The Microtubule Binding of Tau and High Molecular Weight Tau in Apoptotic PC12 Cells Is Impaired because of Altered Phosphorylation*

(Received for publication, July 23, 1999, and in revised form, August 31, 1999)

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Although the importance of the microtubule network throughout cell life is well established, the dynamics of microtubules during apoptosis, a regulated cell death process, is unclear. In a previous study (Davis, P. K., and Johnson, G. V. (1999) Biochem. J. 340, 51–58) we demonstrated that the phosphorylation of the microtubule-associated protein tau was increased during neuronal PC12 cell apoptosis. The purpose of this study was to determine whether the increased tau phosphorylation that occurred during apoptosis impaired the microtubule binding capacity of tau. This study is the first demonstration that microtubule-binding by tau and high molecular weight tau is significantly impaired as a result of altered phosphorylation during a naturally occurring process, apoptosis. Furthermore, co-immunofluorescence studies reveal for the first time that tau populations within an apoptotic neuronal PC12 cell exhibit differential phosphorylation. In control PC12 cells, Tau-1 staining (Tau-1 recognizes an unphosphorylated epitope) is evident throughout the entire cell body. In contrast, Tau-1 immunoreactivity in apoptotic PC12 cells is retained in the nuclear/perinuclear region but is significantly decreased in the cytoplasm up to the plasma membrane. The selective distribution of phosphorylated tau in apoptotic PC12 cells indicates that tau likely plays a significant role in the cytoskeletal changes that occur during apoptosis.

The cytoskeleton and its associated proteins provide the framework of the cell and are important for the facilitation of many critical events such as mitosis, migration, and differentiation. As a major component of the cytoskeleton, microtubules are dynamic structures, essential for many cellular processes, such as process outgrowth, cell polarity, and intracellular trafficking (1–3). Microtubule-associated proteins are critically important modulators of microtubule dynamics. The microtubule-associated protein tau is a family of differentially spliced isoforms (4) that are primarily but not exclusively expressed in neurons (4–8). Tau was first identified by its ability to stabilize microtubules (20, 21). In vitro, tau is a substrate of numerous protein kinases, and phosphorylation by any of these kinases usually but not always decreases the ability of tau to bind microtubules and promote microtubule assembly (for reviews see Refs. 22 and 23). However, the extent to which the microtubule binding capacity of tau is reduced by phosphorylation is highly dependent on which sites are phosphorylated. For example, phosphorylation of tau at just a few sites within the microtubule-binding region virtually abolishes the association of tau with microtubules, whereas phosphorylation of numerous sites outside this region reduces but does not eliminate tau binding to microtubules (24–26). Although it is likely that site-specific phosphorylation also modulates the non-microtubule functions of tau, these putative effects have not yet been examined. Thus far, 25 Ser and Thr residues throughout tau have been identified as phosphorylation sites (27, 28). However, the regulatory implications of phosphorylation at the majority of these residues are unknown.

High molecular weight (HMW)1 tau is produced from an 8-kilobase mRNA generated by alternative splicing from the same gene that encodes for tau (6, 7). HMW tau contains one or two additional inserts compared with tau and migrates on SDS-polyacrylamide gels with an apparent molecular mass of approximately 110 kDa (6, 7). HMW tau is primarily expressed in the peripheral nervous system and in cell lines derived from the neural crest such as PC12 cells (29, 30). Very little is known about the function and regulation of HMW tau. Fractionation of peripheral nerve tissue demonstrated that HMW tau was in

* This work was supported by National Institutes of Health Grant NS35060. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: HMW, high molecular weight; NGF, nerve growth factor; PIPES, 1,4-piperazinediethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; PBS, phosphate-buffered saline; JNK, N-terminal c-Jun kinase.
the tubulin fraction, clearly indicating that it associated with microtubules (31). Given this finding, it is likely that the general functional properties of HMW tau may be similar to those of tau. However, the microtubule binding characteristics of HMW tau have not yet been measured. The regulation of HMW tau by phosphorylation remains mostly unclear, as is the specific intracellular localization of HMW tau compared with tau.

