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Short- and long-term mechanisms of tau regulation in PC12 cells

Einat Sadot[1], Jacob Barg[1,2], David Rasouly[3], Phillip Lazarovici[3] and Irith Ginzburg[1,*]

[1]Department of Neurobiology, The Weizmann Institute of Science, PO Box 26, 76100 Rehovot, Israel
[2]Cardiovascular and Hypertension Research Laboratory, E. Wolfson Medical Center, Tel Aviv University Medical School, Holon, Israel
[3]Department of Pharmacology, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, 91120 Jerusalem, Israel

*Author for correspondence

SUMMARY

Induction by nerve growth factor of neurite outgrowth in PC12 cells is transcription-dependent and is associated with the accumulation of tau protein. It was recently shown that short-term treatment with staurosporine, a protein kinase alkaloid inhibitor, induced an elevation of tau protein levels and outgrowth of stable neurites. In this study, we analyzed the mechanism(s) by which nerve growth factor and staurosporine exert their effects on tau levels. We demonstrate that nerve growth factor affects tau mRNA stability, thus contributing to the observed increase in tau mRNA levels. On the other hand, tau mRNA levels were not affected by the treatment with staurosporine. We also demonstrate that the phosphorylation of tau protein was reduced after treatment of PC12 cells with nerve growth factor or staurosporine, as shown by immunoblot analysis using specific antibodies and alkaline phosphatase treatment. Thus, regulation of tau levels by nerve growth factor appears to be mediated by transcriptional, post-transcriptional and posttranslational steps, whereas the effect of staurosporine on tau levels may be attributed to its effect on the state of phosphorylation of the protein.

Key words: microtubule-associated protein (MAP), nerve growth factor (NGF), staurosporine, tau mRNA half-life, tau protein phosphorylation

INTRODUCTION

PC12 cells, a clonal cell line derived from rat pheochromocytoma, serve as a useful model system for the study of regulated neurite outgrowth (Greene and Tischler, 1976). When PC12 cells are exposed to nerve growth factor (NGF) for several days, they stop proliferating and acquire many features of sympathetic neurons, including outgrowth of long-branching neurites (Greene and Tischler, 1976). The NGF-induced initiation of neurite outgrowth in PC12 cells was shown to be dependent on RNA transcription, while neurite elongation was transcription-independent (Burstein and Greene, 1978). It was suggested that the transcription-dependent step leads to the specific synthesis and accumulation of materials required for neurite outgrowth (Burstein and Greene, 1978). Detailed analysis revealed that tubulin and tau mRNA levels in PC12 cells increased in response to NGF and returned to basal levels when NGF was removed (Drubin et al., 1988). However, although NGF treatment resulted in an increase in total tubulin protein levels, the time course of this increase could not be correlated with an increase in assembled microtubules (MTs) (Drubin et al., 1985). In contrast, the time course of the increase in tau and MT-associated protein 1 (MAP1) levels was identical to those of the induction of MT assembly and neurite outgrowth. These data, as well as studies using tau antisense oligodeoxynucleotides to inhibit tau expression, suggested that tau proteins might act as a key factor regulating neurite elongation and stabilization in PC12 cells (Hanemaaijer and Ginzburg, 1991).

Staurosporine, a protein kinase inhibitor, was recently shown to induce neurite outgrowth in PC12 cells (Rasouly et al., 1992, 1993). Staurosporine treatment causes a rapid increase in tau protein levels, with a time course similar to that of the initiation of its neurotropic effect, which was characterized by rapid outgrowth of stable neurites resistant to colchicine treatment. The increase in tau protein levels was already evident after 1 hour of treatment, reaching a maximal level of 2-3-fold after 5 hours of treatment and declining to the basal level during the next 10-15 hours (Rasouly et al., 1993).

In the present study, we compared the mechanisms that underlie the effects of NGF and staurosporine treatments on tau levels in PC12 cells. We demonstrate that treatment of PC12 cells for 6 days with NGF resulted in tau mRNA stabilization. Staurosporine treatment for 5 hours did not affect tau mRNA levels in the treated cells. Moreover, the short-term effects of staurosporine, namely, the induction of neurite outgrowth and the increase in tau protein levels, were not inhibited by treatments with either transcription or translation inhibitors. The neurotropic effects of both NGF and staurosporine treatment were correlated with decreased levels of tau phosphorylation, which may affect tau binding to MTs and thus result in neurite outgrowth.

MATERIALS AND METHODS

Cell culture

PC12 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 8% horse serum and 8% fetal calf serum (FCS) in 8% CO_2 at 37°C in an incubator. For treatment with NGF, 1.2-1.5×10^6 cells were plated on collagen-coated 90 mm dishes and grown in DMEM supplemented with 1% horse serum and 50 ng/ml 7S NGF (Sigma). NGF was added every 2 days. For treatment with staurosporine, cells were plated as above and grown in DMEM supplemented with 1% horse serum and 50 nM staurosporine. Staurosporine was prepared to a very high purity by Dr Y. Matsuda at Kyowa Hakko Kogyo Research Laboratories, Tokyo.

