Site-specific Dephosphorylation of Tau Protein at Ser\textsuperscript{202}/Thr\textsuperscript{205} in Response to Microtubule Depolymerization in Cultured Human Neurons Involves Protein Phosphatase 2A*

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Tau proteins isolated from paired helical filaments, the major building blocks of Alzheimer's disease neurofibrillary tangle, are abnormally phosphorylated and unable to bind microtubules. To examine the dynamics of tau phosphorylation and to identify specific tau phosphorylation sites involved in the stabilization of microtubules, we treated cultured postmitotic neuron-like cells (NT2N) derived from a human teratocarcinoma cell line (NTera2/D1) with drugs that depolymerize microtubules (i.e. colchicine or nocodazole). This led to the recovery of dephosphorylated tau from the NT2N cells as monitored by a relative increase in the electrophoretic mobility of tau and an increase in the turnover of $^{32}$PPO$_4$-labeled tau. However, not all phosphorylation sites on tau are affected by colchicine or nocodazole. Ser\textsuperscript{202}/Thr\textsuperscript{205} appears to be completely and specifically dephosphorylated by protein phosphatase 2A since this dephosphorylation was blocked by inhibitors of protein phosphatase 2A but not by inhibitors of protein phosphatase 2B. These findings, together with the recent observation that protein phosphatase 2A is normally bound to microtubules in intact cells, suggest that the polymerization state of microtubules could modulate the phosphorylation state of tau at specific sites in the normal and Alzheimer's disease brain.

Paired helical filaments (PHFs)\textsuperscript{1} are the major building blocks of neurofibrillary tangles, neurofibril threads, and senile plaque neurites in Alzheimer's disease brains (reviewed in Refs. 1–3). PHFs are composed of abnormally phosphorylated central nervous system (CNS) tau proteins (PHF-tau) (4–8) that normally promote and stabilize the assembly of microtubules (MTs) (9). Normal adult CNS tau consists of six alternatively spliced isoforms encoded by one gene (10, 11), and all are found in PHF-tau (12), which differs from normal tau in the extent and sites of phosphorylation (2, 8, 13–16). We recently showed that some of the putative “abnormal” phosphorylation sites in PHF-tau are normal sites of phosphorylation in adult rat (17) and adult human brain tau isolated from biopsy samples (18). These studies also showed that rat and human brain tau proteins are phosphorylated to a lesser extent at many of the same sites as in PHF-tau. Significantly, an increase in tau phosphorylation decreases MT binding (19, 20), and enzymatic dephosphorylation PHF-tau restores its ability to bind MTs (20). Several phosphorylation sites in tau (including Ser\textsuperscript{396} and Ser\textsuperscript{262}) modulate binding to MTs (20, 21), and hyperphosphorylation of these sites may decrease the affinity of PHF-tau for MTs leading to the depolymerization of MTs, impaired axonal transport, neuronal degeneration, and the aggregation of tau into PHFs.

The affinity of tau for MTs is regulated by the number of MT binding repeats (22, 23), proline-rich sequences adjacent to the MT binding repeats (24), linker sequences between MT binding repeats (25), and the extent and sites of phosphorylation (19–21). The expression of different tau isoforms and their phosphorylation states are developmentally regulated (2, 11, 17, 18, 20). During human development only the smallest isoform is expressed, and this isoform contains three MT binding repeats and no N-terminal inserts (11). The six adult human brain tau isoforms differ in the number of MT binding repeats and with respect to the presence or absence of N-terminal inserts (11, 22), while fetal tau is more phosphorylated than adult tau. Thus, the number of MT binding repeats and phosphorylation may facilitate reorganization of the cytoskeleton during axonal growth and synaptogenesis by modulating the affinity of fetal tau for MTs.

