Reversible Heat Stress-related Loss of Phosphorylated Alzheimer-type Epitopes in Tau Proteins of Human Neuroblastoma Cells

Marie Fang Chiang, Wan-Kyng Liu, and Shu-Hui Yen

Department of Pathology, Albert Einstein College of Medicine, Bronx, New York 10461

Human neuroblastoma cells, LAN, were used to study the phosphorylation and dephosphorylation of tau proteins. These cells contained mainly a form of tau comparable to fetal brain tau in molecular weight (55 kDa). Neuroblastoma tau reacted with antibodies that recognize epitopes spanning the whole tau molecule (E-1, Alz50, Tau-1, and Tau46), and antibodies (PHF-1, NP8, and T3P) that recognize hyperphosphorylated tau (PHF-tau) in Alzheimer's disease (AD) brains. Exposure of the cells to 45°C heat stress resulted in dephosphorylation of the epitopes recognized by PHF-1, NP8, and T3P. Transfer of the heat-stressed cells to 37°C led to rephosphorylation of the dephosphorylated epitopes. Cells that had been treated with okadaic acid (OA), regardless of whether they were subsequently subjected to heat stress or heat stress and recovery, all contained tau with a molecular weight similar to that of control cells. These tau proteins, similar to tau in control cells, also reacted with antibodies to phosphorylated epitopes. However, unlike the tau from control or heat-stressed cells, the OA-treated and heat-stressed tau had decreased reactivity with Tau-1. Alteration of Tau-1 immunoreactivity has been reported to be an early event in AD neurodegeneration. The reduction of Tau-1 immunoreactivity observed in OA-treated samples could be restored by incubation of electroblots of isolated tau with alkaline phosphatase, indicating an induction of the Tau-1 epitope phosphorylation by OA. The results of our studies suggest that neuroblastoma cells may contain phosphatases/kinases that are comparable to that in AD, and that culture cells may be used for studying the mechanisms involved in AD neurofibrillary formation.

[Key words: human neuroblastoma cells, heat stress, okadaic acid, phosphorylation, dephosphorylation]

Microtubule-associated proteins, tau, in adult brains are heterogeneous in molecular weight and isoelectric charge (Cleveland et al., 1977; Ksiezak-Reding et al., 1988). These proteins, detected by gel electrophoresis as five or six polypeptides ranging between 50 and 60 kDa, are encoded by six transcripts generated

from a single gene by alternative gene splicing (Goedert et al., 1989; Himmler, 1989; Himmler et al., 1989; Goedert and Jakes, 1990). Three of the tau transcripts contain three tandem repeats in the carboxy (C)-terminal half, which are considered to be the microtubule binding domain. The remaining transcripts contain four repeats. Tau transcripts also differ from each other by the presence or absence of 29 or 58 amino acid inserts located in the amino (N)-terminal region (Goedert et al., 1989; Goedert and Jakes, 1990). Besides alternative gene splicing, heterogeneity of adult tau is due to phosphorylation (Lindwall and Cole, 1984). Purified tau contains about 2 mol of phosphate/mol protein, which can be phosphorylated *in vitro* by different kinases, including protein kinase A (Yamamoto et al., 1985), protein kinase C (Baudier and Cole, 1987b), casein kinase II (Diaz-Nido et al., 1988), calcium/calmodulin kinase II (Yamamoto et al., 1983; Baudier and Cole, 1987a), tubulin-stimulated kinase (Ishiguro et al., 1988), ATP-Mg-dependent protein phosphatase activator, F_A (Yang et al., 1991), and microtubule-associated protein (MAP) kinase (Dreux et al., 1992). Phosphorylated tau is a substrate for *in vitro* dephosphorylation by alkaline phosphatase (Lindwall and Cole, 1984), acid phosphatase, and protein phosphatases 1, 2A, and 2B (Goto et al., 1985; Yamamoto et al., 1988).

