INTRODUCTION

Tau is a neuronal microtubule-associated protein found predominantly in axons. Tau is encoded by a single gene but alternative splicing of RNA gives rise to multiple isoforms. These differ by the presence of either three or four repeat regions located toward the carboxy terminus and which form part of the microtubule binding region, and also in the presence or absence of inserts located toward the amino terminus of the molecule. There are six tau isoforms in human brain and these are expressed in a cell- and developmental-specific manner; all six are expressed in adult human brain but only the smallest isoform (containing three carboxy-terminal repeat regions and no amino-terminal inserts) is present in the fetus (see Goedert et al., 1991, for review).

Tau binds to microtubules and promotes microtubule assembly in vitro (Goedert and Jakes, 1990) and probably functions in the formation and maintenance of axons since down-regulation of tau expression in primary cerebellar neurons with antisense oligonucleotides inhibits the ability of these cells to generate axons (Caceres and Kosik, 1989; Caceres et al., 1991). Such observations suggest that tau is involved in assembling neuronal microtubules into the organised arrays required for the elaboration and maintenance of axons. Indeed, tau transfected into SF9 insect cells induces the formation of axon-like processes containing polarised bundles of microtubules such as are found in axons (Knops et al., 1991; Baas et al., 1991), and mammalian non-neuronal cells transfected with tau also often display organised bundles of microtubules (Lewis et al., 1989; Kanai et al., 1989; Lee and Rook, 1992; Kanai et al., 1992; Montejo de Garcini et al., 1994; Lo et al., 1993).

SUMMARY

Tau is a neuronal microtubule-associated protein that appears to function in the formation and maintenance of axons by influencing microtubule organisation. Tau is a phosphoprotein and is more heavily phosphorylated in fetal than in adult brain, and is also hyperphosphorylated in Alzheimer’s disease where it forms the major component of paired helical filaments (PHFs). Tau phosphorylation probably modulates microtubule dynamics since in vitro, phosphorylated tau has a reduced affinity for microtubules and is less potent at promoting microtubule assembly. In order to understand how phosphorylation effects cellular microtubule organisation, we studied 3T3 and CHO cells transfected with tau and the tau kinase GSK-3β. Tau transfected cells displayed prominent bundles of microtubules that did not appear to be nucleated by a microtubule-organising centre. Co-transfection of tau with GSK-3β led to increased phosphorylation of tau and also to a reduction in microtubule bundling such that the microtubule network in many of the tau/GSK-3β transfected cells appeared similar to non-transfected interphase cells. Transfection of a mutant tau, in which five of the known GSK-3β targeted phosphorylation sites were mutated to alanine so as to preclude phosphorylation, also induced microtubule bundling. However, co-transfection of this mutant with GSK-3β did not diminish the bundling effect. Biochemical analyses of microtubule and cytosolic fractions from the transfected cells demonstrated that GSK-3β-mediated phosphorylation of tau reduced its affinity for microtubules. These results suggest that phosphorylation of tau by GSK-3β modulates its ability to organise microtubules into ordered arrays such as are found in axons.

Key words: Tau/GSK-3β, Phosphorylation, Microtubule
and cyclin-dependent kinase-5 (cdk-5) (Drewes et al., 1992; Goedert et al., 1992; Hanger et al., 1992; Mandelkow et al., 1992; Mulot et al., 1994; Baumann et al., 1993; Paudel et al., 1993). Recently, GSK-3α and GSK-3β have been shown to induce cellular phosphorylation of tau so that it resembles fetal/PHF-tau (Lovestone et al., 1994; Sperber et al., 1995).

Phosphorylation effects tau function in vitro; phosphorylated tau has a reduced affinity for microtubules and a reduced ability to promote microtubule assembly (Biemat et al., 1993; Lindwall and Cole, 1984; Lu and Wood, 1993; Trinczek et al., 1995). However, the effect(s) that phosphorylation has on the cellular function(s) of tau is less well understood. Since tau is more heavily phosphorylated in fetal than in adult brain, tau phosphorylation might have a developmental-specific role and hyperphosphorylation of tau in Alzheimer’s disease might be a pathogenic mechanism. Tau phosphorylation therefore has relevance to both developmental and disease processes. In order to study the effect that tau phosphorylation has on cellular microtubules, we studied microtubule organisation in tau transfected cells in which tau phosphorylation status was manipulated by co-transfection with the tau kinase GSK-3β.