Although several kinases and phosphatases have been implicated in regulating the phosphorylation state of tau in vitro (12, 21, 26–38), it is not known which of these enzymes performs this function in vivo, and the mechanisms that lead to the abnormal phosphorylation of PHF-tau in the Alzheimer's disease brain are unknown. In part, this reflects the lack of model systems of Alzheimer's disease neurofibrillary lesions. Moreover, biochemical studies of tau in cultured cells are difficult since tau is expressed almost exclusively in postmitotic neurons. Recently, we have obtained nearly pure cultures of neuron-like cells (NT2N) by treating a human teratocarcinoma cell line (NTera2/D1 or NT2) with retinoic acid (39, 40). Notably, the neuron-like NT2N cells are irreversibly postmitotic and develop the phenotype of late embryonic CNS neurons (40, 41).

Here, we used the NT2N neurons as a model system to study the regulation of the phosphorylation state of human tau, as well as the affinity of tau for MTs, by perturbing the MT network with colchicine or nocodazole, which lead to the site-specific dephosphorylation of tau. Further, we also demonstrated that the site-specific dephosphorylation of tau is due to the action of protein phosphatase 2A (PP2A).
EXPERIMENTAL PROCEDURES

Materials—Reticin acid, GTP, poly-o-lysine, cystine arabinoside, fluoride oxuryridine, uridine, type III Escherichia coli alkaline phosphatase, colchicine, nocardazole, Triton X-100, leupeptin, TPCK, TLCK, phenylmethylsulfonyl fluoride, pepstatin, EDTA, and MES were purchased from Sigma. [35S]Methionine and [32P]PO4 were from ICN; okadaic acid and calyculin-A were purchased from LC laboratories, Woburn, MA; FK506 was a gift from Fujisawa, Inc., Marlrose Park, IL, and FK520 was a gift from Merck. All tissue culture medium was purchased from Life Technologies, Inc. Matrigel was purchased from Collaborative Research. Centricon-10 and Microcon-10 were obtained from Amicon, Inc. Taxol was obtained from Dr. Ven Narayan of the Drug Synthesis and Chemical Branch, Division of Cancer Treatment, NCI.

Cell Culture—NT2 cells were grown and maintained as described (40) with a few modifications. Briefly, NT2 cells were treated with reticin acid for 5 days and then replated at reduced density. After 2 days, the cells were mechanically and enzymatically dislodged for enrichment for NT2 cells (40) from the parent NT2 cells, and these NT2 neurons were replated at a density of 8.0 × 10^5/100 mm^2 on dishes previously coated with poly-o-lysine (10 μg/ml) and Matrigel. The NT2 cells were then maintained in media modified Eagle’s medium high glucose with 5% fetal bovine serum, penicillin/streptomycin, and mitotic inhibitors (1 μM cystine arabinoside, 10 μM fluoride oxuryridine, and 10 μM uridine) for up to 6–8 weeks. Except where noted, 4–6-week-old NT2 cells were used to maximize the amount of fetal tau expressed in these cells (see Fig. 2), and each experiment was repeated at least three times.

Preparation of Human Adult, Human Fetal, and Recombinant Tau Protein Standards—Human adult CNS tau was isolated from normal autopsy brains as described (42). Briefly, high salt-extracted, heat- and acid-stable adult brain tau was further purified by reassembly with exogenous phosphocellulose-purified bovine tubulin in the presence of taxol (43). Human fetal brain tau was prepared in a similar manner except that the final purification step (i.e. reassembly with exogenous MTs) was omitted. Recombinant human tau isoforms were a gift from Dr. M. Goedert of the Medical Research Council, Cambridge, United Kingdom (22).

Preparation of Dephosphorylated Tau Samples—Dephosphorylation of fetal tau and enriched tau preparations obtained from NT2 cells was carried out overnight at 37°C using 10 units/ml type III E. coli alkaline phosphatase in 50 mM Tris-HCl, pH 8.0, containing 0.5 mM ZnSO4 and protease inhibitors but without phosphatase inhibitors as described (42). Control samples were incubated identically except that alkaline phosphatase was omitted from the samples.