Tau immunoblots

Proteins were extracted from PC12 cells as follows: cells were washed twice with phosphate-buffered saline (PBS), collected using a rubber policeman and homogenized in lysis buffer (50 mM Tris, pH 8.5, 1% Triton X-100, 5 mM EDTA, 0.15 M NaCl, 50 μg/ml PMSF, 10 μg/ml leupeptin). The cell lysate was centrifuged for 10 minutes at 14,000 rpm at 4°C and the supernatant was collected. Protein concentrations were quantified using Bradford reagent and samples were analyzed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% acrylamide). Gels were blotted onto 0.2 μm nitrocellulose using a semidry blotting device (E&K Scientific Products, Inc.). The tau protein was analyzed using tau-1 monoclonal antibody (mAb) 1:1,000 (Binder et al., 1985) or undiluted α-tau 7.51 mAb (Novak et al., 1991), that recognizes a phosphorylation-independent epitope; the α-tau 7.51 mAb was kindly provided by Drs C. M. Wischik and J. Ávila. The western blot analysis was performed in 5% low-fat milk powder followed by reaction with a secondary antibody, goat antimouse conjugated to horseradish peroxidase (HRP) (Jackson) 1:5,000. The blots were developed using ECL reagents (Amersham).

Northern blot analysis

Total RNA was extracted from PC12 cells using the guanidinium thiocyanate method (Chomczynski and Sacchi, 1987). RNA samples (25 μg) were analyzed by hybridization to ^{32}P-labeled tau and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) riboprobes transcribed from Bluescript plasmids using T7 RNA polymerase. Hybridization was performed in 5×SSC, 0.1% SDS, 20 mM $NaHPO_4$, pH 6.8, 5× Denhardt's solution, 100 μg/ml denatured salmon sperm and 50% formamide at 68°C for 16 hours, followed by washing in 0.1× SSC containing 0.1% SDS at 68°C. The blots were exposed at −70°C for 20-48 hours.

Quantitative analysis of northern and western blots

The tau proteins or mRNAs on the autoradiograms were quantitatively analyzed with the aid of a computerized video imaging system (Biological Detection Systems, Pittsburgh, PA, USA). Determination of optical density (OD) values of tau proteins or mRNA levels was standardized by defining an OD value for the clear areas, designated as the background level, for each lane and subtracting it from the OD value obtained for tau. The area occupied by the signals was also determined. In the northern blots, OD values for GAPDH mRNA were obtained for each lane. The data for tau mRNA are presented as the OD value multiplied by the area occupied by the signal divided by similarly obtained values for GAPDH, and expressed as per cent of control. For each experiment, a single exponential decay curve was used to obtain the $t_{1/2}$ value, using the mathematical function $f(x) = ae^{-bx}$. The b value was resolved by the Sigma Plot 4.11 computer program (Jandel Scientific) and $t_{1/2}$ was calculated as the ratio of $0.69/b$. The figure and interactions of the function were obtained using the Sigma Plot 4.11 computer program. Data were analyzed by analysis of variance (ANOVA), followed by Bartlett's test for homogeneity of variance. Bonferroni's test of multiple comparisons was applied as the post hoc test.

Treatment of PC12 cells with actinomycin D and with cycloheximide

PC12 cells (1.5×10^6) were plated on collagen-coated 90-mm plates and treated with 50 ng/ml NGF for 6 days. Control and NGF-treated cells were treated with actinomycin D (5 μg/ml) for 1, 5, 20 or 27 hours, or with cycloheximide (60 μg/ml) for 5 hours. Total RNA was then extracted and processed as described above.

Alkaline phosphatase treatment

Alkaline phosphatase treatment was performed as described (Goedert et al., 1992b). Total proteins extracted from PC12 cells (25 μg) were treated with 6 units of calf intestine alkaline phosphatase (Boehringer) in 100 mM Tris buffer, pH 8, containing 1 mM $MgCl_2$ and 50 μg/ml PMSF in a final volume of 20 μl, for 5 hours at 37°C. The reaction was stopped by addition of 5 μl of 5× protein sample buffer (Laemmli, 1970). Samples were subjected to SDS-PAGE (10% acrylamide) and tau proteins were analyzed by immunoblotting.

RESULTS

Treatment with NGF or staurosporine and their effects on neurite outgrowth and on levels of tau protein and tau mRNA

PC12 cells treated with 50 ng/ml of NGF stopped dividing and started to differentiate, as indicated morphologically by extension of neurites. After 6 days in NGF the neurites formed a dense network, which was dependent on the continuous presence of NGF (Fig. 1B) (Drubin et al., 1985). Staurosporine took much less time to exert its effect: after 5 hours of treatment of PC12 cells with 50 nM of staurosporine, short neurites were already observed (Fig. 1C) (Rasouly et al., 1992). The neurites induced by staurosporine differed, both morphologically and physiologically, from those induced by long-term treatment with NGF: they were thinner, and there were fewer processes per cell (Fig. 1C) (Rasouly et al., 1992).