MATERIALS AND METHODS

Construction of tau and mutant tau expression vectors

A cDNA encoding the smallest human tau isoform containing three carboxy-terminal repeat regions and no amino-terminal inserts (tau0N3R) was obtained from Dr Ken Kosik (Harvard University, Boston) and cloned into the mammalian expression vector pSG5 (Stratagene). Five of the known GSK-3β sites (ser199, ser202, ser235, ser396 and ser404, numbering according to the largest human brain tau isoform) were mutated to alanines so as to preclude phosphorylation by overlapping PCR mutagenesis using Pfu polymerase (Stratagene). Ser199 and ser202 were mutated using the same set of mutagenic primers. Ser396 and ser404 were also mutated with the same set of mutagenic primers. Mutagenic primers were: 5'-GGAGTGCCTGGGGCGCCGGGGCGCTTAGCCCGCTG-3' and 5'-CACCGGCTACAGGCCCCCCGCCCCAGGACTCCC-3' (ser199 and ser202); 5'-GCGGGAACAGGGGCTTGGTGGAG-3' and 5'-CTCCACCCCAAGGCGCTCGTCGCCG-3' (ser235); 5'-GCGGCGTCTCCACAGACACACTGGCCCTTGTGACATC-3' and 5'-GGGGCCCTGTTTCTGGGACACGCGCTCAGG-3' (ser396 and ser404). The mutant cDNA was also cloned into the mammalian expression vector pSG5 and sequenced to check for errors. For expression of GSK-3β, cells were transfected with a human GSK-3β cDNA cloned into plMT2 (Lovestone et al., 1994).

Cell culture and transfection

Chinese hamster ovary (CHO) cells were grown in α-MEM containing 10%, v/v, foetal bovine serum supplemented with 2 mM glutamine, 100 i.u. ml⁻¹ penicillin and 100 μg ml⁻¹ streptomycin. 3T3 cells were grown in DMEM containing 10%, v/v, foetal bovine serum supplemented as for CHO cells. CHO cells were transfected by the calcium phosphate method using the Promega Protection kit according to the manufacturer’s instructions. 3T3 cells were transfected by lipofection using the Gibco BRL LipofectAMINE reagent according to the manufacturer’s instructions.

Immunofluorescence microscopy

For immunofluorescence microscopy, cells were grown on glass coverslips in 35 mm dishes, allowed to attach for 24 hours and then transfected with tau and GSK-3β plasmids as described above (4 μg total DNA for 3T3 cells, 7 μg for CHO cells). Cells were fixed 40 hours later in methanol at -20°C for 10 minutes, rehydrated in PBS and incubated with primary antibodies for 1 hour. These were N356, a mouse monoclonal antibody to α-tubulin (Amersham International, UK), TP70, a rabbit polyclonal antibody to tau that recognises both phosphorylated and non-phosphorylated tau (Lovestone et al., 1994) and a monoclonal antibody to GSK-3β (Affiniti, Nottingham, UK). After washing in PBS, the cells were incubated for a further hour with fluorescein-conjugated goat anti-mouse immunoglobulins (Vector Laboratories Ltd Peterborough UK) and/or biotinylated donkey anti-rabbit immunoglobulins (Amersham). To visualise products labelled with the biotinylated antibodies, cells were washed and then incubated with streptavidin Texas red (Vector Labs Ltd). Coverslips were then washed and mounted in Vectashield mounting medium (Vector Labs Ltd).

Biochemical analyses of transfected cells

Transfected cells were studied by western blotting. Cells were washed with PBS, scrapped into SDS-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, heated in a boiling water bath for 10 minutes and proteins resolved on 10%, w/v, SDS-PAGE gels. Proteins were transferred to nitrocellulose membranes using a Bio-Rad TransBlot system, blocked in PBS containing 3% skimmed milk and 0.2% Tween-20, and incubated with primary antibodies in PBS containing 0.6% skimmed milk and 0.2% Tween-20. Following washing in PBS/skimmed milk/Tween-20, the blots were incubated in secondary antibodies, washed again and developed using the Enhanced Chemiluminescence (ECL) detection system (Amersham). Antibodies to tau were TP70, which recognises both phosphorylated and non-phosphorylated tau, AT8 (epitope requires phosphorylated ser202 and thr205) and 8D8 (epitope requires phosphorylated ser396) (Goedert et al., 1994, 1995; Lovestone et al., 1994).

