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Microtubule-stabilizing agent prevents protein accumulation-induced loss of synaptic markers ☆

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Abstract

Synaptic pathology is associated with protein accumulation events, and is thought by many to be the best correlate of cognitive impairment in normal aging and different types of dementia including Alzheimer's disease. Numerous studies point to the disruption of microtubule-based transport mechanisms as a contributor to synaptic degeneration. Reported reductions in a microtubule stability marker, acetylated α-tubulin, suggest that disrupted transport occurs in Alzheimer's disease neurons, and such a reduction is known to be associated with transport failure and synaptic compromise in a hippocampal slice model of protein accumulation. The slice model exhibits accumulated proteins in response to chloroquine-mediated lysosomal dysfunction, resulting in corresponding decreases in acetylated tubulin and pre- and postsynaptic markers (synaptophysin and glutamate receptors). To test whether the protein deposition-induced loss of synaptic proteins is due to disruption of microtubule integrity, a potent microtubule-stabilizing agent, the taxol derivative TX67 (10-succinyl paclitaxel), was applied to the hippocampal slice cultures. In the absence of lysosomal stress, TX67 (100–300 nM) provided microtubule stabilization as indicated by markedly increased levels of acetylated tubulin. When TX67 was applied to the slices during the chloroquine treatment period, pre- and postsynaptic markers were maintained at control levels. In addition, a correlation was evident across slice samples between levels of acetylated tubulin and glutamate receptor subunit GluR1. These data indicate that disruption of microtubule integrity accounts for protein deposition-induced synaptic decline. They also suggest that microtubule-stabilizing drugs can be used to slow or halt the progressive synaptic deterioration linked to Alzheimer-type pathogenesis.

Keywords: Alzheimer's disease; Microtubule integrity; Protein deposition; Synaptic decline; Taxol derivative; TX67

1. Introduction

Synapses are sites of neuronal communication that provide the capacity of memory function and learning. Synaptic pathology is thought to account for the cognitive decline associated with aging and age-related dementias (Coleman et al., 2004). The loss of synapses and/or vital synaptic components indeed has been reported to strongly correspond with the dementia of Alzheimer's disease (see Davies et al., 1987; Terry et al., 1991; Samuel et al., 1994; Heinonen et al., 1995; Sze et al., 1997; Masliah et al., 2001). Synaptic deterioration also correlates with frontotemporal dementias (Lipton et al., 2001) and dementia with Lewy bodies (Masliah et al., 1993; Brown et al., 1998). The normal aging brain is also notable for synaptic alterations and reduced expression of proteins responsible for the function of synapses (see Tamaru et al., 1991; Bahr et al., 1992, 1993). Mechanisms underlying such synaptic changes may contribute to mild cognitive impairment and a growing risk for the onset of age-related neurodegenerative disorders.

Regarding Alzheimer-type pathogenesis, it is apparent that proteins and oligomers with a propensity to accumulate intracellularly can disrupt transport mechanisms responsible for

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replenishing supplies to synapses. The collapse of microtubule integrity and concomitant disruption of somatofugal transport has been implicated in the axonopathy and synaptic pathology related to early stage Alzheimer's disease (Heinonen et al., 1995; Hempen and Brion, 1996; Bendiske et al., 2002; Butler et al., 2005; Stokin et al., 2005; Zhang et al., 2005). One obvious candidate that may be involved in the damage is the microtubule-associated protein tau, which exhibits distinct changes in the aged brain and Alzheimer's disease (Lee, 1995; Alonso et al., 1996; Bahr and Vicente, 1998). Aberrant behavior of tau, including neurofibrillary deposits, has been linked to reduced expression of synaptic markers in age-related diseases and in models of protein accumulation (Bahr, 1995; Sasaki and Iwata, 1996; Callahan et al., 1999; Hall et al., 2000; Bendiske et al., 2002). Tau aggregation events likely disrupt the normal equilibrium between normal tau and phosphorylated tau, thus decreasing the availability of functional tau for stabilizing microtubules and their transport mechanisms (Bahr, 2003; Michaelis et al., 2005b). It has also been proposed that misregulation of tau chemistry causes transport failure and resultant starvation of synapses long before tau aggregates into neurofibrillary tangles (Mandelkow et al., 2003). Together, microtubules and microtubule-based transport mechanisms are important for the maintenance of synapses.