In PC12 cells treated with NGF for 6 days, total tau protein levels were increased by 8-10-fold above control levels, as monitored using tau-1 mAb (Fig. 2A and C) and as previously shown (Drubin et al., 1985). Treatment of PC12 cells with staurosporine resulted in a similar increase of about 8-10-fold above control levels as early as 5 hours of treatment (Fig. 2B and C). The staurosporine-induced increase could range between 3 and 10-fold, depending on the staurosporine batch used and/or the subtype origin, or the passage number of the PC12 cells (Rasouly et al., 1993). Although the increase in total tau levels was similar for both agents, a closer inspection indicates that the effect of NGF or staurosporine may be different on the high-molecular weight (HMW) and low-molecular weight (LMW) tau isoforms. In NGF-treated cells, the HMW tau (110 kDa) was up-regulated by 5-fold and the LMW (47-55 kDa) tau by 9-fold above control levels (Fig. 2A). In contrast, in cells treated with staurosporine the HMW tau was up-regulated by more than 10-fold and the LMW tau by 7-fold (Fig. 2B). The above results may suggest different mechanisms underlying the up-regulation of the LMW and HMW tau protein isoforms.

Previous studies have established that NGF induction of neurite outgrowth in PC12 cells is transcription-dependent (Burstein and Greene, 1978). We found that whereas tau mRNA levels were increased by 7-fold following 6 days of NGF treatment (Fig. 3A and C), in cells treated with staurosporine

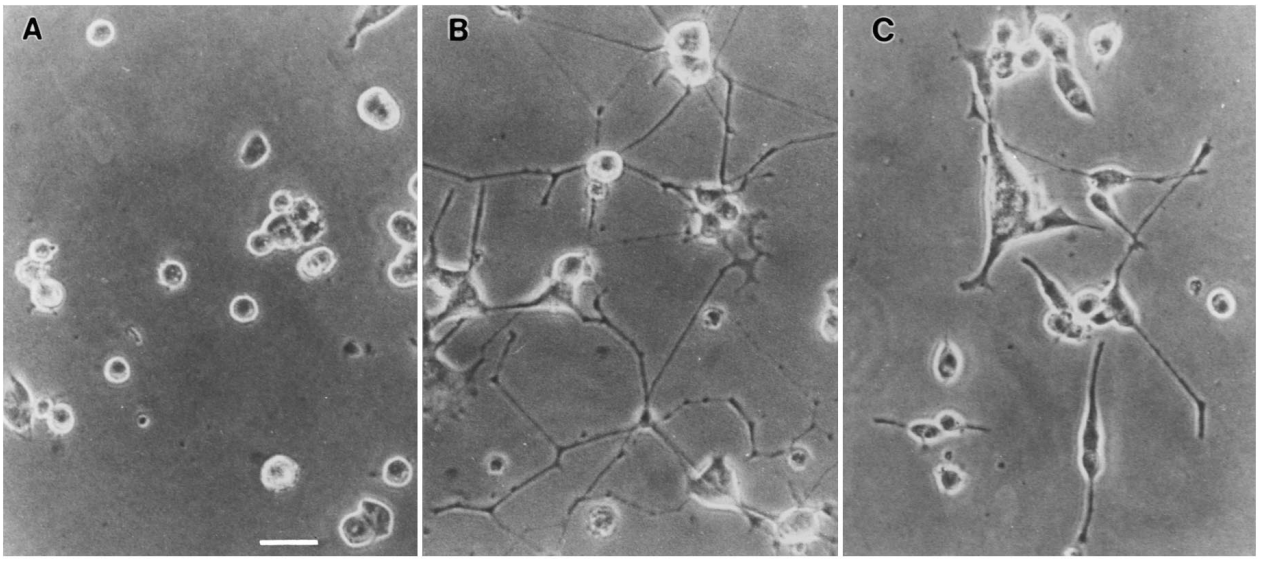
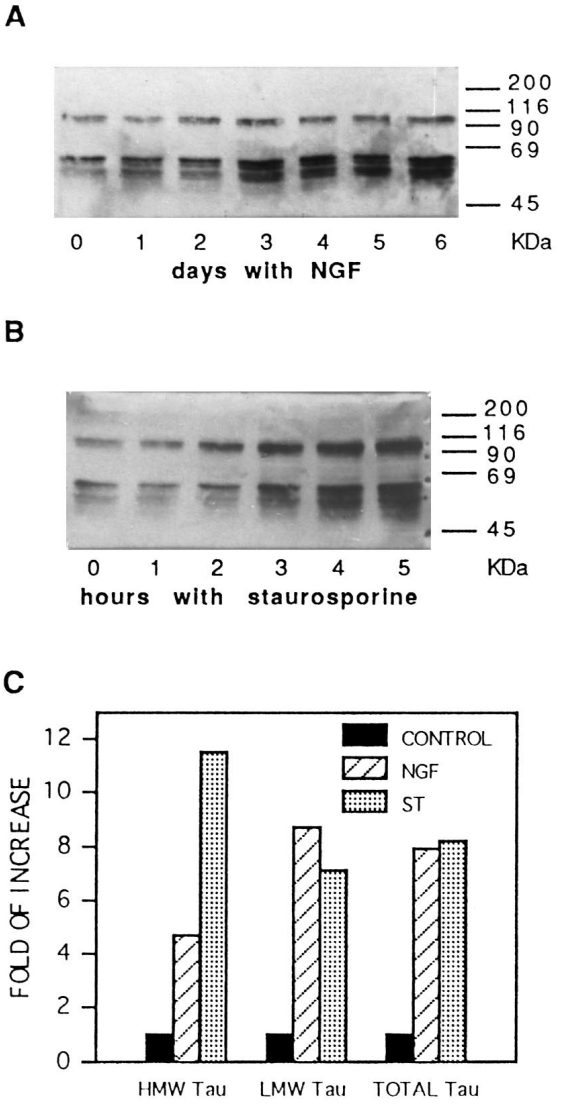