In order to determine the relative proportion of tau bound to microtubules and tau present in the cytosolic fraction (not bound to microtubules), microtubule and cytosolic fractions from transfected cells were studied by western blotting. Microtubule and cytosolic fractions were prepared essentially as described (Solomon et al., 1979). Briefly, cells were collected in microtubule stabilising buffer comprising 80 mM Pipes, pH 6.8, 1 mM MgCl₂, 1 mM EGTA, 0.1% Triton X-100 and 30% glycerol at 37°C. Microtubules were then pelleted by centrifugation at 100,000 g (av) at 37°C and the microtubule and supernatant fractions prepared for SDS-PAGE by addition of SDS-PAGE sample buffer and heating in a boiling water bath for 10 minutes. Equal proportions of the samples representing equivalent numbers of cells were analysed by western blotting.

RESULTS

Microtubule organisation in tau and tau/GSK-3β transfected cells

In order to investigate how phosphorylation of tau by GSK-3β effects cellular microtubule organisation, we transiently transfected mouse 3T3 cells and CHO cells with cDNAs encoding a human tau isoform and GSK-3β, and studied microtubule organisation by double-label immunofluorescence microscopy and tau/microtubule interactions by biochemical methods. Similar results were obtained with both cell-types.

We chose for study, the smallest human tau isoform, which contains three carboxy-terminal repeat regions and no amino-terminal inserts, since this isoform is present in both fetal and adult human brain and hence experimental manipulations designed to change its phosphorylation status have physiological relevance (as stated above, this tau is probably more heavily phosphorylated in fetal brain than in the adult brain).
For immunofluorescence microscopy, we utilised a mouse monoclonal antibody to $\alpha$-tubulin (N356; Amersham) and a rabbit polyclonal antibody to tau (TP70) that detects both phosphorylated and non-phosphorylated tau (Lovestone et al., 1994).

Non-transfected, interphase cells contained typical radiating arrays of microtubules that often could be seen to emanate from a microtubule organising centre when labelled with the tubulin antibody. However, transfection of tau into these cells dramatically altered this pattern of labelling. In cells expressing high levels of tau (as judged by intensity of fluorescence staining), bundles of microtubules could be discerned which often formed a ring, or spiral around the circumference of the cell (Fig. 1A,B). These bundles of microtubules did not appear to emanate from a microtubule organising centre. However, it is possible that a microtubule organising centre is involved in their nucleation but cannot be easily discerned because of the intense immunofluorescence labelling of the microtubule bundles. The tau transfected cells usually had a more rounded appearance and the bundling pattern of labelling was sufficiently marked to enable tau transfected cells to be easily identified on the basis of tubulin immunofluorescence alone. Such a reorganisation of the microtubule cytoskeleton by tau is similar to that previously reported for tau transfected non-neuronal cells (Lewis et al., 1989; Kanai et al., 1989, 1992; Lee and Rook, 1992; Montejo de Garcini et al., 1994). Labelling with antibody TP70 indicated that tau in the transfected cells co-localised with microtubules but that some tau was also cytoplasmic and not associated with microtubules. However, to confirm that a proportion of the tau was indeed associated with microtubules, some preparations were extracted with the non-ionic detergent Triton X-100 prior to immunolabelling. In these preparations, tubulin and tau staining was coincident (data not shown).

Western blot analyses of tau from transfected CHO and 3T3 cells with antibody TP70 demonstrated that it migrated as two major species that are probably the result of differential phosphorylation (Bramblett et al., 1993; Sygowski et al., 1994; Gallo et al., 1992; Kanai et al., 1989; Lovestone et al., 1994) (Fig. 2a, track 1) but that the tau failed to react, or reacted only very weakly with antibodies AT8 and 8D8, which both recognise.

Fig. 1. Immunofluorescence of transfected 3T3 cells. (A,B) Cells transfected with wild-type tau; (C,D) cells co-transfected with wild-type tau and GSK-3$\beta$; (E,F) cells transfected with the mutant tau; (G,H) cells co-transfected with mutant tau and GSK-3$\beta$. (A,C,E,G) Stained for tubulin; (B,D,F,H) the same cells viewed to show tau. $\times$420.
phosphorylated residues that are generated by GSK-3β activity (Lovestone et al., 1994; Sperber et al., 1995) (see Fig. 2B, track 1, for labelling with AT8). Hence the tau present in the transfected cells was largely unphosphorylated at these known GSK-3β sites in both the CHO and 3T3 cells which is similar to that previously reported for tau transfected non-neuronal cells (Lovestone et al., 1994; Preuss et al., 1995; Sperber et al., 1995).