If microtubule disruption accounts for the reduced expression of synaptic proteins during protein accumulation events, then stabilizing microtubules and promoting the integrity of transport systems should prevent the synaptic decline. To address this issue, the present study utilized a derivative of paclitaxel (taxol) in an *in vitro* model of protein accumulation. Taxol is a common cancer chemotherapeutic agent that induces tubulin polymerization and microtubule stabilization (Schiff et al., 1979; Parness and Horwitz, 1981). Microtubule stabilizers are suggested to protect against Alzheimer-type neuropathogenesis (Michaelis et al., 2005a; Zhang et al., 2005; Divinski et al., 2006). Taxol and the taxane analogue TX67 (10-succinyl paclitaxel) have been reported to protect primary neurons against Aβ peptide toxicity and to block Aβ-induced increases in abnormal tau phosphorylation (Michaelis et al., 1998, 2005a; Li et al., 2003).

Here, TX67 was tested in a hippocampal slice model that reproduces protein accumulation events in response to the disruption of lysosomal degradative processes. The induced protein deposition in hippocampal slice cultures is associated with transport failure, axonopathy, and progressive synaptic decline, thus indicating the value of the model system (Bahr et al., 1994, 1998; Bendiske et al., 2002; Butler et al., 2005). The resulting synaptic pathology is gradual and leads to a marked reduction in the number of synapses per neuron (Bahr and Bendiske, 2002), similar to the synapse reduction evident in early stage Alzheimer's disease (Davies et al., 1987). With the use of TX67, microtubule stabilization was found to protect against the synaptic pathology produced by lysosomal disturbances and associated protein deposition. These findings support the idea that microtubule integrity is compromised in protein deposition diseases, and they indicate a plausible therapeutic strategy.

2. Materials and methods

2.1. Tissue preparation and organotypic hippocampal slice cultures

All protocols were approved by the University of Connecticut's institutional animal care and ethics committee. Sprague–Dawley rat litters (Charles River Laboratories; Wilmington, Massachusetts) were housed following guidelines from the National Institutes of Health. The animals were allowed 4–5 days of acclimatization prior to sacrifice. Brains were removed at 11–12 days of age and hippocampi were rapidly dissected under ice-cold conditions. The tissue was sectioned into transverse slices (400 μm), which were placed in groups of 6–10 slices per Millicell-CM insert (Millipore Corporation; Bedford, Massachusetts). Initial maintenance included 15–20 days in culture, periodically supplied with media composed of 50% basal medium Eagle, 25% Earle's balanced salts, 25% horse serum, and defined supplements (Bahr et al., 1994, 1998). For adult mouse brains, tissue was separated in ice-cold buffer consisting of 0.32 M sucrose, 5 mM HEPES (pH 7.4), 1 mM EDTA, 1 mM EGTA, 0.6 μM okadaic acid, 50 nM calyculin A, and a protease inhibitor cocktail containing 4-(2-aminoethyl)benzenesulfonyl fluoride, pepstatin A, E-64, bestatin, leupeptin, and aprotinin. The tissue samples were homogenized in lysis buffer consisting of 15 mM HEPES (pH 7.4), 0.5 mM EDTA, 0.5 mM EGTA and the protease inhibitor cocktail.

2.2. Cultured slice treatment groups

One set of slice cultures was incubated with media containing vehicle or 60 μM chloroquine (Sigma Chemical Co.; St. Louis, Missouri) for 3–9 days (media changed every 1–2 days). Chloroquine is a weak base that accumulates in acidic compartments, leading to the disruption of lysosomal protein degradation. The level of chloroquine used influences lysosomal processes without affecting protein synthesis, glycosylation, or secretion. A second set of slices was treated daily for 3–6 days with 0–300 nM TX67, prepared by parallel solution phase synthesis, to determine dose-dependent effects. In a third set of slice cultures, chloroquine treatment continued for 6 days followed by subsequent daily incubations with media alone or with 100–200 nM TX67 for 2 days. The last set was treated with chloroquine and TX67 together for 3–6 days. After the experimental treatments, slices were gently harvested with ice-cold buffer followed by homogenization in lysis buffer (15 mM HEPES, 0.5 mM EDA, 0.5 mM EGTA, and protease inhibitors).

