral cortex and underlying white matter from several regions of each brain from which fresh frozen tissue

Table 1. Quantitative analysis of τ on immunoblots of rat cerebrum 6 h after heat shocking

	Female			Male		
	^{125}I-protein A bound			^{125}I-protein A bound		
Tau-1 epitope	Co (n = 5)	HS (n = 5)	P	Co (n = 6)	HS (n = 8)	P
Nonphosphorylated*	2.94 ± 1.29	1.83 ± 0.92	<0.001 (ν = 23)	3.58 ± 1.11	3.00 ± 0.94	<0.001 (ν = 23)
Total†	4.99 ± 1.84	5.73 ± 2.36	<0.05 (ν = 20)	8.78 ± 2.79	8.13 ± 2.91	NS (ν = 22)
Total/nonphosphorylated ratio	2.06 ± 0.68	3.95 ± 2.35	<0.001 (ν = 30)	2.57 ± 0.76	2.74 ± 0.53	NS (ν = 22)

Data for ^{125}I-labeled protein A (^{125}I-protein A) bound are cpm × 10^{-3}/100 μg of extract protein (mean ± SD). HS, heat-shocked; Co, control; n, number of animals; ν, degrees of freedom; NS, $P > 0.05$;* and †, defined as the epitope recognized by Tau-1 without and with alkaline phosphatase treatment of transferred proteins prior to immunostaining, respectively.

Table 2. Quantitative analysis of τ on immunoblots of rat cerebrum 3 and 6 h after heat shocking

Tau-1 epitope	^{125}I-labeled protein A, cpm $\times 10^{-3}$/100 μg of extract protein: 3 h (n = 2)	6 h (n = 2)	P
Nonphosphorylated	3.75 ± 1.91	3.24 ± 1.58	<0.01 (ν = 11)
Total	10.70 ± 3.67	10.57 ± 3.10	NS (ν = 11)
Total/nonphosphorylated ratio	3.05 ± 0.57	3.38 ± 0.63	<0.005 (ν = 11)

Definitions and abbreviations are as in Table 1; data are mean ± SD.

Table 3. Quantitative analysis of τ on immunoblots of female rat peripheral axons 6 h after heat shocking

Tau-1 epitope	^{125}I-labeled protein A, cpm $\times 10^{-3}$/300 μg of extract protein: Co (n = 4)	HS (n = 5)	P
Nonphosphorylated	0.26 ± 0.13	0.15 ± 0.11	<0.001 (ν = 13)
Total	0.60 ± 0.20	0.42 ± 0.12	<0.005 (ν = 11)
Total/nonphosphorylated ratio	2.17 ± 0.49	2.52 ± 0.76	NS (ν = 7)

Definitions and abbreviations are as in Table 1; data are mean ± SD.

was procured were prepared and analyzed as described above. The immunohistochemical and immunoblot data obtained from the same region were compared. Anti-τ monoclonal antibodies, Tau-2 (19) (provided by L. I. Binder) and T46 also were used. The proteins' approximate molecular masses were calculated on immunoblots by using prestained standards (Bio-Rad). All quantitative data were analyzed statistically using Student's two-tailed t test.

RESULTS

τ Immunoreactivity Is More Intense in Heat-Shocked than in Control Rats. In both Vibratome and paraffin-embedded sections, there was more intense staining in heat-shocked than in control rats. Also, although not morphometrically analyzed, nuclei appear to be larger in heat-shocked than in control rats (Fig. 1). However, there were no differences at the electron microscopic level in the morphology and in the distribution of staining between the control and heat-shocked rats (data not shown).

Altered Phosphorylation of τ in Heat-Shocked Female Rats. As we have shown (19), a phosphorylation event in the somatodendritic compartment of neurons of young adult rats almost always prevents the binding of Tau-1 without prior dephosphorylation. This finding in the perikaryon and dendrites of neurons contrasts with that in axons where a significant amount of τ is immunodetectable without pretreatment with phosphatase. Thus, Tau-1 most likely recognizes a phosphorylation-dependent nonphosphorylated epitope. In this study, we defined nonphosphorylated τ and total (phosphorylated plus nonphosphorylated) τ as the forms of τ recognized by Tau-1 without and with prior dephosphorylation, respectively.