Tau proteins from fetal brains are smaller in size than those from adult brains. Fetal human tau proteins are polypeptides of molecular weight 50–55 kDa (Liu et al., 1992). These polypeptides are encoded by transcripts with three tandem repeats and without N-terminal inserts (Goedert et al., 1989).

Hyperphosphorylated tau proteins are a major component of paired helical filaments (PHF), which are abnormal structures characteristic of degenerating neurons in Alzheimer's disease (AD) (Kidd, 1963; Terry et al., 1964; Wisniewski et al., 1976; Braak et al., 1986). These modified tau proteins, named PHF-tau or A68, differ from normal adult tau protein in the extent and site(s) of phosphorylation (Grundke-Iqbal et al., 1986; Kosik et al., 1988; Greenberg and Davies, 1990; Ksiezak-Reding et al., 1990b, 1992; Brion et al., 1991; Lee et al., 1991; Liu et al., 1991). Several subregions (or sites) of the tau molecule have been reported to be phosphorylated in PHF-tau, but not in normal adult tau. These subregions are recognized by antibodies E-2 (Liu et al., 1993), Tau-1 (Wood et al., 1986; Kosik et al., 1988), PHF-1 (Greenberg et al., 1992), TP30 (Brion et al., 1991), T3P (Lee et al., 1991), NP8 (Ksiezak-Reding et al., 1987), anti-ptau1, and anti-ptau2 (Kanemaru et al., 1992). With the exceptions of the E-2, TP30, and Tau-1 epitopes, all of the phosphorylated sites are at the C-terminal half of the tau molecule. Of the phosphorylated sites, those that are recognized by PHF-1, anti-ptau1, or anti-ptau2 also have been detected by immunoblotting in tau from fetal brains (Greenberg et al., 1992;

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Correspondence should be addressed to Shu-Hui C. Yen, Department of Pathology, F-538, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

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Kanemaru et al., 1992). These findings raise the possibility that the activation of kinases or inhibition of phosphatases unique to AD and fetal neurons or the imbalance of normal kinases/phosphatases in AD tissue leads to hyperphosphorylation of PHF-tau. The significance of the reexpression of fetal proteins in the pathogenesis of AD is also supported recently by the finding of a 70 kDa fetal protein unrelated to tau and phosphorylated MAP5 in Alzheimer brains (Hasegawa et al., 1990; Morishima-Kawashima et al., 1991).

To understand the dynamics of tau phosphorylation and also the mechanism(s) involved in abnormal phosphorylation of PHF-tau, we have subjected cultured human neuroblastoma cells to heat stress and/or okadaic acid (OA), an inhibitor specific to phosphatases 1 and 2A (Bialojan and Takai, 1988). Tau proteins were isolated from cultured cells and probed with different tau antibodies. We demonstrated that (1) more than 90% of the tau proteins in cultured neuroblastoma cells are similar to fetal brain tau in molecular weight and immunochemical properties, (2) neuroblastoma tau shares phosphorylated epitopes with PHF-tau, (3) heat stress induces dephosphorylation of neuroblastoma tau, (4) heat stress-induced dephosphorylation occurs at PHF-1, NP8, and T3P epitopes, (5) OA protects tau from heat stress-induced dephosphorylation, and (6) OA induces phosphorylation of tau proteins at the Tau-1 epitope. Our results demonstrate that cultured human neuroblastoma cells may be a source for identification and isolation of enzymes related to the phosphorylation and dephosphorylation of PHF-tau and fetal tau. Furthermore, these cultured cells may be useful for understanding the mechanisms involved in PHF formation.

Materials and Methods

Cell culture. LAN cells, obtained originally from Dr. J. L. Beidler (Sloan Kettering Institute, NY), were cultured in 175 cm² flasks, in an incubator at 37°C containing 8% CO₂. The cells were grown in RPMI 1640 medium supplemented with 0.4 mM serine, 0.4 mM asparagine, 1 mM glutamine, 1% nonessential amino acids, 10% fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 µg/ml).