Fig. 1. Phase microscopy of PC12 cells treated with NGF and with staurosporine. (A) Control cells. (B) Cells treated with 50 ng/ml NGF for 6 days. (C) Cells treated with 50 nM staurosporine for 5 hours. Cells were plated on collagen-coated plates (1.2×10^6 cells/plate) and examined under a light microscope. ×400. Bar, 20 μm.



for 1, 2, 3, 4 or 5 hours the tau mRNA levels remained constant (Fig. 3B and C). The quantitative results shown in Fig. 3C are derived following normalization of the signal obtained for tau mRNA with the GAPDH probe. These findings suggest that, in contrast to the increase in NGF-treated cells, the observed increase in tau protein levels in response to short-term staurosporine treatment is transcription-independent.

The observed increase in tau mRNA following NGF treatment might reflect increased transcription (Burstein and Greene, 1978; Drubin et al., 1988) and/or an increase in the stability of tau mRNA. To examine the latter possibility, PC12 cells were grown in the absence or presence of NGF for 6 days. Thereafter, 5 μg/ml of actinomycin D, which inhibits RNA synthesis was added to control and differentiated cells that were harvested 5, 20 or 27 hours after addition of the inhibitor (Fig. 4A). Tau mRNA levels were measured by hybridization with a tau-specific probe, and the levels of GAPDH mRNA were measured as an internal control for equal RNA loading (Fig. 4A). Analysis of the decay curves of tau mRNA isolated from control and NGF-treated cells at the various time points (Fig. 4B) revealed that the half-life of tau mRNA in NGF-treated cells was significantly higher than in control cells, namely, 17.2±2.2 and 6.8±3.0 hours, respectively. It thus appears that NGF treatment results in stabilization of tau mRNA, an effect that may contribute by more than 2-fold to the observed 7-fold elevation of tau mRNA levels in induced cells.

Fig. 2. Tau protein levels following treatment with NGF and with staurosporine. Western blot analysis of proteins extracted from control PC12 cells and from PC12 cells treated with NGF or staurosporine and separated on SDS-PAGE (10% acrylamide). Blots were analyzed with 1:1,000 diluted tau-1 mAb. (A) Increase in tau protein level after treatment with NGF for 1, 2, 3, 4, 5 or 6 days. (B) Increase in tau protein level after treatment with staurosporine for 1, 2, 3, 4 or 5 hours. (C) Quantitative analysis of the fold of increase of HMW, LMW and total tau levels after NGF treatment for 6 days or staurosporine treatment for 5 hours. Western blots were quantified by optical scanning densitometry. Plotted values represent percent of control cells in the above representative experiments (A,B).

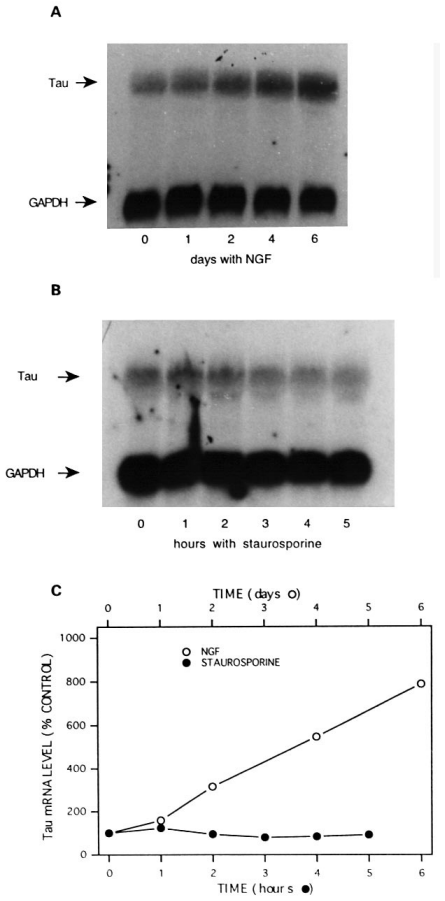


Fig. 3. Tau mRNA levels following treatment with NGF and with staurosporine. Northern blot analysis of total mRNA (25 μg) extracted from control PC12 cells and from PC12 cells treated with NGF or with staurosporine. Samples were separated on formaldehyde 1% agarose gels and hybridized to tau and GAPDH probes. (A) Levels of tau mRNA extracted from PC12 cells treated with NGF for 1, 2, 4 or 6 days. (B) Levels of tau mRNA extracted from PC12 cells treated with staurosporine for 1, 2, 3, 4 or 5 hours. (C) Quantitative analysis of the changes in tau mRNA after treatment with NGF and with staurosporine. At each time point, the OD of the tau signal was divided by that of GAPDH. Plotted values represent percent of control cells.