2.3. Immunoblot analysis

Hippocampus slice samples were homogenized in groups of 6–8 by sonicating in ice-cold lysis buffer, and protein concentration was assessed with a BSA standard. Equal aliquots of the samples were denatured in sodium dodecyl sulfate (SDS) at 100 °C, separated by SDS-polyacrylamide gel electrophoresis, and blotted to nitrocellulose. Immunodetection was

achieved by incubating blots overnight at 4 °C with affinity-purified antibodies against α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor subunit GluR1 (Bahr et al., 1996), antibodies against *N*-methyl-D-aspartate (NMDA) receptor subunit NR1 (Chemicon; Temecula, California), monoclonal anti-synaptophysin (Boehringer Mannheim; Indianapolis, Indiana) and anti-acetylated tubulin (ICN Biomedicals; Costa Mesa, California), anti-synapsin I (CalBiochem; San Diego, California), and anti-actin (Sigma). Anti-IgG-alkaline phosphatase conjugates were used for secondary antibody incubation. Development of immunoreactive species used the 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium substrate system and was terminated prior to maximum intensity in order to avoid saturation. Integrated optical density of the bands was determined at high resolution with BIOQUANT software (R & M Biometrics; Nashville, Tennessee).

3. Results

In order to study the synaptopathogenesis associated with protein accumulation, slice cultures prepared from hippocampus were allowed to stabilize for 2–3 weeks under normal media conditions, then were incubated with the acidotropic agent chloroquine. Chloroquine disrupts lysosomal protein degradation and is well known to induce protein accumulation events and related ultrastructural changes (Ivy et al., 1984; Bahr, 1995; Bahr and Bendiske, 2002; Butler et al., 2005), similar to those that occur in Alzheimer's disease and in the aged brain. As shown in Fig. 1, hippocampal slice cultures treated with chloroquine clearly exhibit time-dependent reductions in the postsynaptic glutamate receptor subunit GluR1 and the presynaptic vesicle marker synaptophysin. This is consistent with reports showing that chloroquine-induced protein accumulation events are linked to synaptic decline (Bahr et al., 1994; Bendiske et al., 2002) and that conditions that decrease protein deposition lead to synaptic recovery (Bendiske and Bahr, 2003; Butler et al., 2005, 2006). The decline in GluR1 and synaptophysin occurred while actin concentrations were unchanged (Figs. 1 and 2C–E), indicating an early phase of synaptic pathology preceding further levels of neuronal atrophy.

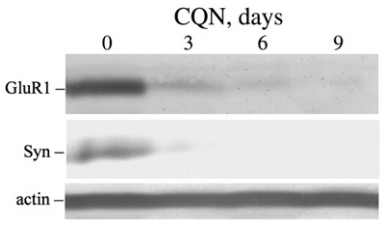

Fig. 1. Loss of synaptic markers through the disruption of lysosomal protein processing. Cultured hippocampal slices were treated with the lysosomal inhibitor chloroquine (CQN) for the days indicated, after which they were harvested in groups of six to eight for immunoblot analyses. Chloroquine treatments were staggered in order to assure all slices were harvested on the same culture day. Actin was assessed as a load control on the same immunoblots stained for the AMPA receptor subunit GluR1 and the synaptic vesicle protein synaptophysin (Syn).