Heat-shock treatment. The cells were subjected to heat stress at 45°C for 1 hr. Some cells were harvested immediately, while others were harvested after 1, 2, 4, 6, or 16 hr of recovery at 37°C. In some studies, the cells were heat stressed for 15, 30, and 45 min and then harvested. In other studies, 1 µM emetine dihydrochloride (a protein synthesis inhibitor) was added to the cultures before heat stress, or 30 min before the recovery. The amount of emetine was effective in the inhibition of protein synthesis in LAN cells and other human neuroblastoma cells as well (Casper and Davies, 1989).

Okadaic acid treatment. Cells were treated with 1 µM of OA (Moana Bioproducts Inc., HI), followed by heat stress at 45°C and recovery at 37°C. In some studies, the cells were treated with 500 nM, 100 nM, 50 nM, and 10 nM OA. For controls, OA-treated cells were kept at 37°C for 1 and 5 hr.

Cell harvesting. The cells were collected in 5.0 ml of 10 mM Tris-HCl, pH 8.0, 0.14 M NaCl, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 0.2% azide, and recovered by centrifugation at 300 × g for 10 min. They were then lysed in a solution containing 20 mM 2-[N-morpholino]ethanesulfonic acid (MES), pH 6.8, 80 mM NaCl, 1 mM MgCl₂, 2 mM EGTA, 10 mM NaH₂PO₄, 20 mM NaF, 1 mM PMSF, and 10 µg/ml leupeptin, and stored at -70°C or used immediately. In some experiments, additional phosphatase inhibitors were added to the lysis solution mentioned above. These inhibitors include 50 mM NaF, 25 mM β-glycerolphosphate, 0.1 mM sodium vanadate, and 1 µM OA. Samples extracted with both conditions showed similar immunoblotting pattern with anti-tau antibodies.

Preparation of tau-enriched fraction. The lysed cell suspensions were homogenized with a Potter-Elvehjem type homogenizer (20 strokes), and centrifuged at 11,000 × g for 15 min. The supernatants were removed and mixed with mercaptoethanol (final concentration, 2%) and NaCl (2%) for 30 min at 4°C. The samples were then boiled for 5 min,

followed by centrifugation at 11,000 × g for 15 min. The heat-stable supernatants thus obtained were enriched in tau proteins. In two studies, tau proteins were further purified by treatment with 2.5% perchloric acid as described before (Lindwall and Cole, 1984; Ksiezak-Reding et al., 1990b). The acid-soluble proteins were precipitated with methanol.

Gel electrophoresis, electroblotting, and immunoblotting. The heat-stable or heat and perchloric acid-stable tau preparations were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). The amount of protein in these preparations was determined (Lowry et al., 1951). A similar amount of protein from different experimental conditions was loaded on acrylamide gel. After separation with 10% SDS-PAGE and electroblotting to nitrocellulose paper, proteins were immunostained with various anti-tau antibodies. The bound immunoglobulins were detected by the avidin-biotin-peroxidase method with a Vectastain ABC kit (Vector Inc.), by a secondary antibody linked to alkaline phosphatase (Calbiochem), or by the chemiluminescent method (Amersham). In some studies the electroblots were double labeled with two anti-tau antibodies. In such instances, Vectastain was used for detecting one antibody, and alkaline phosphatase for the other antibody. The intensity of the immunostaining was determined by scanning the immunoblots with a Shimadzu TLC Scanner.

For studying Tau-1 epitope phosphorylation, a five-tooth comb was used in gel electrophoresis. The electroblot obtained from each lane was divided in halves. One half of the strip was incubated with *Escherichia coli* alkaline phosphatase (10 IU/ml; Sigma) for 2 hr at 37°C in 100 mM Tris-HCl buffer, pH 8.1, with 0.1 mM ZnCl₂, 1 mM MgCl₂, 1 mM PMSF, and 0.2 mM dithiothreitol. The other strip was not treated with the enzyme. Both strips were then incubated with Tau-1 antibody. To demonstrate the specificity of alkaline phosphatase, 50 mM phosphate buffer was mixed with phosphatase in some studies.