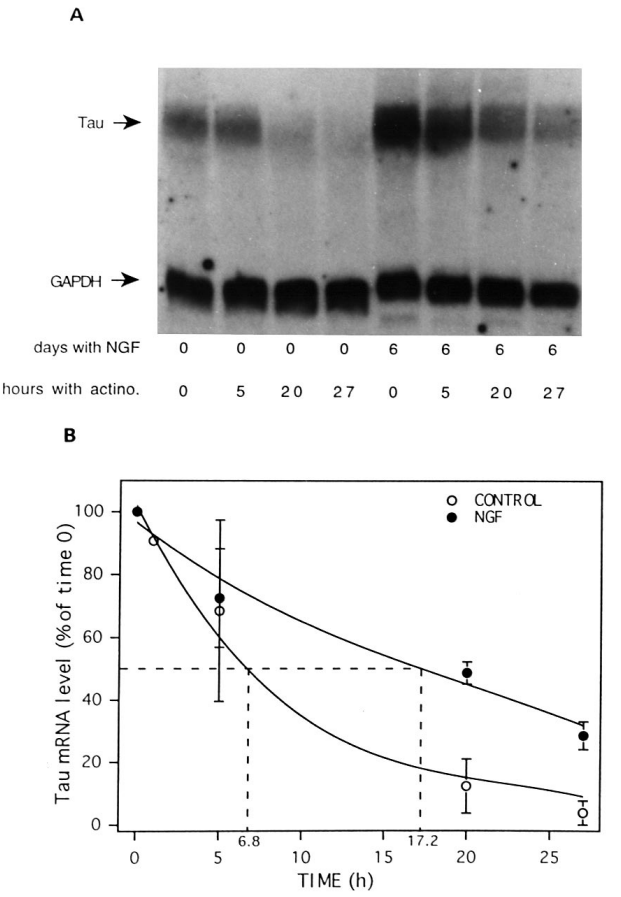


Fig. 4. Tau mRNA following treatment with actinomycin D. (A) Northern blot analysis of total mRNA (25 μg) extracted from control PC12 cells and from PC12 cells treated with NGF with or without actinomycin D. Samples were separated on formaldehyde 1% agarose gels and hybridized to tau and GAPDH probes. Control cells and cells treated with NGF for 6 days were treated with 5 μg/ml actinomycin D for 5, 20 or 27 hours. The blot shown is representative of three experiments. (B) Quantitative analysis of the results of three experiments, showing the decay of tau mRNA following actinomycin D treatment. The $t_{1/2}$ values for control cells (6.8±3.0) differ significantly from those obtained for NGF-treated cells (17.2±2.2). $P<0.05$.

To further analyze the effect of staurosporine on the tau protein level, PC12 cells were treated for 5 hours with staurosporine in the presence of 5 μg/ml actinomycin D, which inhibits RNA synthesis or 60 μg/ml cycloheximide, which inhibits protein synthesis. Our results showed that neither the transcription nor the translation inhibitor abolished the effect of staurosporine on neurite outgrowth in the treated cells (Fig. 5B and C) or the increase in tau protein levels (data not shown). These results support the above suggestion that the short-term effect of staurosporine on tau protein levels is transcription-independent and does not result from accumulation of newly synthesized proteins.

Effects of NGF and staurosporine treatments on the state of phosphorylation of tau proteins

Staurosporine, being a nonselective protein kinase C inhibitor, may affect the detection of tau protein levels by acting at the posttranscriptional level, e.g. by modulating its state of phosphorylation. Tau is a phosphoprotein, and the tau-1 mAb used for its analysis is sensitive to the state of tau phosphorylation and preferentially recognizes nonphosphorylated forms of tau (Papasozomenos and Binder, 1987; reviewed by Mandelkow and Mandelkow, 1993). Proteins extracted from control PC12 cells or from PC12 cells treated with staurosporine or NGF were incubated with alkaline phosphatase and then subjected to western blot analysis using tau-1 mAb (Fig. 6A and B). Treatment of tau protein with alkaline phosphatase was used to demonstrate the abnormal phosphorylation form of tau present in paired helical filaments (PHFs) (reviewed by Mandelkow and Mandelkow, 1993). The results demonstrated that after the phosphatase treatment, tau immunoreactivity was similar in untreated cell extracts and in extracts prepared from cells treated with staurosporine for 5 hours (Fig. 6B, lanes 2 and 3), suggesting that