Tau and other cytoskeletal regulatory proteins may play an important role in facilitating the cytoskeletal reorganization that occurs during apoptotic cell death (32–35). In a previous study, our laboratory demonstrated that during apoptosis of neuronal PC12 cells induced by trophic factor withdrawal, tau and HMW tau were hyperphosphorylated at residues within the Tau-1 antibody epitope and potentially at other Ser and/or Thr residues (35). The present study focused on the functional and localization alterations associated with tau and HMW tau hyperphosphorylation during apoptosis of neuronal PC12 cells. Intriguingly, the microtubule binding capacity of both tau and HMW tau from apoptotic neuronal PC12 cells was significantly less than that of tau and HMW tau isolated from control cells. Furthermore, the microtubule binding function of tau and HMW tau from apoptotic cells was restored to almost control levels following dephosphorylation. Examination of Tau-1 immunoreactivity in apoptotic cells indicated that tau hyperphosphorylated at the Tau-1 epitope was limited to specific subcellular locations. These novel findings demonstrate functional and localization consequences of tau and HMW tau phosphorylation during a physiological process, apoptosis, and suggest that the phosphorylation of tau may play an important role in microtubule reorganization during the apoptotic process of neuronal cells.

EXPERIMENTAL PROCEDURES

Cell Culture—PC12 cells were grown on rat tail collagen-coated dishes in RPMI 1640 medium containing 10% horse serum, 5% Fetal Clone II (Hyclone, Logan, UT), 20 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin as described previously (36). To induce differentiation to a neuronal phenotype, PC12 cells were maintained in serum-free medium with 5% serum and 100 ng/ml nerve growth factor (NGF) for 3–4 days. For differentiation to a neuronal phenotype, PC12 cells were maintained in medium containing 5% serum and 100 ng/ml nerve growth factor (NGF) for 9–10 days (36). Serum and NGF were removed by replacing the differentiated PC12 cells onto new collagen-coated dishes as described previously (37), and cells were maintained in serum-free medium with or without 100 ng/ml NGF for the times indicated (38). For co-immunofluorescence studies, cells were reseeded onto glass coverslips in 24-well plates coated with 12 μg/ml laminin (Life Technologies, Inc.) and 100 μg/ml poly L-lysine (Sigma).

Immunoblot Analysis—Cell homogenates were prepared, and immunoblot analysis was carried out as described previously (38). Briefly, proteins were separated by electrophoresis on a 7.5% SDS-polyacrylamide gel, transferred to nitrocellulose, and probed with either Tau-1 or Tau-5. After incubation with horseradish peroxidase-conjugated secondary antibody, immunoblots were developed using Enhanced Chemiluminescence (Amersham Pharmacia Biotech). Immunoblots were quantified using a Bio-Rad GS-670 imaging densitometer.

Preparation of Taxol-stabilized Microtubules—Rat brain tubulin was purified from once-cycled microtubules by phosphocellulose chromatography as described previously (9). Tubulin containing fractions were pooled, and taxol was added to a final concentration of 20 μM. Following incubation at 37 °C for 30 min, the assembled microtubules were pelleted by centrifugation at 130,000 × g for 1 h. Microtubules were resuspended in stabilization buffer (50 mM PIPES/KOH, pH 6.9, 1 mM EGTA, 0.5 mM MgSO₄, 20 μM taxol, and 0.1 mM GTP), aliquoted, and frozen at −80 °C until use.