Only the heat-shocked rats expressed the 72-kDa protein induced by heat shocking (Fig. 2, lane 2). The immunoblot analyses of cerebral extracts are shown in Fig. 2 and in Table 1. In heat-shocked females, the nonphosphorylated amount of all three τ polypeptides (52, 60, and 66 kDa) was markedly reduced (Fig. 2*A*, lane 4). In males, this reduction was much less, though constant, and thus also was statistically significant. After dephosphorylation, there was a marked increase in total τ when compared to the nonphosphorylated τ in all rats, but only heat-shocked females showed a slightly significant increase ($P < 0.05$) of total τ when compared to the controls. More importantly, the very statistically significant increase ($P < 0.001$) in the ratio of total τ to nonphosphorylated τ in heat-shocked rats in comparison with control rats and in the presence of a marked reduction in nonphosphorylated τ was indicative of increased phosphorylation only in females, as further exemplified by an upward mobility shift, the appearance of the slowest moving polypeptide (68 kDa), and changes in the banding of τ polypeptides by SDS/PAGE (Fig. 2*B*, lane 4). In addition, the changes described above progressively increased in females from 3 to 6 h after heat shocking (Table 2). In heat-shocked males, there was a mild increase in phosphorylation, but it never achieved significant levels. Generally, in this age group, the trend of changes in males was similar but less dramatic than in females. No significant differences between control and heat-shocked rats were found when T46 was used (heat-shocked vs. control, 80,728 ± 2314 vs. 81,392 ± 5986 cpm, respectively).

Because the cerebral extracts contained both cerebral cortex and white matter, we analyzed the peripheral nerves to determine the contribution of axons to the changes described above (Fig. 2 and Table 3). Only a single τ polypeptide of ≈115 kDa was recognized by Tau-1 (Fig. 2 *E* and *F*), which was present in the central nervous system only in regions that received peripheral afferents (e.g., brain stem and spinal cord). τ is exclusively axonal in peripheral nerves (23). Control axons contained approximately equal amounts of phosphorylated and nonphosphorylated τ; after heat shocking, there was a significant reduction in both nonphosphorylated τ and total τ (Table 3 and Fig. 2 *E* and *F*). In addition, the peripheral nerves contained 30–40 times less τ than the cerebral extracts, indicating that the changes seen in cerebral extracts could not be solely axonal.

Two Phosphorylated 62- and 66-kDa τ Polypeptides in Cerebrum Render τ Immunodetectable in Histologic Preparations of AD. In routinely processed histologic preparations of autopsy and biopsy specimens of human brain tissue, the mere presence of τ immunoreactivity indicates a pathologic process (1). To investigate the biochemical bases of these findings, we analyzed cerebral cortex and the underlying white matter of many regions (e.g., superior frontal and middle temporal gyri, inferior parietal lobule, hippocampus, primary visual area, and basal ganglia) of five AD-affected brains with and without

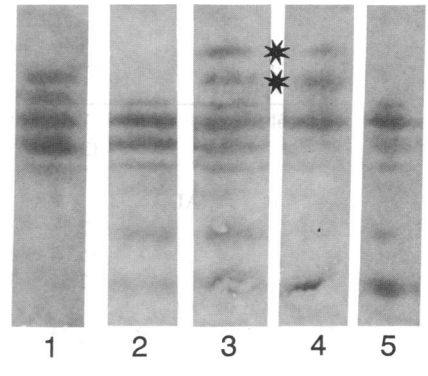

FIG. 3. In a 77-year-old white woman with AD and dementia for 5 years (lanes 2–4), the two slowest moving τ polypeptides (62 kDa and 66 kDa, respectively; asterisks) are revealed by both Tau-1 and Tau-2 but are absent from a control (lane 5) with no neurologic disease. Immunoblots were prepared from SDS extracts of the temporal lobe and stained using the peroxidase-antiperoxidase technique. Lanes: 1, purified bovine brain τ stained with Tau-1; 2 and 3, AD stained with Tau-1 without and with pretreatment with alkaline phosphatase, respectively; 4, AD stained with Tau-2; 5, control without neurologic disease stained with Tau-2. Note that in lanes 2–4 the proteolytic products of τ are similar.