Drug Treatments of NT2N Cells—Stock solution of the drugs used in this study included: 2.5 mM colchicine, 1 mg/ml nocardazole, 500 μM okadaic acid, 100 μM calyculin-A, and 1 mM each FK506 and FK520 (L-683,590–000X-012) dissolved in dimethyl sulfoxide or 95% ethanol. Stock solutions were diluted in cell culture medium to achieve final concentration steps. Protein analysis was determined using bicinchoninic acid as a dye reagent with bovine serum albumin as the standard (46). Samples were run on 8.5% SDS-PAGE gels and then electroblotted to nitrocellulose membranes for probing with different tau or tubulin antibodies. Antibody binding was either detected with the peroxidase antiperoxidase method (47) or enhanced chemiluminescence or quantitatively determined using [125I]-labeled goat anti-mouse IgG (42), and the nitrocellulose membranes were exposed to PhosphorImager plates in order to quantitate the amount of radioactivity. Quantification of the amount of radioactivity in a given protein band was performed with the ImageQuant software provided with the PhosphorImager.

Isolation of Tau and Tubulin from NT2N Cells—Total tau was extracted from NT2N cultures by homogenization in 1 ml of ice-cold reassembly buffer (0.1 mM MES, 0.5 mM MgSO4, 1 mM EGTA, and 2 mM dithiobisethanol, pH 6.8) containing 0.75 M NaCl and a mixture of protease and phosphatase inhibitors (see above) as described previously (20, 42). To obtain tau proteins in the NT2N cells that are either bound to MTs or unbound in the cytosol, cultures were scraped into MT stabilization buffer (i.e. reassembly buffer containing 2 mM GTP and 20 μM taxol to stabilize MTs (43, 45) plus 0.3 mM Triton X-100 (W/V), 2 mM dithiothreitol, and a mixture of protease and phosphatase inhibitors) and processed as described (20, 42). Tubulin subunits were either assembled into MTs and recovered in the pellet or remained unpolymerized in the supernatant and were analyzed by removing an aliquot from the homogenized cell pellets and processing through the first spin before the boiling or concentration steps. Protein analysis was determined using bicinchoninic acid as a dye reagent with bovine serum albumin as the standard (46). Samples were run on 8.5% SDS-PAGE gels and then electroblotted to nitrocellulose membranes for probing with different tau or tubulin antibodies. Antibody binding was either detected with the peroxidase antiperoxidase method (47) or enhanced chemiluminescence or quantitatively determined using [125I]-labeled goat anti-mouse IgG (42), and the nitrocellulose membranes were exposed to PhosphorImager plates in order to quantitate the amount of radioactivity. Quantification was performed with the ImageQuant software provided with the PhosphorImager.

Radiolabeling and Immunoprecipitation—The radiolabeling and immunoprecipitation procedures were as described previously (44) with some modifications. Briefly, cultured NT2 cells were starved by incubation in serum-free medium without methionine or without phosphore for 20 min before the addition of the same medium containing 100 μCi/ml [35S]Met or [32P]PO4, respectively (ICN). For the pulse-chase experiments, cell cultures were washed twice with complete medium, and incubated an additional 10 min to allow for the continued incorporation of labeled methionine that occurs following removal of labeled medium before initiating the pulse. For the [32P]PO4 incorporation studies cells were labeled continuously for 4 h, washed three times with complete medium, and chased in complete medium with and without colchicine for 0–8 h. At the end of the chase, cell cultures were rinsed twice in phosphate buffer/saline, scraped into cell lysis buffer (50 mM Tris-HCl, pH 7.4, 0.45 mM NaCl, 2% Triton X-100 (v/v), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 5 mM EDTA) containing a mixture of protease and phosphatase inhibitors (2 mM phenylmethylsulfonyl fluoride, 20 μM NaF, 0.5 mM sodium orthovanadate, and 10 μg/ml each of TPCK, TLCK, leupeptin, and soybean trypsin inhibitor). Cell lysates were sonicated and then immunoprecipitated using a sandwiched secondary antibody in which protein A-garosge beads were complexed with rabbit-anti-mouse IgG (5:1) before the addition of the primary mouse monoclonal antibodies (mAbs). Immunoprecipitates were separated by 10% Tris/Tricine gels, stained with Coomassie Blue R250, treated with TCA/HANCE, dried, and then placed on PhosphorImager (Molecular Dynamics, Inc.) plates for 24 h to quantitate the amount of radioactivity. Quantitation of the amount of radioactivity in a given protein band was performed with the ImageQuant software provided with the PhosphorImager.