Purification of Tau and HMW Tau—Because tau and HMW tau are heat-stable proteins (9, 39), enrichment of tau and HMW tau from control and apoptotic PC12 cells was carried out by preparing a heat-stable fraction as described previously (38). For each purification, 7–9 100-mm dishes of control or apoptotic PC12 cells were used. Tau and HMW tau were separated using size exclusion chromatography on a column (20 × 1 cm) containing Toyopearl HW-55S chromatographic medium with a globular protein size exclusion limit of 10,000–700,000 kDa (TosoHaas, Montgomeryville, PA). Proteins were eluted with column buffer (20 mM Mes, pH 7.2, 1 mM EGTA, 1 mM EDTA, 2 mM diethiothreitol, 750 mM NaCl, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 2 μg/ml peptatin A, 10 μg/ml soybean trypsin inhibitor, and 0.1 mM phenylmethylsulfonyl fluoride) at a flow rate of 0.1 ml/min. Fractions containing Tau and HMW tau were identified by immunoblot analyses using the phosphate-independent antibody Tau-5 (41). To ensure that non-tubulin-associated protein 2 was not present in HMW tau fractions, the immunoblots were also probed with AP14 antibody (42). Fractions containing tau or HMW tau were combined and dialyzed against buffer containing 50 mM PIPES/KOH, pH 6.9, 1 mM EGTA, and 0.5 mM MgSO₄ using Slide-A-Lyzer (Pierce). Proteins were concentrated using Centric-10 concentrators (Amicon, Beverly, MA), and the concentration was determined using the bicinchoninic acid assay (Pierce).

Microtubule Binding Assay—The microtubule binding assay was carried out as described previously with modifications (24). To remove unpolymerized tubulin, taxol-stabilized microtubules were incubated for 30 min at 37 °C, centrifuged at 110,000 × g for 10 min, and resuspended in reaction buffer containing 50 mM PIPES, pH 6.9, 1 mM EGTA, 0.5 mM MgSO₄, 20 μM taxol, and 0.1 mM GTP at 37 °C. Microtubules (30 μM) were reincubated for 10 min at 37 °C. Tau at 4.5 μM or HMW tau at 2 μM was then added, and incubation continued for 10 min at 37 °C. After incubating each sample with a 10% sucrose (prepared in reaction buffer) cushion, the samples were centrifuged at 110,000 × g for 10 min in a Beckman Airfuge. Microtubule-bound tau or HMW tau present in the pellets was determined by quantitative immunoblot analysis using appropriate secondary antibody, and statistical analysis was carried out using Student’s t-test. To dephosphorylate tau or HMW tau prior to use in the microtubule binding assay, the proteins were incubated in the absence or presence of lambda protein phosphatase (New England Biolabs Inc., Beverly, MA) in reaction buffer for 4 h at 37 °C. Following incubation, microtubules were added, and the assay was carried out as described above.

Co-immunofluorescence—For fixation, cells were rinsed once with PEM (80 mM PIPES/KOH, pH 6.8, 1 mM EGTA, 2 mM MgCl₂, 10 μM taxol, and 1 mM GTP) and incubated with 2% (w/v) paraformaldehyde in PEM for 30 min at 37 °C. After rinsing with phosphate-buffered saline (PBS; pH 7.4, 1.5 mM KH₂PO₄, 8.0 mM NaHPO₄, 2.7 mM KCl, 137 mM NaCl), cells were incubated with 0.1 M glycine for 20 min and permeabilized for 90 s in 0.5% (w/v) Triton X-100 in PBS (16). Staining was carried out essentially as described by Brandt et al. (16). Before staining, cells were blocked for 1 h with PBS containing 0.3% bovine serum albumin. All antibodies were diluted in blocking solution before use. The primary antibodies used were: a tau polyclonal antibody (Dako Corp.), the Tau-1 monoclonal tau antibody (8) (a gift from Dr. L. Binder), and an active e-jun N-terminal kinase polyclonal antibody (Camago, WI). Tau-1 antibody recognizes tau or HMW tau only when Ser195, Ser198, Ser199, Ser202, and Thr205 (numbered according to the longest human brain tau isoform (43)), are not phosphorylated (8, 44). To increase sensitivity, Tau-1 was biotinylated with sulfo-NHS-biotin according to the manufacturer’s instructions (Pierce) and was visualized using Texas Red-conjugated streptavidin (Jackson Immunoresearch Labs, West Grove, PA). Secondary antibodies were fluorescein isothiocyanate-isothiocyanate-conjugated anti-rabbit, fluorescein isothiocyanate-isothiocyanate conjugated rabbit anti-mouse, or Texas Red-conjugated goat anti-rabbit (Jackson Immunoresearch Labs). Cells were incubated for 1 h at room temperature with the primary antibodies, followed by a 30-min incubation at room temperature with the appropriate secondary antibodies. Coverslips were then incubated with 10 μg/ml Hoechst in PBS for 1 min at room temperature. After extensive washing with PBS, coverslips were mounted using Immuno-Mount (Shandon, Pittsburgh, PA) and stored at −20 °C. Cells were photographed using the SPOT cooled color digital camera (Diagnostic Instruments Inc., Sterling Heights, MI) on a Nikon Diaphot 200 microscope with a 100× CF/CF N plan apochromat objective. The images of the Hoechst stained nuclei were pseudocolored for increased clarity with the SPOT camera software.