Antibodies. Eight tau-reactive antibodies were used, which included monoclonal antibodies Alz50, Tau-1, PHF-1, NP8, and Tau46 and polyclonal antibodies E-1, E-2, and T3P. Alz50 and NP8, provided by P. Davies (Albert Einstein College of Medicine), were raised against AD brain homogenates (Wolozin et al., 1986; Ksiezak-Reding et al., 1987). The Alz50 epitope is phosphate independent (Greenberg et al., 1992) and is located at the N-terminus of tau molecule amino acids (aa) 2–10 (Ksiezak-Reding et al., 1990a; Goedert et al., 1991). The NP8 epitope is phosphate dependent and is present in neurofilament proteins and bovine and rat tau proteins, but not normal adult human tau. E-1 and E-2 (Liu et al., 1992) were raised to synthetic peptides corresponding to amino acid residues 19–33, and 44–55, respectively, of the longest human tau isoform (Goedert et al., 1989). Tau-1, obtained from L. I. Binder (University of Alabama), recognizes an epitope located at amino acid residues 189–207. The Tau-1 epitope and some of the E-2 epitopes are more phosphorylated in PHF-tau than in normal tau (Liu et al., 1992). PHF-1 was raised against a relatively soluble population of PHF (Greenberg et al., 1992). This antibody reacts most intensely with PHF-tau, less intensely with fetal human tau, and weakly with adult human tau. The PHF-1 epitope is phosphate dependent. T3P was raised against a phosphosynthetic peptide (Lee et al., 1991), whose amino acid sequence corresponds to aa 389–402 of tau, and the phosphate was at Ser³⁹⁶. The antibody reacts with PHF-tau, but has no reactivity with normal adult tau. Tau46 recognizes an epitope located at the C-end of tau (aa 404–441) (Kosik et al., 1988), which is also shared by microtubule-associated protein 2 (MAP2) (Ksiezak-Reding et al., 1990a). Both T3P and Tau46 were obtained from V. Lee (University of Pennsylvania).

Results

Tau isoforms in neuroblastoma cells

Immunoblotting of tau-enriched fractions from LAN cells demonstrated the presence of a major tau-immunoreactive band at 55 kDa region, two minor bands at 53 kDa and 58 kDa (Fig. 1, lanes marked C), and smaller-molecular-weight bands. The detection of minor tau isoforms depended on the amount of protein loaded on gels. The immunoblots of tau extracted by different lysis solutions were similar.

The 58 kDa, 55 kDa, and 53 kDa bands all reacted with tau-reactive antibodies that recognize epitopes spanning the entire tau molecule (Alz50, Tau-1, Tau46, and E-1, not shown). These tau proteins also contained phosphorylated epitopes (PHF-1,

Figure 1. Immunoblotting of a tau-enriched fraction from neuroblastoma cells with Alz50, Tau-1, and Tau46. Samples from untreated cells (C), cells with 15–60 min (m) of heat stress at 45°C, cells with 2–6 hr (h) of recovery from heat stress, and cells treated with 1 μ M OA were compared. C_{OA} represents preparations from cells that were treated with OA alone for 5 hr. Untreated cells contain tau of molecular weight 58, 55, and 53 kDa (marked by arrows), and smaller tau fragments. Tau46 reacts with both tau and MAP2C (marked by arrowheads), due to the sharing of a common amino acid sequence. Heat stress resulted in a reduction of tau molecular weight. OA-treated cells contained tau with a molecular weight similar to that of untreated cells. The treatment reduced the reactivity of tau with Tau-1 antibody.