To determine how phosphorylation of tau by GSK-3β might influence the tau-induced bundling of microtubules, we co-transfected cells with both tau and a GSK-3β cDNA. As previously reported (Lovestone et al., 1994), transient transfection of GSK-3β into fibroblasts increases GSK-3β activity in the population of cells. Western blot analyses of these GSK-3β co-transfected cells with antibody TP70, demonstrated that a proportion of the tau had a reduced electrophoretic mobility such that two, slower migrating tau species could be discerned (Fig. 2A, track 2). These are probably the result of different degrees of phosphorylation of the tau in the co-transfected pool of cells. Indeed, individual transfected cells in this co-transfected pool express differing levels of GSK-3β as judged by brightness of immunofluorescence staining with the GSK-3β antibody (data not shown) and this suggests that within the pool, phosphorylation of tau might be somewhat heterogeneous. These new, slower migrating tau species were strongly reactive with the phosphorylation-dependent antibodies AT8, and 8D8 (see Fig. 2B, track 2, for labelling with AT8) which was shown by cutting blots in half and probing one half with TP70 and the other half with AT8/8D8 as previously described (Lovestone et al., 1994). Hence, tau in the GSK-3β co-transfected CHO and 3T3 cells is hyperphosphorylated in a similar fashion to that reported for tau/GSK-3β transfected COS cells (Lovestone et al., 1994; Sperber et al., 1995).

We determined by double immunofluorescence staining with tau antibody TP70 and the GSK-3β antibody that over 95% of the cells expressing tau also expressed transfected GSK-3β. Since the majority of cells in the tau+GSK-3β cotransfected pool expressed both of these proteins, we therefore reasoned that any differences in microtubule organisation which we detected between tau and tau+GSK-3β transfected cells could be attributed to co-transfection of GSK-3β. We therefore, performed double immunofluorescence staining with the tubulin and tau antibodies on the tau/GSK-3β co-transfected cells so as to determine the effect that phosphorylation of tau by GSK-3β has on microtubules.

In contrast to cells transfected with tau alone, microtubules in many cells co-transfected with both tau and GSK-3β were organised into a network that was more characteristic of that observed in non-transfected cells. Microtubules in these co-transfected cells could also often be seen to originate from a microtubule organising centre (Fig. 1C,D) and bundling of microtubules was less pronounced. These results suggest that phosphorylation of tau by GSK-3β inhibits its ability to re-organise cellular microtubule networks but do not eliminate the possibility that GSK-3β influences microtubule organisation directly. We therefore studied microtubule arrays in cells transiently transfected with GSK-3β alone. These cells appeared identical to non-transfected cells (data not shown).

To demonstrate further, that co-transfection of tau with GSK-3β altered microtubule organisation by a direct effect on tau, we created a mutant of tau in which five of the known GSK-3β sites (ser199, ser202, ser215, ser396 and ser404) were mutated to alanine (so as to preclude phosphorylation) and studied the properties of this mutant tau in transfected cells. Western blots of cells transfected with this mutant tau revealed that it migrated differently to the wild-type tau. Instead of the two major tau species observed in wild-type tau transfected cells, one major mutant tau species, which co-migrated with the fastest migrating wild-type tau, and a much less abundant slower migrating tau species, detectable only following extended exposure of the blots, were detected with antibody TP70 (Fig. 2A, track 3). Co-transfection of cells with the mutant tau and GSK-3β did not alter this pattern of labelling (Fig. 2A, track 4) and as expected, did not generate epitopes for any of the phosphorylation-dependent antibodies (see Fig. 2B, tracks 3 and 4, for labelling with AT8).

Cells expressing high levels of the mutant tau appeared similar to cells expressing high levels of wild-type tau; the microtubule network was rearranged into organised bundles of microtubules and the cells often took on a more rounded appearance (Fig. 1E,F). However, in contrast to the wild-type tau, co-transfection of GSK-3β did not alter this pattern of microtubule organisation; cells transfected with both mutant tau and GSK-3β still displayed organised arrays of bundled microtubules (Fig. 1G,H). Thus the reduced bundling of microtubules observed in the tau/GSK-3β co-transfected cells appears to be a direct effect of tau phosphorylation by GSK-3β.