RESULTS

Tau and HMW Tau from Apoptotic Cells Have Reduced Microtubule Binding Capacity—As demonstrated previously by our laboratory (38) and others (45, 46), neuronal PC12 cells become apoptotic following removal of serum and NGF. Depriving neuronal PC12 cells of serum and NGF for 48 h resulted in a significant decrease in cell viability and an increase in the presence of condensed chromatin (38). Additionally, based on quantitative immunoblot analysis, significant increases in the

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phosphorylation of tau and HMW tau from apoptotic cells within the Tau-1 epitope occurred at this time point (35). Therefore, all subsequent studies were carried out on PC12 cells 48 h after removal of serum and NGF or of serum alone (as a control). Chromatin morphology determined by Hoechst staining demonstrated that after 48 h of serum and NGF deprivation ~25% of the cells exhibited condensed chromatin (38). However, the majority of the cells exhibited activated N-terminal c-Jun kinase (JNK) staining in the nucleus (data not shown, but see Fig. 4, D and F), an event in the apoptotic process that precedes condensation of the chromatin (47–49).

The function of tau and HMW tau isolated from apoptotic and control PC12 cells was assessed using an in vitro microtubule binding assay. Equivalent amounts of tau or HMW tau purified from control and apoptotic cells were incubated with taxol-stabilized microtubules. The amount of tau and HMW tau from apoptotic cells that bound to the microtubules was significantly less (37 and 27% of control, respectively) than that of tau and HMW tau isolated from control cells (Fig. 1). These findings demonstrate that the microtubule binding capacity of tau and HMW tau from apoptotic neuronal PC12 cells is significantly impaired.

**Restoration of the Microtubule Binding Capacity of Tau and HMW Tau from Apoptotic Cells by Dephosphorylation**—Tau and HMW tau from apoptotic and control cells were dephosphorylated with lambda protein phosphatase prior to assessing their function using the microtubule binding assay. To determine whether incubation with lambda protein phosphatase resulted in tau dephosphorylation, equivalent amounts of tau from control (+ NGF) and apoptotic (− NGF) cells deprived of serum for 48 h were incubated in the presence (+) or absence (−) of lambda phosphatase and phosphorylation within the Tau-1 epitope was analyzed by immunoblotting (A). Following incubation in the presence (+) or absence (−) of lambda phosphatase, tau or HMW tau from control (+ NGF) and apoptotic (− NGF) neuronal PC12 cells deprived of serum and NGF for 48 h were incubated with taxol-stabilized microtubules. Microtubule bound tau or HMW tau was determined by immunoblot analysis using the phospho-independent antibody, Tau-5 (B). Immunoblots are representative of at least three independent experiments. Molecular masses (kDa) are indicated at the left.