T3P, and NP8) known to be present in PHF-tau (Fig. 2, lanes marked C). The 58 kDa tau differed from other tau bands in reactivity with E-2, an antibody that recognizes a subregion of tau known to be expressed in the N-terminal half of adult brain tau, but not fetal brain tau. Unlike the 58 kDa band, the 55 kDa and 53 kDa bands were not recognized by E-2 (Fig. 3; compare the blot stained with E-2 with the blot stained with both E-2 and Tau-1). By densitometric scanning of immunoblots stained with Tau-1 or Tau46, we estimated that the 53–55 kDa tau accounted for at least 90% of the neuroblastoma tau.

Besides tau proteins, Tau46 also reacted with bands at 68 kDa (Fig. 1, band marked with arrowheads) and above 200 kDa (not shown). These non-tau proteins are probably related to the MAP2 family, since Tau46 has been reported to recognize epitopes shared by tau and MAP2 (Ksiezak-Reding et al., 1990a). NP8 also reacted with proteins of molecular weight higher than tau and different from MAP2 (not shown). The NP8-reactive proteins are most likely phosphoproteins that share epitopes with tau and neurofilament proteins.

Effect of heat stress on the electrophoretic mobility and immunoreactivity of tau

Incubation of neuroblastoma cells at 45°C for 15–60 min resulted in an increase in the electrophoretic mobility of tau (Fig. 1, Stress). Instead of detecting tau at the 53–58 kDa region, three

or four tau-immunoreactive bands were detected between 55 kDa and 45 kDa. Similar to untreated tau, these polypeptides reacted with antibodies that recognize epitopes at the N-terminus (Alz50, E-1), middle region (Tau-1), and the C-terminus (Tau46) of tau molecule. The samples with longer heat stress proportionally contained more tau with high electrophoretic mobility (Fig. 1; compare band a with bands b and c). The heat-stressed tau reacted weakly with E-2, and they were detected as two bands migrating around the 55 kDa region (Fig. 3).

Heat stress changed the immunoreactivity of tau with PHF-1, T3P, and NP8 (Fig. 2, Stress). In cells that were heat stressed for 15 min, there was an apparent decrease of the immunoreactivity of tau with antibodies to phosphorylated epitopes. After 30–60 min of heat stress, the immunoreactivity of phosphorylated epitopes was no longer detectable, indicating that the loss of immunoreactivity of phosphorylated epitopes may be due to dephosphorylation. Addition of a protein synthesis inhibitor did not modify the response to heat stress. Also, the inclusion of additional phosphatase inhibitors in the lysis buffer did not alter results (data not shown).

Tau immunoreactivity in cells recovering from heat stress

Similar to heat-stressed cells, recovering cells also contained three or four undegraded tau polypeptides of 55–45 kDa that were reactive with Alz50, Tau-1, Tau46, and E-1 (Fig. 2). Recovery was slow; by 16 hr the cells still contained some tau with

Figure 2. Effect of heat stress, recovery, and OA treatment on the immunoreactivity of neuroblastoma tau to PHF-1, NP8, and T3P. The 58 kDa, 55 kDa, and 53 kDa tau proteins (marked by arrows) were recognized by the antibodies. Heat stress for 30–60 min (*m*) abolished the immunoreactivity of tau with antibodies to phosphorylated epitopes. During recovery, the tau proteins regained their immunoreactivity. OA treatment protected the cells from heat stress-induced alteration of immunoreactivity to antibodies to phosphorylated epitopes.

molecular weight smaller than that of control cells (data not shown).

Unlike the stressed cells, there was a reappearance of immunoreactivity with PHF-1, T3P, and NP8 in recovering cells (Fig. 2, Recovery). Whether protein synthesis inhibitors were present or not had no effect on the immunoreactivity of phosphate-dependent epitopes. By densitometry, tau with 6 hr of recovery was about 15, 12, and 3 times more immunoreactive with PHF-1, T3P, and NP8, respectively, than tau with 2 hr of recovery. The results indicate that the epitopes dephosphorylated by heat stress can be rephosphorylated during recovery.