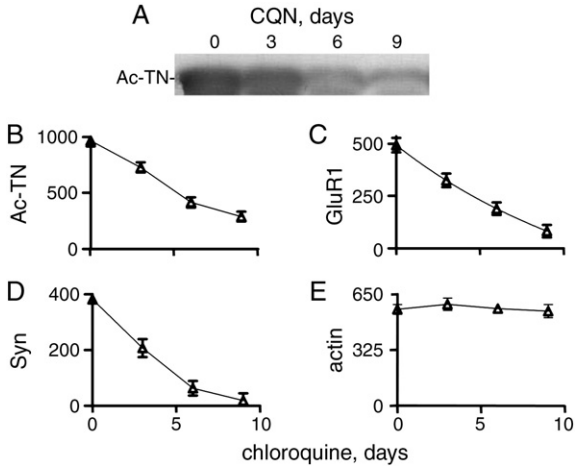

Fig. 2. Gradual loss of acetylated tubulin (Ac-TN) and synaptic markers induced by lysosomal dysfunction. Hippocampal slice cultures were treated with chloroquine (CQN) for up to 9 days, then harvested into groups of 6 to 8 and prepared for immunoblotting. Parallel slice samples were immunostained for acetylated tubulin (A, B), GluR1 (C), synaptophysin (Syn, D), and actin as a control (E). Immunoreactivity profiles were determined across the treatment period, the plotted data representing mean integrated optical densities (±S.E.M.) from 5–8 separate immunoblot samples. Analyses of variance in B–D: $P<0.0001$ for each.

The early synaptic pathology was associated with evidence of microtubule destabilization in the slice model. In Fig. 2A, chloroquine was found to cause a steady decrease in acetylated tubulin immunostaining. The immunoreactivity levels were reduced by 65–75% over the 9-day treatment period (Fig. 2B; ANOVA: $P<0.0001$). The decrease was specific for the acetylated form of tubulin since total tubulin was unchanged by the chloroquine treatment (106±8% of control; NS). Tubulin acetylation is a specific marker of stable microtubules, and measures of this post-translational modification are significantly reduced in Alzheimer's disease neurons containing neurofibrillary tangles (Hempen and Brion, 1996). These results support the idea that microtubule compromise is involved in

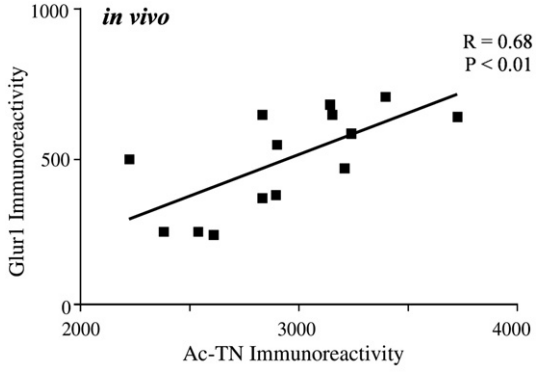

Fig. 3. The microtubule stability marker acetylated tubulin (Ac-TN) correlates with GluR1 levels *in vivo*. Neocortical tissue homogenates were quickly prepared from p25 transgenic mice and background control mice. Equal protein aliquots were analyzed by immunoblot, and linear regression was conducted on within-sample measures of acetylated tubulin and GluR1. The obtained correlation coefficient (R) was 0.68.

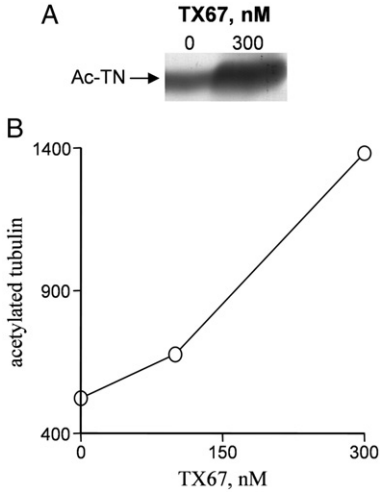

Fig. 4. Effects of taxane analogue TX67 on a microtubule integrity measure in hippocampal slice cultures. Cultured slices were treated with or without TX67 for 6 days (A). The representative immunoblot was probed for the microtubule stability marker acetylated tubulin (Ac-TN), showing a 3-fold increase in staining. Mean integrated optical density values exhibit a dose-dependent increase in acetylated tubulin immunoreactivity (B). Similar results were found with a 3-day treatment period.

Alzheimer-type degeneration including the characteristic synaptic pathology.