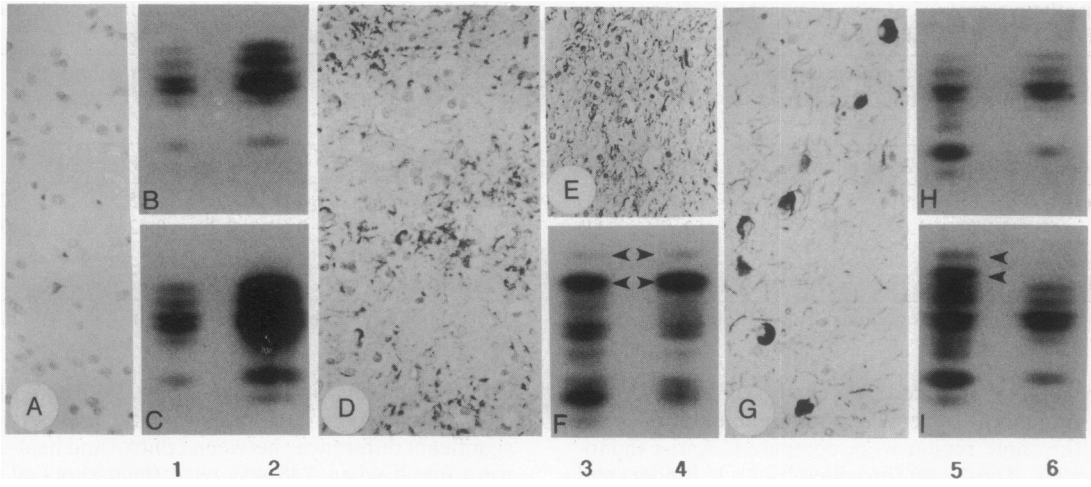

FIG. 4. Only regions with τ immunoreactivity in histologic preparations contain the two slowest moving phosphorylated τ polypeptides (62 kDa and 66 kDa, respectively). Paraffin-embedded sections prepared from the superior frontal gyrus of 10% (vol/vol) formalin-fixed autopsy brain tissue of a control without neurologic disease (*A*), a 59-year-old white man with a 4-year history of dementia (*D* and *E*), and an 82-year-old white woman with a 6-year history of dementia (*G*) were stained with Tau-1 after pretreatment with alkaline phosphatase. (*A*, *D*, and *G*) Cortex. (*E*) White matter. (×180.) Autoradiographs of immunoblots of SDS extracts of fresh frozen cortex and underlying white matter prepared from the same region as the above histologic preparations and stained with Tau-1 and ^{125}I-labeled protein A are shown in *B* plus *C*, *F*, and *H* plus *I*, respectively, without (*B* and *H*) and with (*C*, *F*, and *I*) pretreatment with alkaline phosphatase. Lanes: 1, 3, and 5, cortex; 2, 4, and 6, white matter. The quantitative analyses of immunoblots from the above individuals, designated as Co, AD_1, and AD_2, respectively, are shown in Table 4. Note that the 62- and 66-kDa τ polypeptides (arrowheads) correlate with the presence of histologic immunoreactivity; out of the 27 AD patients, τ immunoreactivity in white matter to the extent shown in *E* is exceptional. Note also the lack of immunoreactivity in *A*, neurites and almost no neurofibrillary tangles in *D* and *E*, and neurites and many neurofibrillary tangles in *G*.

histologic τ immunoreactivity and of three control brains by immunoblot analysis (Figs. 3 and 4). Whereas the cortex and white matter of the controls and the white matter and cortex without τ immunoreactivity of AD-affected brains contained only four τ polypeptides of 52–60 kDa, the cortical regions and, exceptionally, perhaps in the early stages, the white matter of AD-affected brains with τ immunoreactivity contained two additional slowest moving τ polypeptides of 62 and 66 kDa. The latter two polypeptides were revealed by Tau-1 after pretreatment with alkaline phosphatase and also by Tau-2 and T46 without prior dephosphorylation. In addition, the amount of these two polypeptides was proportional to the amount of τ immunoreactivity seen in immunohistochemical preparations from the same regions of the brain (data not shown). However, when gels were overloaded (200 μg of protein per lane) these two polypeptides were faintly revealed by Tau-1 without prior dephosphorylation.

Altered Phosphorylation Impairs the Amount of τ that Enters the Axon in AD. τ was analyzed quantitatively in cerebral cortex and the underlying white matter of the superior frontal gyrus in one control and two AD (AD_1 and AD_2) individuals, chosen because of their differences in immunohistochemical staining and duration of dementia (Table 4 and Fig. 4). In AD, the amount of phosphorylated τ progressively increased in the cortex as indicated by the progressive increase in the total τ to nonphosphorylated τ