Their function using the microtubule binding assay. To determine whether incubation with lambda protein phosphatase resulted in tau dephosphorylation, equivalent amounts of tau from control (+ NGF) and apoptotic (− NGF) cells deprived of serum for 48 h were incubated in the presence (+) or absence (−) of lambda phosphatase and phosphorylation within the Tau-1 epitope was analyzed by immunoblotting (A). Following incubation in the presence (+) or absence (−) of lambda phosphatase, tau or HMW tau from control (+ NGF) and apoptotic (− NGF) neuronal PC12 cells deprived of serum and NGF for 48 h were incubated with taxol-stabilized microtubules. Microtubule bound tau or HMW tau was determined by immunoblot analysis using the phospho-independent antibody, Tau-5 (B). Immunoblots are representative of at least three independent experiments. Molecular masses (kDa) are indicated at the left.
Loss of Tau-1 Immunoreactivity in Apoptotic PC12 Cells—To examine the localization of tau associated with changes in Tau-1 phosphorylation (35) within apoptotic cells, co-immunofluorescence studies were carried out using Tau-1 and polyclonal tau antibodies. Total tau in control cells was distributed throughout the neurites and cell body (Fig. 3A). In control cells Tau-1 reactivity, which recognizes only an unphosphorylated epitope of tau and HMW tau (8), was present throughout the cell body, including the nuclear and perinuclear regions as evidenced by colocalization with the Hoechst stain. In contrast, Tau-1 staining was almost undetectable in the neurites (Fig. 3B). Although total tau staining of apoptotic cells was still present throughout the cell body, including the nuclear and perinuclear regions (Fig. 3D), a dramatic loss of Tau-1 immunoreactivity was observed in the cell body and at the plasma membrane indicating hyperphosphorylation within the epitope, and was present only within the nucleus and/or perinuclear region (E). Open arrowheads point to specific cells (D–F) and are used for orientation purposes between panels. In E and F the solid arrowheads indicate a nucleolus and emphasize the lack of Tau-1 staining in this area. Bar, 5 μm.

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To clearly demonstrate that cells possessing noncondensed chromatin and decreased Tau-1 immunoreactivity in the cultures deprived of serum and NGF were undergoing apoptosis, cells were co-stained with Tau-1 and an antibody to activated JNK. Several studies have demonstrated previously that JNK activity increases during apoptosis of neuronal PC12 cells (48, 49) and localizes to the nucleus (47). JNK is activated by phosphorylation of Thr183 and Tyr185 (51), and a JNK antibody recognizing an epitope containing these phosphorylated residues was used. In control cells, a diffuse, light staining pattern of active JNK was observed throughout the cytoplasm but was virtually absent in the nucleus as indicated by the lack of colocalization with the Hoechst staining (Fig. 4A and C). Tau-1 staining in control cells was, as before, primarily in the cell body (Fig. 4B). In cells deprived of NGF and serum, staining of active JNK was intensely bright and localized to the nucleus in cells exhibiting normal chromatin morphology, indicating that these cells are apoptotic (Fig. 4D and F). Cells possessing nuclear active JNK also exhibited the alterations in the Tau-1 staining, which was localized to the nucleus and perinuclear regions (Fig. 4E).
DISCUSSION

Microtubule dynamics are essential for numerous processes during cell life, and they most likely play an integral role during apoptotic cell death. This is implied by several studies demonstrating alterations of cytoskeletal components during apoptosis (33, 52, 53). Tau acts as a regulator of microtubule dynamics through its ability to stabilize microtubules and promote microtubule assembly (9–11), a function that is modified by its phosphorylation state (20, 21, 54). However, abnormal alterations in the phosphorylation state of tau may lead to impaired function and thus negatively affect microtubule stability and cytoskeletal dynamics (21, 55, 56).

During apoptosis of neuronal PC12 cells tau and HMW tau are hyperphosphorylated at Ser and/or Thr residue(s) within the Tau-1 antibody epitope and potentially at other Ser/Thr residues (35). The purpose of this study was to investigate the outcome of altered phosphorylation of tau and HMW tau during neuronal PC12 cell apoptosis in terms of function and localization. In this study, two novel findings are presented. First, alterations in the phosphorylation state of tau and HMW tau during neuronal apoptosis significantly reduced the microtubule binding capacity of tau and HMW tau. Second, in apoptotic cells Tau-1 staining was significantly reduced throughout the cell body with the exception of the nucleus and perinuclear region, indicating hyperphosphorylation of tau and HMW tau at this epitope in the cell body but not in the nucleus and perinuclear region. Thus the intracellular localization of tau is an important determinant of whether or not it is hyperphosphorylated during apoptosis.