As acetylated tubulin levels gradually decreased in the slice model, a corresponding loss of synaptic markers occurred as actin remained at a constant level (see Fig. 2C–E). A similar correspondence to acetylated tubulin decline was previously linked to the disruption of microtubule-based transport mechanisms (Bendiske and Bahr, 2003), providing further evidence that microtubule destabilization contributes to the induced synaptopathogenesis. Note also that, *in vivo*, in those tissue samples with lower acetylated tubulin levels there was a corresponding reduction in the postsynaptic marker GluR1 (Fig. 3; $P=0.007$, one-sample *t*-test of slope). This was found across cortical homogenates prepared from control and transgenic mice, the latter over-expressing human p25 which has been shown to cause increased cdk5 activity and hyperphosphorylation of tau (Ahlijanian et al., 2000). Together these data indicate that the concentration of acetylated tubulin, and hence the status of microtubule stability, impacts on synaptic integrity.

In order to test whether the protein deposition-induced loss of synaptic markers is due to microtubule destabilization, the succinylated taxol derivative TX67 was utilized. TX67 is a

Table 1
Long-term effect of TX67 alone on a synaptic marker in hippocampal slice cultures

Treatment group	GluR1 immunoreactivity
Control	421±75
TX67, 6 days	371±43

Hippocampal slice cultures were treated with 300 nM TX67 daily for 6 days, and levels of GluR1 were determined by immunoblot. Mean integrated optical density values (±S.E.M.) were measured by image analysis from 6–8 slice groups. The mean GluR1 level in TX67-treated slices was 88% of the level found in untreated control slices (difference was not statistically significant).

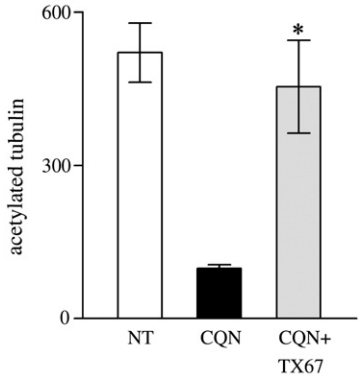

Fig. 5. Effects of TX67 in hippocampal slices with pre-existing microtubule alteration due to chloroquine-induced lysosomal disturbance. Following a 6-day chloroquine exposure, slices were supplied with media alone (CQN) or with the addition of 200 nM TX67 for 2 days. Control slices not treated with chloroquine or TX67 were maintained in culture in parallel (NT). The compromised acetylated tubulin level was markedly increased by the TX67 treatment (*unpaired *t*-test: $P<0.001$). Plotted are mean integrated optical densities ±S.E.M. ($n=4–8$).

potent microtubule-stabilizing agent designed for improved blood–brain barrier permeability (Rice et al., 2005). First, TX67 was assessed for promoting microtubule stabilization in