**Tau-microtubule interactions in tau/GSK-3β transfected cells**

The immunofluorescence studies demonstrate that phosphorylation of tau by GSK-3β reduces its ability to organise microtubules into bundled arrays. Since this is likely to involve changes in the affinity of tau for microtubules, we prepared microtubule and cytosolic fractions from the transfected cells and by analysing equivalent proportions of each fraction (representing equal numbers of cells) on western blots, determined whether elevation of GSK-3β activity affects the relative amount of tau that is bound to microtubules.

Such western blots with antibody TP70 demonstrated that the wild-type and mutant tau were present in both microtubule and cytosolic fractions which is consistent with the immuno-
fluorescence observations that demonstrated some non-microtubular cytoplasmic tau staining; the two major wild-type and the single major mutant tau species detected in western blots of total tau were present in both fractions (Fig. 3A,B, tracks 2 and 4). However, in cells co-transfected with the wild-type tau and GSK-3\( ^{3\beta} \), the relative proportion of tau in the microtubule pellet was markedly reduced and a corresponding increase in cytoplasmic tau was observed (cf. Fig. 3A,B, tracks 3). In contrast, no changes in the proportions of microtubule and cytosolic tau were observed in cells co-transfected with the mutant tau and GSK-3\( ^{3\beta} \) (cf. Fig. 3A,B, tracks 4, and 5).

The slower migrating wild-type tau species generated by GSK-3\( ^{3\beta} \) phosphorylation were more abundant in the cytosolic fraction than the microtubule fraction, which suggested that these species in particular had a lower affinity for microtubules (cf. Fig. 3A,B, tracks 3). In order to further confirm that phosphorylation of tau by GSK-3\( ^{3\beta} \) induced a shift in this tau from the microtubule to cytosolic fraction, we probed blots of these fractions with the phosphorylation-dependent antibodies AT8 and 8D8; these antibodies only labelled tau in the cytosolic fraction (Fig. 3C,D,E,F, tracks 3). The relative position of the cytosolic tau species recognised by AT8 and 8D8 with respect to the microtubule-bound tau species was determined by processing one half of a blot with AT8/8D8 and the other with TP70 (data not shown). These experiments demonstrated that the cytosolic tau species recognised by AT8 and 8D8 had a reduced electrophoretic mobility. Cellular phosphorylation of tau by GSK-3\( ^{3\beta} \) therefore reduces its electrophoretic mobility and its affinity for microtubules.

**DISCUSSION**

Tau is a phosphoprotein and phosphorylation of tau reduces its affinity for microtubules and its ability to promote microtubule assembly in vitro (Lindwall and Cole, 1984; Biernat et al., 1993; Lu and Wood, 1993). Many of the residues that are phosphorylated on tau during development and in PHF-tau in Alzheimer’s disease are serines and threonines preceding a proline. Since GSK-3\( ^{3\beta} \), a proline-directed kinase, induces cellular phosphorylation of tau on at least some of these residues, we investigated how GSK-3\( ^{3\beta} \)-mediated tau phosphorylation influences cellular microtubule organisation by studying fibroblast cells transfected with tau, and GSK-3\( ^{3\beta} \). Co-transfection of tau with GSK-3\( ^{3\beta} \) elevates GSK-3\( ^{3\beta} \) activity in the cells so as to phosphorylate tau (Lovestone et al., 1994; Sperber et al., 1995).

The phosphorylation status of transfected tau has now been studied in a variety of different cell types including 3T3 and CHO cells (Gallo et al., 1992; Bramblett et al., 1993; Sygowski et al., 1994; Preuss et al., 1995; Sperber et al., 1995). A proportion of transfected tau is phosphorylated at ser\(^{396}\) and ser\(^{202}\) in stably transfected 3T3 cells (Sygowski et al., 1994) and ser\(^{396}\) is phosphorylated in CHO cells stably transfected with tau isoforms containing four carboxy-terminal repeat regions but not three repeat regions (Bramblett et al., 1993). Recent studies have demonstrated that phosphorylation of some ser/pro and thr/pro sites, including the GSK-3\( ^{3\beta} \) targeted residues ser\(^{202}\), ser\(^{235}\), ser\(^{396}\) and ser\(^{404}\), is regulated in a cell cycle-dependent manner in CHO cells and that the majority of tau is not phosphorylated at these residues in interphase cells (Preuss et al., 1995). Our observations on 3T3 and CHO cells transiently transfected with the smallest human tau isoform containing three carboxy-terminal repeat regions are therefore in broad agreement with these earlier studies and demonstrate that the majority of the tau present in the pool of 3T3 or CHO cells is not phosphorylated on ser\(^{202}\) or ser\(^{396}\). However, as previously reported for COS cells, co-transfection of tau with GSK-3\( ^{3\beta} \) led to a dramatic increase in the proportion of tau phosphorylated on these sites; tau is therefore phosphorylated by GSK-3\( ^{3\beta} \) in CHO and 3T3 cells also.