RESULTS

NT2N Cells Express Only the Fetal Tau Isoform—Tau isoforms from human fetal brain consists of a single isoform, whereas in the adult human brain six alternatively spliced isoforms are present. To determine whether the NT2 cells express a tau isoform similar to fetal human tau, we compared tau in NT2 cells with tau isolated from human fetal brain (Fig. 2). Our data show that the tau in NT2 cells co-migrates with human fetal tau (Fig. 2A, compare lanes 3 and 5) and that it is recognized by the mAb PHF1, an antibody that recognizes phosphorylated Ser396/404 (Fig. 2B, lanes 3 and 5). Further-
More, when tau from the NT2N cells and fetal human tau were enzymatically dephosphorylated the multiple phosphoisoforms in each preparation were reduced to a single band (Fig. 2A, lanes 2 and 4) that co-migrated with the smallest isoform of the six recombinantly expressed human brain isoforms (Fig. 2A, compare lane 1 with lanes 2 and 4). Additionally, dephosphorylated tau from the NT2N cells and fetal human tau were no longer recognized by the phosphorylation-dependent mAb PHF1 (Fig. 2B, lanes 1, 2, and 4). These results indicate that NT2N cells express only the smallest fetal tau isoform and that the tau proteins isolated from NT2N cells are indistinguishable from human fetal tau based on their Mr and immunological properties (see also Figs. 4 and 6).

Synthesis and Phosphorylation of Tau in NT2N Cells—To further characterize the tau expressed in NT2N cells, we examined the synthesis and turnover of tau in these cells. Cultures were radiolabeled with [35S]Met, and then tau was immunoprecipitated. Continuous labeling for up to 2 h showed that tau initially appears as a single band, and this band is gradually modified by incremental phosphorylation. Continuous labeling for up to 2 h showed that tau initially appears as a single band, and this band is gradually modified by incremental phosphorylation. By the end of the chase period, tau becomes modified as indicated by an upward shift in mobility and diffusion of the signal into a wider band. C. NT2N cultures were pulse-labeled with [32P]PO4 for 15 min and chased with complete medium for 1–4 h before immunoprecipitation with T14/46 and electrophoresis on 10% Tris-Tricine gel. Note that during the chase period tau becomes modified as indicated by an upward shift in mobility and diffusion of the signal into a wider band.

Colchicine Treatment Results in the Dephosphorylation of Tau Protein in NT2N Cells—Since the binding of tau to MTs is regulated by phosphorylation and the increased phosphorylation of tau decreases the binding of tau to MTs, we examined the effects of MT-depolymerizing drugs (colchicine) on the phosphorylation state of tau. NT2N cells were pulse-labeled with [35S]Met for 30 min and then chased in medium with or without colchicine for up to 4 h. By the end of the chase period, tau became phosphorylated in the control cultures, resulting in a decrease in the electrophoretic mobility of tau and a broadening of the tau band (Fig. 4A, lanes 1-3). In contrast, tau from the colchicine-treated NT2N cultures migrated faster, suggesting that colchicine treatment either led to the degradation of tau or reduced the level of phosphorylation (or at least increased the turnover of phosphate groups) in newly synthesized tau in the NT2N cells (Fig. 4A, compare lane 4 with lanes 5 and 6). To examine this further, radioactivity in the [35S]-labeled tau bands was quantified before and after a 4-h chase. Our data revealed that 35S-labeled tau was extremely stable and that there was negligible turnover or degradation of tau (Fig. 4C). Indeed, there was no significant difference between the amount of 35S-labeled tau that was recovered before or after the 4-h chase in NT2N cultures that were or were not treated with colchicine. Other studies using antibodies that recognized epitopes at the N or C termini of tau (i.e., T1, T3, ALZ50, T60, and T46) revealed no immunoreactive bands corresponding to tau degradative products in the NT2N cultures after colchicine treatment (data not shown).