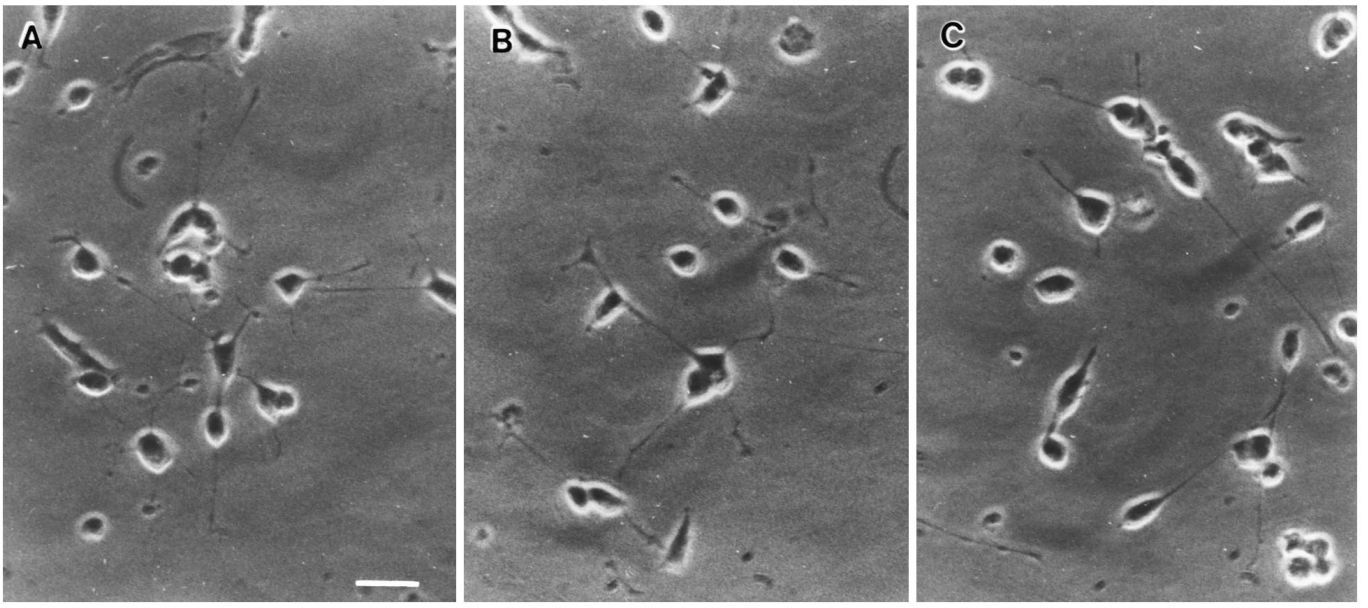


Fig. 5. Phase microscopy of PC12 cells treated with staurosporine and actinomycin D or cycloheximide. PC12 cells were plated on collagen-coated plates (1.2×10^6 cells/plate) and treated with: (A) 50 nM staurosporine for 5 hours; (B) staurosporine with 5 μg/ml actinomycin D for 5 hours; and (C) staurosporine with 60 μg/ml cycloheximide for 5 hours. Cells were examined under a light microscope. ×400. Bar, 20 μm.

the observed elevation in tau protein levels after staurosporine treatment may be the result of its phosphorylation state and hence its differential recognition by tau-1 mAb (Fig. 6B, lanes 3 and 4).

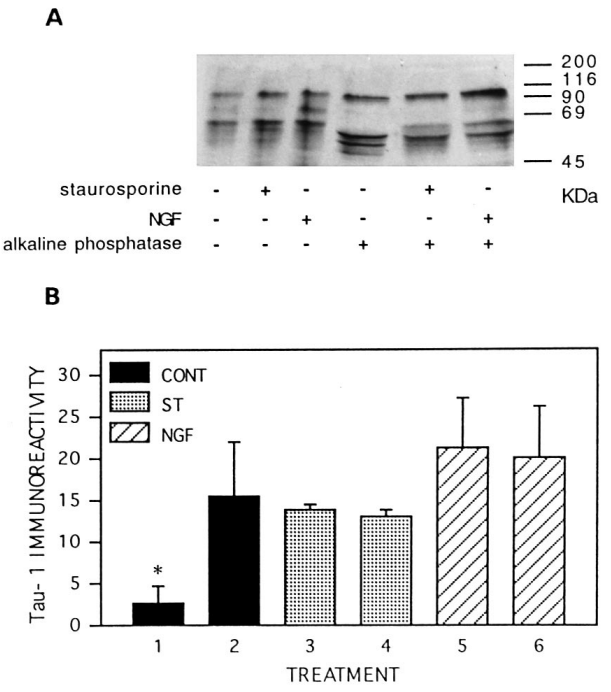


Fig. 6. Analysis of protein extracts treated with alkaline phosphatase. Western blot of proteins extracted from control PC12 cells and from PC12 cells treated with NGF for 6 days or with staurosporine for 5 hours. Samples were subjected to treatment with alkaline phosphatase (6 units) for 5 hours. (A) Western blot analyzed with tau-1 mAb. (B) Quantitative analysis of three repeated experiments showing the effects of the above treatments on tau protein levels before (lanes 1, 3 and 5) and after (lanes 2, 4 and 6) alkaline phosphatase treatment.