Cells expressing high levels of transfected wild-type tau, or a mutant tau in which five serine residues known to be phosphorylated by GSK-3\( ^{3\beta} \) were mutated to alanine so as to preclude phosphorylation appeared similar; the cells took on a more rounded appearance and the microtubules were reorganised into bundled arrays that did not appear to be nucleated from a microtubule-organising centre. These observations are similar to those previously described for both tau and MAP2 transfected cells (Kanai et al., 1989, 1992; Lewis et al., 1989; Lee and Rooke, 1992; Weisshaar et al., 1992; Lo et al., 1993; Montejo de Garcia et al., 1994). However, co-transfection of wild-type tau with GSK-3\( ^{3\beta} \) reduced microtubule bundling so that many cells appeared similar to non-transfected interphase...
cells and displayed typical radiating cytoplasmic networks of microtubules that emanated from a microtubule organising centre. In contrast, co-transfection of GSK-3β with the mutant tau did not noticeably diminish microtubule bundling, the cells still displayed prominent arrays of organised microtubule bundles. Since transfection of GSK-3β alone had no apparent effect on microtubule organisation, we conclude from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation. We observe from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation. We conclude from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation. We conclude from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation. We conclude from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation. We conclude from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation. We conclude from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation. We conclude from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation. We conclude from these studies that phosphorylation of tau by GSK-3β alone had no apparent effect on microtubule organisation.

Analyses of microtubule and cytosolic fractions from the transfected cells demonstrated that phosphorylation of tau by GSK-3β reduced the affinity of the wild-type but not the mutant tau for microtubules. Furthermore, antibodies AT8 and 8D8, which recognise phosphorylated tau epitopes, only labelled tau in the cytosolic fraction and did not label tau bound to microtubules. Bramblett et al. (1993) have also shown that tau phosphorylated on ser396 in CHO cells has a reduced affinity for microtubules. Hence, the tau-induced microtubule bundling that is inhibited by GSK-3β phosphorylation, appears to be mediated (at least in part) by tau-microtubule interactions.

Experiments to down-regulate tau expression in primary neuronal cultures and neuroblastoma cells using antisense approaches suggest that tau functions in the formation and maintenance of axons (Caceres and Kosik, 1989; Caceres et al., 1991; Esmaeli-Azad et al., 1994). In vitro studies indicate that the microtubule assembly promoting activity of tau is modulated by phosphorylation (Biernt et al., 1993; Lindwall and Cole, 1984; Lu and Wood, 1993). Although recent studies have demonstrated that several ser-pro/thr-pro sites in tau are phosphorylated in adult brain, a greater proportion of these sites appear to be phosphorylated in the fetus (Goedert et al., 1993; Bramblett et al., 1993; Brion et al., 1993; Mawal-Dewan et al., 1994). Together, these observations suggest that phosphorylation influences tau function so as to fulfil some developmental-specific requirement. One possibility is that phosphorylation might modulate tau’s ability to stabilise axonal microtubules into organised arrays and this might permit greater plasticity during development when circuitry is being established. Likewise, hyperphosphorylation of tau in Alzheimer’s disease might destabilise axonal microtubules and indeed, microtubules are conspicuously absent in PHF containing neurones in Alzheimer’s disease (Flament-Durand and Couck, 1979; Gray et al., 1987). The results presented here, which demonstrate that phosphorylation of tau by GSK-3β can indeed alter cellular microtubule organisation, provide experimental evidence to support these notions.

This work was supported by grants from the Wellcome Trust, MRC and Nuffield Foundation to C.C.J.M; U.W. is supported by a donation from British American Tobacco and M.U. is supported by a Wellcome Trust Prize studentship. We thank Dr Ken Kosik, Harvard Medical School for the gift of tau cDNA, Jim Woodgett, Ontario Cancer Research Institute for the gift of GSK-3β cDNA and our colleagues Diane Hanger, for samples of PHF-tau, and Brian Anderton for antibody TP70 and for many helpful discussions.

REFERENCES


(Received 22 February 1996 - Accepted 4 April 1996)