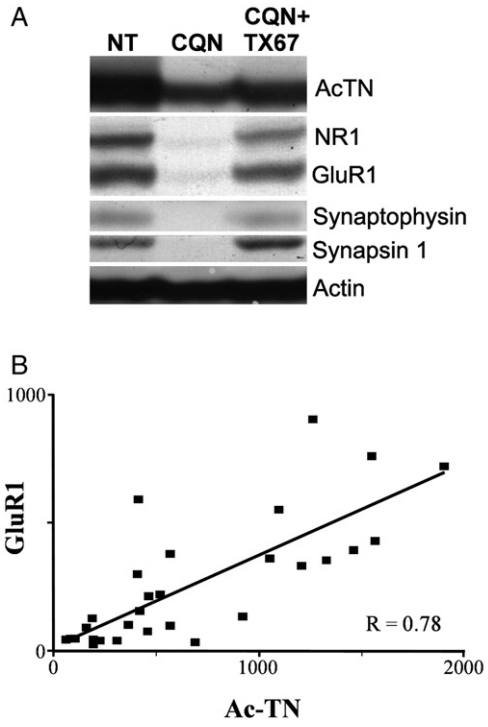

Fig. 6. Microtubule stabilization via TX67 protects against chloroquine-induced synaptic decline. Homogenate samples of 6–8 slices each were prepared from cultures that received no treatment (NT), chloroquine for 6 days (CQN), or chloroquine in the presence of TX67 for 6 days. A: On parallel immunoblots, the slice samples were immunostained for acetylated tubulin (Ac-TN), NR1, GluR1, synaptophysin, synapsin 1, and actin as a control. Preserved levels of acetylated tubulin correspond with preserved levels of the synaptic markers. B: Linear regression was conducted on the immunoreactivity measures of acetylated tubulin and GluR1 across individual samples from the different treatment groups. The obtained correlation coefficient was 0.78 ($P<0.0001$).

hippocampal slice cultures in the absence of any lysosomal stressor, using acetylated tubulin as an indicator of microtubule integrity. After 6 daily treatments with 300 nM TX67, the slice cultures exhibited 2- to 3-times more acetylated tubulin than control slices (Fig. 4A). This marked increase indicates enhancement of microtubule integrity, corresponding with the taxol-induced effects on microtubules. The 6-day treatment with TX67 alone had no effect on the sensitive synaptic marker GluR1 (Table 1) or on neuronal morphology in Nissl-stained slices (not shown). The lower concentration of 100 nM TX67 also produced microtubule-stabilizing effects, causing a 30–40% increase in acetylated tubulin as part of a dose-dependent response (Fig. 4B). In chloroquine-treated slices with compromised microtubule integrity, subsequent application of TX67 increased acetylated tubulin levels 4- to 5-fold, restoring them to near control levels after 2 days of exposure (Fig. 5).

Next, we tested whether TX67's ability to restore microtubule integrity is associated with protection of synaptic integrity in the slice model. The consequences of chloroquine-induced lysosomal dysfunction included marked reductions in synaptic proteins (Fig. 6A). Immunoblots stained for the NMDA receptor subunit NR1 and the AMPA receptor subunit GluR1 indicated reduced levels of the postsynaptic components by 70–80%. Presynaptic components synaptophysin and synapsin 1 also exhibited pronounced declines. When TX67 was administered to the slice cultures during the 6-day chloroquine treatment period, the microtubule-stabilizing agent preserved the levels of acetylated tubulin as well as of all synaptic markers tested (see third lane in Fig. 6A). The TX67-mediated protection provided maintenance of pre- and postsynaptic proteins to levels at or near those found in control slices.

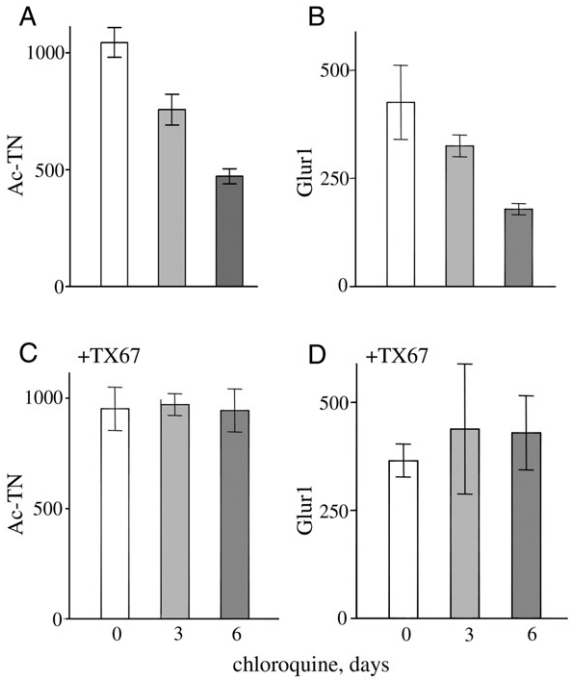

Fig. 7. TX67 prevents the gradual loss of acetylated tubulin and GluR1. Hippocampal slice cultures were treated with chloroquine for 0–6 days in the absence (A, B) or presence of TX67 (C, D). Slice samples were harvested into groups of 6 to 8 and assessed for acetylated tubulin and GluR1 by immunoblot. Immunoreactivity levels were determined by image analysis, and mean integrated optical densities ±S.E.M. are shown. Analyses of variance: A, $P<0.0001$; B, $P=0.001$; C and D, not significant.