The strongest effect of alkaline phosphatase treatment was observed in tau proteins isolated from untreated control cells, in which tau-1 immunoreactivity was increased by 5-fold (Fig. 6B, lanes 1 and 2). In contrast, the tau-1 immunoreactivity of proteins extracted from cells treated with staurosporine or NGF did not change markedly following alkaline phosphatase treatment (Fig. 6B, lanes 3-6). Moreover, analysis of the extracted proteins using α-tau 7.51 mAb, revealed no marked difference between untreated control and staurosporine-treated cells, while in NGF-treated cells tau levels were more than 2-fold higher than control cells for both the HMW tau and LMW tau (Fig. 7A and B). This increase in total tau levels is the result of NGF induction. The 7.51 mAb recognizes segments of tau in the last two repeats, is not sensitive to protein phosphorylation, recognizes all forms of tau proteins, and thus serves as a generic tau marker (Novak et al., 1991).

DISCUSSION

Previous studies have established a key role for MAPs in neurite outgrowth in PC12 cells induced to differentiate via NGF (Black et al., 1986; Drubin et al., 1985; Greene et al., 1982). Moreover, the assembly of MTs and their subsequent stabilization were directly linked to the expression and accumulation of MAP1, MAP2, MAP3, MAP5 and tau (Black et al., 1986; Brugg and Matus, 1988; Drubin et al., 1985).

In the present study, we show that the stability of tau mRNA in PC12 cells is increased by more than 2-fold after 6 days of treatment with NGF. This factor may contribute to the 7-fold increase in the total amount of tau mRNA observed in the cells following NGF treatment. We suggest therefore that the increase in tau expression in PC12 cells in response to NGF

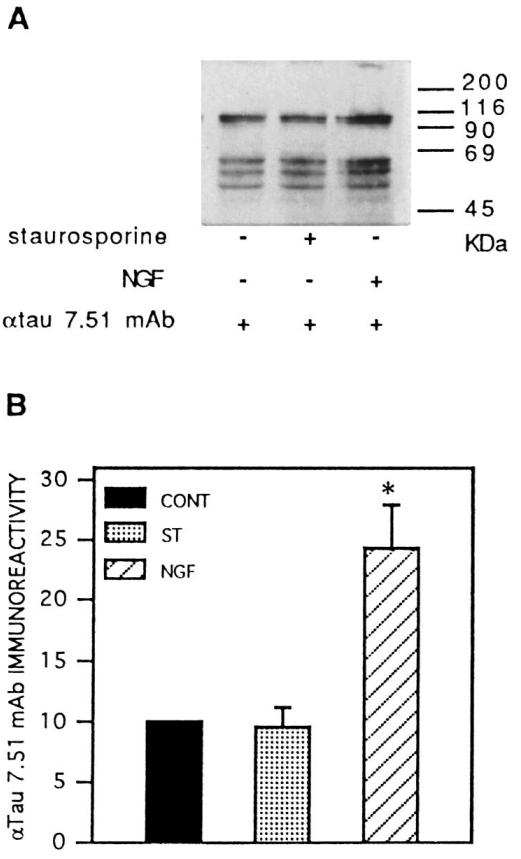


Fig. 7. Tau protein levels following NGF and staurosporine treatments, analyzed by α-tau 7.51 mAb. (A) Proteins extracted from control PC12 cells and from PC12 cells treated with NGF for 6 days or with staurosporine for 5 hours were separated on SDS-PAGE (10% acrylamide) and analyzed using α-tau 7.51 mAb.
(B) Quantitative analysis of three repeated experiments showing the effects of the above treatments on tau protein levels, as revealed by α-tau 7.51 mAb. Tau protein level is more than 2-fold higher in NGF-treated PC12 cells, as compared to control cells. $P<0.05$.

may be due to an increase in both transcription and mRNA stabilization. Stability of mRNA is an important mechanism for regulating gene expression; however, the detailed steps involved in the determination of individual mRNA stability are not yet clear (Peltz and Jacobson, 1992). An example of transcription and stabilization mechanisms controlling mRNA levels was demonstrated in the case of hormonal and substratum induction of β-casein protein in a mammalian epithelial cell line (Eisenstein and Rosen, 1988): a total induction of about 70-fold was contributed to a 2-3-fold stimulation of transcription and a 30-fold increase in β-casein mRNA stability. Stabilization has been attributed to signals in the 3′-untranslated (UTR) region of proto-oncogenes (Bonnieu et al., 1988; You et al., 1992) and in GM-CSF lymphokine (Shaw and Kamen, 1986), all of which contain A+U-rich elements (AREs) that may promote poly(A)$^+$ removal and subsequent mRNA destabilization. Some trans-acting factors that bind to these AREs have been characterized, and several properties of some of these ARE-binding factors point to their possible participation in mRNA turnover (Brewer, 1991; Vakalopoulou et al., 1991; You et al., 1992). A 52-57 kDa protein, which specifically binds to the 3′-UTR region of ribonucleotide reductase R1, was shown to be involved in reduction of the rate of R1 mRNA degradation in response to TPA treatment (Chen et al., 1993). The tau mRNA exhibits a very long 3′-UTR region of 3,848 bp (Sadot et al., 1994), which may suggest that this region contains regulatory signals and among them sequences that play a role in mRNA stability. Observations accumulating in our laboratory indicate that the tau 3′-UTR region can bind specific proteins and may contain mRNA-stabilizing elements (our unpublished data). These observations may shed some light on the mechanism by which NGF affects tau mRNA stability.