To assess whether colchicine treatment of NT2N cells resulted in the dephosphorylation of tau, cultures were labeled with [32P]PO4 for 4 h and then chased in the presence or absence of colchicine. In the absence of colchicine about 40% of the [32P]PO4 associated with tau was turned over by 4 h of chase (Fig. 4B, lanes 1-4, and Fig. 4D). However, colchicine treatment increased the rate of phosphate turnover such that about 75% of the [32P]PO4 disappeared by 4 h and most of the [32P]PO4 was associated with tau isoforms that migrated more rapidly than their counterparts in the control NT2N cultures (Fig. 4B, lanes 5-8, and Fig. 4D). These observations suggest that colchicine treatment results in the dephosphorylation of tau rather than
proteolysis or some other changes, and this was confirmed in other studies using quantitative Western blotting (data not shown).

Dephosphorylated Tau Is Recovered in the Supernatant in NT2N Cells after Colchicine or Nocodazole Treatment—Both colchicine and nocodazole depolymerize MTs such that the majority of the tubulin subunits are recovered in the supernatant. To examine the effects of these agents on the ability of tau to bind MTs, NT2N cultures were treated with either colchicine or nocodazole for up to 4 h. It is evident that after 2 h of drug treatment about 80% of the MTs were recovered as tubulin subunits in the supernatant (Fig. 5B, lanes 1–4, and Fig. 5D) and that the level of soluble tau was increased from about 45 to 90% of the total tau (Fig. 5A, lanes 1–4, and Fig. 5C). This indicates that as MTs disassemble increased amounts of tau are released into the supernatant fraction. Further, tau from nocodazole-treated cultures appeared to be dephosphorylated since it displayed a more rapid electrophoretic mobility in both the soluble and cytoskeletal fractions. Similarly, MAP2C (which can be detected by the mAb T46) appeared to be dephosphorylated, and it was also released into the supernatant (Fig. 5A). These results suggest that both tau and MAP2C become dephosphorylated and are recovered in the supernatant following drug-induced depolymerization of MTs.

Sitespecific Dephosphorylation of Tau by Colchicine—Since each phosphorylation site in tau may have a unique function and some sites may play a role in regulating the binding of tau to MTs, we examined the effects of drug treatments on the selective dephosphorylation of several sites in tau using phosphorylation site-specific antibodies (see Fig. 1). Nitrocellulose replicas of enriched tau fractions obtained from cultures treated with colchicine were generated and probed with a large number of anti-tau antibodies that recognize specific tau epitopes in a phosphorylated or nonphosphorylated state (Fig. 6). Colchicine treatment resulted in the selective and complete dephosphorylation of tau at Ser202/Thr205 with a concomitant reduction of several sites in tau using phosphatases in response to colchicine. An aliquot of the homogenized cells was removed for tubulin analysis. Equal protein, as determined by bicinchoninic acid (BCA) assay, was loaded onto 10% polyacrylamide gels and subjected to SDS-PAGE. Nitrocellulose replicas were probed with anti-tau mAbs T14/46 (panel A) and with anti-β-tubulin mAb (panel B). Lanes 1–6, nocodazole-treated; lane 7 shows positive controls. Panel A, human fetal tau (F, 15 μg); panel B, phosphocellulose-purified bovine tubulin (PC, 5 μg). Protein bands were quantified using ImageQuant software. Colchicine significantly reduced the 32P signal for every time point, and after 4 h the signal was 50% less for colchicine-treated than control (Student’s two-tailed t test assuming equal variance; significance, p < 0.05).