The dual effect TX67 has on microtubule integrity and synaptic maintenance is also exemplified by the highly significant correlation between increased acetylated tubulin levels and preserved GluR1 measures ($P<0.0001$), assessed across individual slice samples from the different treatment groups (Fig. 6B). Across increasing days of chloroquine treatment, the steady reduction in acetylated tubulin (Fig. 7A) corresponded with a steady GluR1 decline in the same slice samples (Fig. 7B). This was not the case, however, when chloroquine was applied regularly to slice cultures in the presence of TX67. The microtubule stabilizer allowed acetylated tubulin levels to remain unchanged during the chloroquine treatment period (Fig. 7C), and this was associated with the maintenance of normal GluR1 levels (Fig. 7D). Together, the experiments indicate that microtubule destabilization is part of the gradual synaptic decline expressed in the slice model of protein accumulation.

4. Discussion

This report shows that in addition to the accumulation of amyloidogenic fragments and phosphorylated tau species (Bahr et al., 1994; Bendiske et al., 2002; Butler et al., 2005), the hippocampal slice model of lysosomal dysfunction exhibits loss of microtubule integrity at a level that leads to synaptic compromise. Corresponding with microtubule destabilization was reduced expression of presynaptic vesicle proteins and postsynaptic neurotransmitter receptor subunits. As expected, the slice model has also been shown to express functional compromise as indicated by decreases in the size of evoked EPSPs (Bahr et al., 1994; Bendiske et al., 2002). Note that declines in synaptic markers indicate deterioration of synapses in Alzheimer's disease (see Masliah et al., 1989; Honer et al., 1992). The synaptic decline in the slice model was ameliorated by a microtubule-stabilizing agent, attributing the loss of synapse maintenance to the disruption of vital microtubule functionality. A close correspondence was in fact evident between recovery of microtubule integrity and synaptic recovery. Together, these findings indicate that loss of microtubule integrity is responsible for synaptic decay early in the pathogenic cascade triggered by abnormal protein accumulation.

Lysosomal perturbation in the slice model produced a gradual loss of tubulin acetylation, a marker of stable microtubules found reduced in Alzheimer's disease (Hempen and Brion, 1996). In the Alzheimer's disease study, loss of acetylated tubulin was most consistent in neurons containing intracellular neurofibrillary deposits. Interestingly, the degree of microtubule compromise in the slice model has been previously shown to correspond with the extent of intracellular tau deposition (Bahr and Bendiske, 2002). Using acetylated tubulin as a good indicator of microtubule integrity, we also found that it closely relates to synaptic integrity *in vitro* and *in vivo*. The correlation between decreased levels of acetylated tubulin and synaptic markers, across chloroquine exposure times, is likely

related to the disruption of important microtubule-based transport processes since transport failure occurred with a similar temporal profile in our slice model (Bendiske et al., 2002). Related synaptic pathology is associated with early clinical stages of Alzheimer's disease, especially the loss of synaptic markers in the hippocampus (Heinonen et al., 1995; Callahan et al., 1999; Coleman et al., 2004).

Microtubule destabilization and transport failure are early signs of neuronal dysfunction in neurodegenerative diseases (see Hempen and Brion, 1996; Sasaki and Iwata, 1996; Trushina et al., 2003), and the two events have been linked to synaptic decline in the slice model. Promoting microtubule integrity with taxane analogue TX67 resulted in the expected restoration of acetylated tubulin levels, and also resulted in restored levels of pre- and postsynaptic proteins. Treating slice cultures with TX67 alone resulted in no change in synaptic or neuronal integrity over a 6-day period. TX67 is part of a family of taxol compounds known to promote microtubule chemistry including tubulin polymerization. Another pharmacological condition that re-established microtubule-based transport also led to restored levels of tubulin acetylation and synaptic markers (Bendiske and Bahr, 2003; Butler et al., 2005). Conversely, when microtubules are pharmacologically disrupted, the result is a loss of synaptic proteins and distinct synaptic vesicles (Tandon et al., 1996; Van Zundert et al., 2004; Charrier et al., 2006). Together, these studies indicate that breakdown of microtubule integrity and related transport systems is a major factor in synaptic pathology.