In apoptotic PC12 cells, total tau staining appeared similar to control cells. In contrast, the pattern of Tau-1 immunoreactivity in apoptotic cells differed significantly from that of control PC12 cells, indicating subcellular changes in the phosphorylation of tau within this epitope. Tau-1 staining in control cells was observed mostly throughout the cell body and to a much lesser extent in the neurites. Similar observations have been reported previously in immature, cultured hippocampal neurons where Tau-1 staining was predominantly in the cell body and only to a minor extent in the developing processes (57). Tau-1 staining was also present in the nucleus and perinuclear region of the control and apoptotic PC12 cells. Tau has previously been identified within the nucleus using the Tau-1 antibody (58–60). In dividing cultured cells tau localizes to the nucleoli during interphase and to the nucleolar organizing regions during mitosis (59). Given these findings it has been hypothesized that tau may be involved in nucleolar reformation and/or rRNA synthesis during cell division (50, 59, 60). In contrast to these observations in dividing cultured cells, tau is present in the nucleus but not in the nucleoli in differentiated, nondividing cells (50). In the present study Tau-1 immunoreactivity was present in nuclei but not in nucleoli of differentiated, neuronal PC12 cells, and thus is in agreement with the previous findings. Because tau in differentiated cells does not localize to the nucleolus, it is likely that the functions
tau mediates in the nucleus of these cells differs from those it regulates in dividing cells. In differentiated cells tau may be involved in signaling or regulation of nuclear proteins. However, the functional roles of nuclear tau in both dividing and differentiated cells remain to be elucidated.

During apoptosis, Tau-1 staining throughout the cell body is significantly diminished, although total tau staining is maintained, indicating an increase in tau phosphorylation within this epitope (35). In agreement with these findings, increased tau phosphorylation was indicated in a previous report that showed increased immunoreactivity of apoptotic PC12 cells with AT8, an antibody that recognizes a phosphoepitope within the Tau-1 epitope (61). Furthermore, it is interesting that Tau-1 immunoactivity (which recognizes only the unphosphorylated epitope) is maintained in the perinuclear region of apoptotic cells. This finding is most likely due to differential alterations in the activity of protein kinases and/or protein phosphatases in specific subcellular compartments during apoptosis. The selective increase in tau phosphorylation in the vicinity of the plasma membrane during apoptosis is indicative of a specific role for tau in this area of the cell. Given that tau likely mediates microtubule-plasma membrane interactions through specific actin-binding proteins (16), it is tempting to speculate that the increase in tau phosphorylation during apoptosis may result in a disruption of these interactions and hence facilitate the membrane blebbing that occurs (38, 62). Microtubule destabilization contributes to apoptotic blebbing (63, 64), and therefore the decrease in the microtubule-binding of tau in apoptotic cells may also play a role in this process. The selective maintenance of Tau-1 immunoactivity in the perinuclear region of apoptotic neuronal PC12 cells indicates that tau in a “normal” phosphorylation state in this subcellular compartment may be required for appropriate progression of the apoptotic process. For example, tau may mediate interactions between the cytoskeleton and the nuclear envelope.

In contrast to the findings presented here and in our previous study (35) that demonstrated increased tau phosphorylation during PC12 cell apoptosis, an earlier report presented data suggesting that there was a general dephosphorylation of tau during apoptosis based on immunocytochemical findings (65). However the study of Mills et al. (65) differed in several aspects from the present one. First, a subclone of the PC12 cell line called PC6–3 (46) was used that displays significant differences from the parental PC12 cell line with respect to sensitivity to apoptotic stimuli and the timing of apoptotic events. Second, the protocol used by Mills et al. (65) to induce apoptosis differed significantly from the procedure used in this study (37, 38). Lastly, it should be noted that unlike the present study, Mills et al. (65) did not examine co-immunofluorescence for total tau and Tau-1 staining, and it was difficult to assess the increases in Tau-1 immunoreactivity relative to the total tau levels in individual cells because the levels of tau in the apoptotic cultures showed significant cell-to-cell variability. In support of the findings presented here, Nuydens et al. (61) reported an increase in tau phosphorylation in apoptotic PC12 cells. Furthermore, during apoptosis there is a decrease in the activity of specific protein phosphatases that are known to dephosphorylate tau (38). Finally, the fact that the microtubule binding capacity of tau and HMW tau from apoptotic cells was restored by dephosphorylation (see below) clearly indicates that tau phosphorylation is increased in the apoptotic paradigm used in the current study.