Fig. 4. Radiolabeled NT2N cultures treated with colchicine. A, NT2N cultures were labeled with [35S]Met for 30 min and chased with or without colchicine (25 μM) in complete medium for 0–4 h. Tau was immunoprecipitated with T14/46 and analyzed on 10% Tris/Tricine gel. Lanes 1–3 contain tau from untreated cultures for 0, 2, and 4 h of chase; lanes 4–6 contain tau from colchicine-treated cultures for 0, 2, and 4 h of chase. Note that tau from colchicine-treated cultures migrates faster and appears dephosphorylated (compare lanes 3 and 6). B, NT2N cultures were labeled with [32P]PO4 for 4 h and chased with or without colchicine (25 μM) in complete medium for 0–4 h. Tau was immunoprecipitated with T14/46 and analyzed on 10% Tris/Tricine gel. Lanes 1–4 contain tau from untreated cultures chased for 0, 2, and 4 h; lanes 5–8 contain tau from colchicine-treated cultures chased for 0, 1, 2, and 4 h. Note that the turnover of phosphates was greater for colchicine-treated cultures as indicated by the decrease in signal (compare lanes 3 and 4 with 7 and 8) and that after 4 h of colchicine treatment only the fastest migrating tau band is apparent, whereas after 4 h of chase without colchicine all isoforms are present (compare lanes 4 and 8). The positions of 66- and 45-kDa molecular mass standards are shown on the right. C, quantitation of the effect of colchicine on total 35S-labeled tau after a 4-h chase. Experiments were performed as described for panel A. Gels were exposed to PhosphorImager plates, and the radioactivity of every lane was quantitated using the ImageQuant software. Colchicine had no significant effect on the amount of tau detected by quantitative analysis (Student’s t test assuming equal variance; significance, p < 0.05). D, quantitation of the effect of colchicine on phosphate incorporation of tau. Experiments were performed as described for panel B. Gels were exposed to PhosphorImager plates and tau bands were quantitated using the ImageQuant software. Colchicine significantly reduced the 32P signal for every time point, and after 4 h the signal was 50% less for colchicine-treated than control (Student’s two-tailed t test assuming equal variance; significance, p < 0.05). Bars indicate the standard error of the mean values from five experiments.

Fig. 5. Time course of the effect of nocodazole on tau. Western blot analyses of tau and tubulin proteins are shown. NT2N cultures were treated with 10 μg/ml nocodazole for 0–4 h. Cultures were processed with MT stabilization buffer to examine the soluble (S) tau fraction and the fraction of tau associated with the cytoskeletal pellet (P). An aliquot of the homogenized cells was removed for tubulin analysis. Equal protein, as determined by bicinchoninic acid (BCA) assay, was loaded onto 10% polyacrylamide gels and subjected to SDS-PAGE. Nitrocellulose replicas were probed with anti-tau mAbs T14/46 (panel A) and with anti-β-tubulin mAb (panel B). Lanes 1–6, nocodazole-treated; lane 7 shows positive controls. Panel A, human fetal tau (F, 15 μg); panel B, phosphocellulose-purified bovine tubulin (PC, 5 μg). Protein bands were quantified using ImageQuant software. Colchicine treatment resulted in the complete and selective dephosphorylation of tau at Ser202/Thr205 with a concomitant downshift in the Mr of tau (compare lanes 1 and 2 with lanes 3 and 4, panel B), and most of the tau was present in the soluble fraction and was downshifted (compare lanes 1 and 2 with lanes 3 and 4, panel A). The positions of 66- and 45-kDa molecular mass standards are shown on the right.
DISCUSSION

In this study we used cultured NT2N cells as an effective model in which to study how the phosphorylation state of tau is regulated in human CNS neurons. Tau isolated from NT2N cells is indistinguishable from human fetal tau since both migrate together on SDS-PAGE gels before and after enzymatic dephosphorylation with alkaline phosphatase. Further, tau isolated from NT2N cells and human fetal tau are phosphorylated on the same sites (i.e. Thr181, Ser202, Thr205, Thr231, Ser396, and Ser404) as detected by site-specific phosphorylation-dependent mAbs. However, the phosphorylation and dephosphorylation of tau at these sites in NT2N cells are dynamic since 40% of the mAbs. When NT2N cultures were treated with colchicine plus specific phosphatase inhibitors (Fig. 7). When NT2N cultures were treated with colchicine plus inhibitors of PP2A (i.e. okadaic acid or calyculin-A), the dephosphorylation of tau at Ser202/Thr205 was blocked as indicated by the prevention of the downshift in M, and preservation of AT8 immunoreactivity (Fig. 7, A and B, compare lane 2 with lanes 4 and 6). However, when NT2N cultures were treated with colchicine plus inhibitors of PP2B (i.e. FK506 or FK520) (61–63) the dephosphorylation of tau at Ser202/Thr205 as determined by a loss of AT8 immunoreactivity was not prevented (Fig. 7, A and B, compare lane 2 with lanes 8 and 10). Since PP1 was shown previously to be incapable of dephosphorylating tau protein in vitro (12) these data suggest that colchicine treatment leads to the complete dephosphorylation of Ser202/Thr205 by activating PP2A.