Table 4. Quantitative analysis of τ on immunoblots of human cerebrum

	^{125}I-labeled protein A bound to Tau-1 epitope			
	Total/nonphosphorylated ratio		Cortex/white matter ratio	
Autopsy	Cortex	White matter	Nonphosphorylated	Total
Control (Co)	1.86 ± 0.68	2.20 ± 1.08	0.35 ± 0.05	0.33 ± 0.12
	(3.56 ± 1.93/	(12.13 ± 6.98/	(1.87 ± 0.44/	(3.56 ± 1.93/
	1.87 ± 0.44)	5.44 ± 1.74)	5.44 ± 1.74)	12.13 ± 6.98)
P (Co vs. AD_1)	NS	NS	<0.001	<0.001
AD_1	2.23 ± 0.23	3.05 ± 0.14	1.60 ± 0.15	1.16 ± 0.08
	(5.68 ± 0.83/	(4.92 ± 0.89/	(2.54 ± 0.19/	(5.68 ± 0.83/
	2.54 ± 0.19)	1.61 ± 0.24)	1.61 ± 0.24)	4.92 ± 0.89)
P (Co vs. AD_2)	<0.02	NS	<0.002	<0.001
AD_2	2.33 ± 0.51	1.65 ± 0.26	1.41 ± 0.22	1.96 ± 0.20
	(6.74 ± 2.92/	(3.46 ± 1.55/	(2.82 ± 0.72/	(6.74 ± 2.92/
	2.82 ± 0.72)	2.06 ± 0.71)	2.06 ± 0.71)	3.46 ± 1.55)
P (AD_1 vs. AD_2)	NS	<0.001	NS	<0.001

AD patients AD_1 and AD_2 had 4- and 6-year histories of dementia, respectively. Immunohistochemically, both patients were characterized by a multitude of abnormal neuropil neurites, but AD_1 had fewer neurofibrillary tangles than AD_2 and exceptionally intense staining in white matter (Fig. 4). Numbers in parentheses are the cpm × 10^{-3} (mean ± SD) of nonphosphorylated τ and total τ from which the ratios were calculated. *P* values refer to ratios. When the cpm data were statistically analyzed for the eight pairs derived from control vs. AD_1 and control vs. AD_2, all were significant, except for the white matter total τ in control vs. AD_1. In the four pairs derived from AD_1 vs. AD_2, only the white matter total τ was significant. The significant *P* values ranged from $P < 0.05$ to $P < 0.01$. For all, $\nu = 3$. All other definitions and abbreviations are as for Table 1.

ratio, which was statistically significant ($P < 0.02$) in specimen AD_2. Concomitantly with the altered phosphorylation of τ in the cortex, a proportionally lesser amount of τ entered the white matter axons, as exemplified by the highly significant increase in the cortex/white matter ratio of both total and nonphosphorylated τ.

DISCUSSION

Grundke-Iqbal *et al.* (24) showed for the first time that dephosphorylation enhances the immunostaining of the 55- to 62-kDa τ polypeptides present in a microtubule preparation from AD-affected brain, especially the two slowest moving τ polypeptides, but not in microtubules prepared from normal brain tissue. Also, aberrant phosphorylation of neurofilaments (25) has been described in AD. Phosphorylation of τ by a calcium- and calmodulin-dependent kinase is known to produce an upward mobility shift in gels by SDS/PAGE (26). Phosphorylation makes τ longer and stiffer (27), but we have observed no changes in its morphology at the electron microscopy level. Nonetheless, our data suggest that in AD the phosphorylated 62- and 66-kDa τ polypeptides undergo conformational changes that allow them to withstand the rigors of immunohistochemical processing and thus are immunodetectable.

Altered phosphorylation of τ both in the heat-shock model and in AD suggests that τ may play a role in an organism's response to stressful stimuli. In this regard, τ exhibits similarities to the low molecular mass (28 kDa) heat shock protein, a phosphoprotein of at least four isoforms, which responds to a variety of stressful stimuli with increased phosphorylation (6, 28). The more vigorous response of females to heat shocking when compared with age-matched males is intriguing, especially since the incidence and prevalence rates of AD are higher in women than in men (29), although this finding is controversial. Glucocorticoids mediate an organism's adaptation to stress by inhibiting the use of glucose and are toxic to hippocampal neurons *in vitro* (30), which, together with the rest of the limbic system, have the highest concentration of glucocorticoid receptors (31). Because the limbic system is among the regions involved early with AD and most vulnerable to it (1) and because a shift from aerobic to glycolytic metabolism takes place after heat shocking (32), the role that life stressors may play in the etiopathogenesis of AD and the modifying effects, if any, of sex hormones on this process deserve further investigation.

We have shown in this study that cortical τ becomes progressively phosphorylated and proportionally less τ enters the white matter axons in AD. τ is associated not only with abnormal filaments but also with ribosomes (1, 2) and nucleoli (5). After heat shocking, the ribosomes undergo a conformational change such that a cytoplasmic protein becomes completely ribosome bound (33), a finding not entirely unexpected since ribosomes are thought to be heat shock sensors (34). Microtubule-associated proteins also have been shown to interact with ribosomes (35). Based on the above findings, our working hypothesis is that, in AD, alterably phosphorylated τ binds to polysomal mRNA and blocks its translation after stressful stimuli. This hypothesis is reminiscent of the group of four phosphoproteins (50–60 kDa) found in *Xenopus laevis* oocytes that throughout oogenesis bind to stored mRNA and prevent its translation until early embryogenesis (36).

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