Because the phosphorylation state of tau and HMW tau was increased during apoptosis, the functions of tau and HMW tau purified from apoptotic and control neuronal PC12 cells were examined in vitro by assessing their ability to bind taxol-stabilized microtubules. The ability of both tau and HMW tau from apoptotic cells to bind to microtubules was impaired significantly compared with tau and HMW tau from nonapoptotic cells. However, after dephosphorylation the microtubule binding capacity of both tau and HMW tau from apoptotic cells was almost fully restored. These findings clearly demonstrate that the phosphorylation state of tau and HMW tau is increased during apoptosis resulting in functional impairment. Further, to our knowledge, this is the first study to demonstrate that phosphorylation of HMW tau diminishes its binding to microtubules. At the present time, little is known about the effects of phosphorylation of specific residues, alone or combination, on tau function. However, it is unlikely that hyperphosphorylation of the Tau-1 epitope is solely responsible for the impairment of tau and HMW tau microtubule-binding during apoptosis. The mechanisms leading to alterations in the phosphorylation of tau and HMW tau during apoptosis may involve increased protein kinase activity, decreased protein phosphatase activity, aberrant expression of a protein kinase, or any combination of these events. For example, increases in Cdc2 protein kinase activity (38, 66, 67) and decreases in protein phosphatase 2B activity (38) occur during apoptosis of neuronal PC12 cells and may potentially contribute to altered tau and HMW tau phosphorylation.

Nuclear changes, including DNA fragmentation and chromatin condensation, have been shown to occur in the late stages of the apoptotic process (68, 69). Therefore, the extremely low levels of total tau, as well as tubulin and actin (data not shown), in apoptotic cells containing condensed chromatin are likely due to late proteolytic events that occur immediately prior to the demise of the cell. In contrast, the alterations in the Tau-1 staining pattern occurred in apoptotic cells prior to nuclear condensation, suggesting that these changes are coincident with earlier phases of the apoptotic process. We hypothesize that hyperphosphorylation within the Tau-1 epitope and coincident impairment of the microtubule binding capacity of tau and HMW tau occurs prior to the late stages of apoptosis and probably during early stages apoptosis when the cell is preparing to irreversibly enter the cell death process (70). This implies that increased phosphorylation of tau and HMW tau at the Tau-1 epitope and at other putative sites resulting in impaired microtubule binding function may contribute to the cytoskeletal alterations and membrane blebbing that occur during apoptotic cell death. Although it cannot be ruled out that changes in tau phosphorylation are nonspecific downstream events that occur as a result of the apoptotic process, the early and selective changes in tau phosphorylation (35) and impairment of microtubule-binding strongly suggest that the increase in tau phosphorylation may facilitate the cytoskeletal rearrangements that occur during apoptosis.

The microtubule binding capacity of tau isolated from Alzheimer’s disease brain is significantly decreased as a result of abnormal phosphorylation (55, 71). Numerous hyperphosphorylated Ser and Thr residues throughout tau from Alzheimer’s disease brain have been identified including the those within the Tau-1 epitope (27, 28), and alterations of phosphorylation within the Tau-1 epitope of tau represent an early neuropathological event of Alzheimer’s disease (72). Although the study of tau hyperphosphorylation in Alzheimer’s disease has been extensive, the mechanisms contributing to the hyperphosphorylated state of Alzheimer’s disease tau are unknown. Interestingly, several studies have presented evidence that neuronal apoptosis may contribute to the neurodegeneration that occurs in Alzheimer’s disease (73–77). Thus, further study of the mechanisms involved in tau hyperphosphorylation at the Tau-1 site and in the incompetence of tau microtubule binding...
function during neuronal PC12 cell apoptosis may contribute to our understanding of events responsible for the hyperphosphorylation and dysfunction of tau during Alzheimer's disease. Finally, the findings of this study have provided important and novel information regarding the function and regulation of HMW tau and the regulation and function of the cytoskeletal proteins tau and HMW tau during neuronal apoptotic cell death.

Acknowledgments—We thank Dr. R. S. Jope for critically reading and editing the manuscript.