One function of tau that is regulated by phosphorylation is the binding affinity of tau to MTs. For example, an increase in the phosphorylation of tau reduces the binding of tau to MTs in vitro (19). Using an MT binding assay, we showed here that about 45% of tau in the NT2N neurons did not bind to MTs and remained in the supernatant. This unbound tau migrated slower on SDS-PAGE gels and was more highly phosphorylated than the tau that remained bound to MTs. Thus, it is likely that the increased phosphorylation of tau at specific sites reduces the binding of tau to MTs in intact neurons. Indeed, recent studies showed that Ser396 and Ser262 are two phosphorylation sites that regulate the binding of tau to MTs (20, 21). However, it is unclear at the present time whether Ser202/Thr205 also is involved in regulating the binding of tau to MTs.

Examination of the effects of depolymerizing the MT network with colchicine or nocodazole in the NT2N cells showed that these agents induced the dephosphorylation of tau. This effect was demonstrated by an acceleration in the electrophoretic mobility of tau on SDS-PAGE gels, an increase in the turnover of [32P]PDP2A-labeled tau, and the site-specific dephosphorylation of tau including the complete dephosphorylation of tau at Ser202/Thr205. Our data support the findings of Drubin et al. (64) in which tau from differentiated PC12 cells treated with colchicine showed an increased electrophoretic mobility on SDS-PAGE gels. In addition to the dephosphorylation of tau, treatment of the NT2N cells with colchicine or nocodazole resulted in the depolymerization of MTs and the release of tau bound to MTs, as shown by an increase in the level of soluble tubulin monomers concomitant with an increase in the levels of dephosphorylated tau in the supernatant. Although this increase in the level of tau in the supernatant most likely results from the drug-induced depolymerization of MTs, the concomitant dephosphorylation of tau may not be due to the direct effects of colchicine or nocodazole on tau. Instead, it could be due to the indirect activation of phosphatases that specifically dephosphorylate tau and MAP2C as a result of the depolymerization of MTs. Indeed, recent studies demonstrated that substantial amounts of the trimeric PP2A are normally bound to MTs in intact cells (65), suggesting that PP2A might be activated during the depolymerization of MTs. Thus, we speculate that the dephosphorylation of tau and MAP2C by phosphatases such as PP2A may reflect an attempt by the neuron to counteract the drug-induced depolymerization of MTs, since the dephosphorylation of tau and MAP2C enhances their ability to bind to MTs.
Our data on colchicine- and nocodazole-induced dephosphorylation of tau differ from the results of Mattson (66), who described an increase in the phosphorylation state of tau in primary cultures of CNS neurons treated with colchicine. The reasons for this discrepancy are unclear, but this may reflect differences between the antibodies and the method used to monitor changes in the phosphorylation state of tau before and after drug treatment.

Our observation that treatment of the NT2N cells with colchicine and nocodazole leads to the dephosphorylation of a subset of the phosphate acceptor sites on tau suggests that different phosphorylated forms of tau may be activated to dephosphorylate tau at different sites. Indeed, treatment of NT2N cells with a subset of the phosphate acceptor sites on tau suggests that this dephosphorylation can be inhibited by inhibitors of PP2B.2 These results with NT2N cells are in agreement with previous observations that tau can be dephosphorylated in vitro by highly purified PP2A and PP2B (32, 67, 68). Furthermore, our recent study of biopsy-derived human tau also implicates PP2A as the phosphatase that dephosphorylates Ser202/Thr205 (18). Thus, future studies will identify the functional significance of tau phosphorylation at Ser202/Thr205.

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