OA protects tau from the effect of heat stress

As shown in Figure 2, OA-treated cells, regardless of whether they were subsequently subjected only to heat stress or to both heat stress and recovery, all contained tau proteins similar to those of control cells in molecular weight and in reactivity with PHF-1, T3P, and NP8. The protection of tau by OA from heat stress was also shown by immunoblotting with antibodies that recognize non-phosphorylated epitopes, Alz50, and Tau46.

Five hundred nanomolar OA was able to block the heat stress-induced changes of tau molecular weight and immunoreactivity (data not shown).

OA treatment induces phosphorylation of the Tau-1 epitope

OA-treated cells contained tau that were less reactive with Tau-1 antibody than tau proteins from untreated control cells (Fig. 1, OA). The 58 kDa band was not stained, and the staining of 55–53 kDa region was weaker than the corresponding region in the

control samples (Fig. 1; compare C_{OA} with C). Tau proteins from 4 or 6 hr recovering cells, as estimated by densitometry, were 70% less labeled by the Tau-1 antibody than those from cells after 2 hr recovery. This change was not due to differences in the loading of protein, since both samples contained similar levels of Alz50 and Tau46 immunoreactivity. Instead, it was most consistent with phosphorylation of the Tau-1 epitope (Fig. 4). Tau-1 immunoreactivity of control cells was not changed by

Figure 3. Immunoblots show E-2 binding to the 58 kDa neuroblastoma tau, but not the 55–53 kDa tau. As shown in the right panel, the 55–53 kDa bands (marked by arrows) not detected by E-2 (see left panel) were positive with Tau-1. The electrophoretic mobility of E-2-reactive tau was increased by heat stress (2, 4 hr).

Figure 4. OA treatment induces phosphorylation of the Tau-1 epitope. Electrophoretograms containing tau from untreated cells (C), from cells treated for 3 hr with OA (C_{OA}), and from cells with OA, heat stress, and recovery were each cut into two lanes: without alkaline phosphatase treatment (a) and with phosphatase treatment (b). Note that alkaline phosphatase treatment increases the immunoreactivity of OA-treated samples but has very little effect on the sample without OA treatment.

alkaline phosphatase treatment. In contrast, phosphatase treatment enhanced Tau-1 immunoreactivity of proteins from OA-treated control cells (Fig. 4, C_{OA}) by 1.8-fold, and from cells subjected to OA + heat stress + recovery by 4.7-fold (Fig. 4). The results indicate that OA treatment was accompanied by phosphorylation of the Tau-1 epitope. Similar alkaline phosphatase treatment did not alter immunoreactivity of tau proteins with Tau46, an observation that is consistent with the fact that Tau46 is not phosphate dependent (data not shown). The induction of phosphorylation of the Tau-1 epitope was observed in cells as early as 1 hr after OA treatment.

Discussion

In this study, we demonstrated that cultured human neuroblastoma cells contained tau of molecular weight about 55 kDa, 53 kDa, and 58 kDa. The 55 kDa and 53 kDa tau had no detectable reactivity with E-2, whereas the 58 kDa tau was reactive with E-2. The 55–53 kDa tau represented more than 90% of the tau from neuroblastoma cells and was comparable to fetal brain tau in molecular weight and in immunoreactivity with anti-tau antibodies (Greenberg et al., 1992; Kenessey and Yen, unpublished observations). Both the major and minor forms of neuroblastoma tau shared phosphorylated epitopes (PHF-1, T3P, and NP8) with PHF-tau. Thus, our results strongly suggest that cultured cells and possibly fetal brains contain kinase/phosphatase systems that may be operative in neurons of AD. Moreover, neuroblastoma cells or fetal brains may be a good source for isolation and characterization of enzymes capable of affecting phosphorylation of tau similar to that in PHF-tau.