Treatment of PC12 cells with staurosporine for short-time intervals induces a rapid elevation in tau-1 immunoreactivity, but with no change in tau mRNA levels. Moreover, neither actinomycin D nor cycloheximide, which inhibit RNA and protein synthesis, respectively, affected the staurosporine-induced neurite outgrowth or increase in tau-1 immunoreactivity. On the other hand, analysis with tau-1 mAb after alkaline phosphatase treatment, as well as analysis with α-tau 7.51 mAb, revealed similar tau immunoreactivity in protein extracts prepared from control and staurosporine-treated cells. Thus, the effects observed following staurosporine treatment can be explained mainly by the change in the phosphorylation state of the protein at the tau-1 epitope (serine 199-202) during the short term of the staurosporine-induced effect. Although total increase in tau-1 immunoreactivity was similar for NGF and staurosporine, an indication for differential regulation of HMW tau and LMW tau is suggestive. Independent regulation of HMW and LMW tau isoforms was observed by Teng et al. (1993) in PC12-C41, a subclone of PC12 cells exhibiting enhanced neurite outgrowth capacity. Comparison of the NGF-induced effects on tau in PC12-C41 and in the native PC12 cells revealed that while NGF treatment caused a similar increase in the total level of tau in both cell types, the increase in the level of HMW tau proteins was 3-fold greater in PC12-C41 than in PC12 cells (Teng et al., 1993).

Dephosphorylated tau was found to be more efficient than phosphorylated tau in promoting MT assembly, although the exact phosphorylation sites are unknown (Lindwall and Cole, 1984). In addition, it was recently demonstrated that phosphorylation of tau by MAP2 kinase decreases its affinity for the MT lattice and reduces its ability to promote MT stabilization (Drechsel et al., 1992). It was also suggested that both calcineurin (protein phosphatase 2B) (Goto et al., 1985) and protein phosphatase 2A (Goedert et al., 1992a) may be able to dephosphorylate tau in vitro. Moreover, it was shown that calcineurin, which is a calmodulin-dependent serine-threonine phosphatase abundant in the brain and is associated with the cytoskeleton, regulates tau phosphorylation in cultured neurons (Ferreira et al., 1993). The above data may suggest that staurosporine, being a protein kinase inhibitor, disrupts the delicate balance between kinases and phosphatases acting in PC12 cells, resulting in reduction of the extent of tau phosphorylation and leading to rapid neurite outgrowth. A lower phosphorylation of tau in this case yields a similar effect to the high expression of tau observed following NGF treatment of PC12 cells or following overexpression of tau in sf9 cells (Baas et al., 1991).

Our experiments using alkaline phosphatase treatment suggest that the level of phosphorylated tau in control PC12

cells is higher than in PC12 cells treated with NGF for 6 days, as analyzed at the tau-1 epitope, while other phosphorylation sites may be affected as well. These results are in agreement with the observation indicating that fetal rat tau is more extensively phosphorylated than mature tau, as evaluated by the change in mobility following alkaline phosphatase treatment (Goedert et al., 1991). Detailed analyses confirmed these observations by identifying specific tau phosphopeptides (Watanabe et al., 1993). Interestingly, in Alzheimer's disease, tau becomes abnormally phosphorylated and self-associates to form the PHF, the principal fibrous component of the neurofibrillary pathology (Lee et al., 1991; Wille et al., 1992). Tau proteins isolated from PHF are unable to bind to MTs, yet following their in vitro dephosphorylation, the PHF-derived tau proteins regain the ability to bind to MTs, indicating the inhibitory consequences of the abnormal phosphorylation (Bramblett et al., 1993). These results are therefore in accord with the fact that fetal tau shares phosphorylation sites with PHF tau, which has been shown to be overphosphorylated as compared to normal tau (Bramblett et al., 1993; Goedert et al., 1993; Kanemaru et al., 1992; Watanabe et al., 1993). The exact phosphorylation sites of tau in PC12 cells remain to be elucidated.

The above data may suggest that PC12 cells can be employed as a model system in order to study the mechanism responsible for overphosphorylation of tau proteins in Alzheimer's disease patients. According to the data presented here, staurosporine may have the capacity to reduce the level of tau phosphorylation, thus possibly supporting viability of neuronal cells, and may therefore be tested as a potential therapeutic drug for Alzheimer's disease.

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