Exposure of cultured cells to heat stress for 30–60 min led to the loss of immunoreactivity of tau proteins with antibodies to phosphorylated epitopes (PHF-1, T3P, and NP8). Exposure of cultured cells to heat stress also led to a decrease in the molecular weight of tau from 53–58 kDa to 55–45 kDa. The changes were unlikely due to proteolysis of the N-end of the tau molecule, since these regions (Alz50 epitope, aa 2–10; E-1 epitope, aa 19–33) were detected in the heat-stressed tau. Involvement of pro-

teolysis at the C-terminus was also unlikely, since the stressed tau retained the Tau46 epitope (aa 404–441). According to previous studies (Ksiazek-Reding et al., 1990a), the C-terminal of tau is less sensitive to proteolysis than the N-terminus. Further evidence that argues against the involvement of proteolysis was obtained from the studies of cells recovering from heat stress, in which the electrophoretic mobility and immunoreactivity of tau were partially restored after the cells were transferred from the heat-stress condition to 37°C. Moreover, addition of OA to cells prior to heat stress protected the tau proteins in these cells from changes in electrophoretic mobility and immunoreactivity induced by heat stress. The results indicate that the increase of electrophoretic mobility of heat-stressed tau is related at least in part to dephosphorylation.

Rephosphorylation of dephosphorylated epitopes was found in cells after 2 hr of recovery at 37°C. At this point in time, only one of the 55–45 kDa bands reacted with antibodies to phosphorylated epitopes. We do not know whether the heat stress-induced dephosphorylation and the recovery-associated rephosphorylation are limited to PHF-1, T3P, and NP8 epitopes. This issue may be resolved by further studies of the immunoreactivity of heat-stressed tau with other antibodies raised to defined phosphorylated epitopes, or analysis of the phosphate content of tau in cells with and without heat stress. The increase of PHF-1, NP8, and T3P immunoreactivities in the recovering state was not likely due to protein synthesis, since similar results were obtained in the presence of protein synthesis inhibitors.

The effect of heat stress on cultured cells was different from that on intact animals. While heat stress apparently did not affect the phosphorylation of Tau-1 epitope in neuroblastoma cells, it has been reported to cause a reduction of Tau-1 immunoreactivity in young adult rats (Papazosomenos and Su, 1991). We do not know the basis for these differences. One possibility might be the difference in species (human vs. rat). Alternatively, this might be a result of differences in the kinase/phosphatase system between neuroblastoma cells and adult rat brains.

Tau from LAN cells or normal human brains, in contrast to PHF-tau, can readily be detected by Tau-1 antibody without pretreatment with alkaline phosphatase. This difference might be due to a rapid dephosphorylation of the Tau-1 site in non-AD samples. In support of this view is our finding of the phosphorylated Tau-1 epitope in cells that were treated with OA, an inhibitor to protein phosphatases 1 and 2A. These phosphatases have been shown to dephosphorylate tau in *in vitro* studies. The effect of OA on the Tau-1 epitope, however, might also be due to activation of kinase(s), which either directly catalyzes the Tau-1 epitope phosphorylation or acts indirectly through a cascade of actions. OA treatment has been shown to increase protein phosphorylation in cultured cells (Olsson and Belfrage, 1987; Haavik et al., 1989; Haystead et al., 1989) and to activate MAP2 kinases in PC12 cells (Miyasaka et al., 1990). Moreover, tau proteins can be phosphorylated *in vitro* by MAP kinases (Dreus et al., 1992). It remains to be determined if the activation of MAP kinases and/or other kinases *in vivo* is required for the phosphorylation of Tau-1 epitope in PHF.

OA treatment prevented the PHF-1, T3P, and NP8 epitopes from the dephosphorylation induced by heat stress. Unlike tau from heat-stressed cells (45–60 min), which did not react with PHF-1, T3P, and NP8, tau proteins from OA-treated and heat-stressed cells did not lose their reactivity with these antibodies. The results suggest that heat stress might exert its effect by

stimulation of phosphatase 1 and/or phosphatase 2A. Alternatively, it might involve inhibition of kinases involved in phosphorylating the sites mentioned above. Comparative analysis of the activity of different kinases and phosphatases in control and stressed cells may resolve this issue.

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