The use of the microtubule-stabilizing agent TX67 established that stable microtubules are a key aspect of synaptic maintenance. The succinate-modified taxol analogue has improved permeability, perhaps allowing efficient penetration in the three-dimensional organotypic slice model. This class of microtubule stabilizers has also been shown to protect against excitotoxic insults (Furukawa and Mattson, 1995), pathogenic events induced by Aβ peptides (Michaelis et al., 1998, 2005a; Li et al., 2003; Sponne et al., 2003), mutant huntingtin toxicity (Trushina et al., 2003), and against the action of a tau mutation associated with frontotemporal dementia (Furukawa et al., 2003). Microtubule-stabilizing agents may represent a strategy to support transport and cytoskeletal functions in order to offset protein accumulation pathology and the associated synaptic decline.

Microtubules require tau for stability and functionality. Irregular tau turnover in the slice model, in response to the lysosomal disturbance, alters the availability of normal tau necessary for stable microtubules and their transport capability as hyperphosphorylated tau species transform into paired helical filaments. The hippocampal slice model has been shown to exhibit gradual deposition of material immunopositive for paired helical filaments, corresponding with gradual transport failure (Bendiske et al., 2002). Hyperphosphorylated tau isoforms destabilize microtubules, and intracellular aggregates of paired helical filament-tau are linked to transport failure and synaptic compromise (Lee, 1995; Alonso et al., 1996, 1997; Bendiske et al., 2002; Bendiske and Bahr, 2003). Corresponding with the slice model data, indicators of lysosomal stress and decreased expression of synaptic mRNAs were evident in Alzheimer's disease hippocampal neurons containing neurofibrillary tangles, but not in neighboring tangle-free neurons (Callahan and Coleman, 1995; Callahan et al., 1999, 2002). Thus, similar cellular changes occur in the slice model and the age-related neurodegenerative disorder.

The current study supports the hypothesis that microtubule mechanisms are disrupted during episodes of lysosome stress or dysfunction, leading to axonopathy, loss of presynaptic integrity, and down-regulation of neurotransmitter receptors. Such a cascade of cellular compromise would have a dramatic effect on neuronal communication and plasticity. Presynaptic vesicle components may have increased vulnerability in the slice model and in Alzheimer's disease due to early axonopathy and disruption of axonal transport (Bendiske et al., 2002; Butler et al., 2005; Stokin et al., 2005). The types of pre- and postsynaptic proteins reduced in the slice model are known to play important roles in plasticity mechanisms underlying learning and memory (see Schmitt et al., 2004; Kushner et al., 2005). Of particular interest are the reduced levels of AMPA receptor subunit GluR1 and their correlation to microtubule destabilization in the slice model. Expression of GluR1 and other AMPA receptor subunits is also reduced in aging and Alzheimer's disease (Bahr et al., 1992; Ikonomovic et al., 1997; Wakabayashi et al., 1999). Such reductions could be detrimental for cognitive function since GluR1-3 concentrations in hippocampal synaptic compartments evidently need to be up-regulated for plasticity and learning (Bevilaqua et al., 2005; Whitlock et al., 2006). Microtubule function and overall cytoskeletal integrity are important for neurotransmitter receptor trafficking, synaptic vesicle organization, and structural domains essential for synaptic modification and modulated signaling. Protein accumulation stress in the slice model indicates that subsequent microtubule destabilization can have a negative impact on synapses in several ways, thus adding to the numerous studies showing that synapses rely heavily on microtubule-based functions. The present study points to the loss of microtubule integrity as facilitating the link between protein deposition pathology and the disruption of vital synaptic machinery for cognition.

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References

Ahlijanian, M.K., Barrezueta, N.X., Williams, R.D., Jakowski, A., Kowsz, K.P., McCarthy, S., Coskran, T., Carlo, A., Seymour, P.A., Burkhardt, J.E., Nelson, R.B., McNeish, J.D., 2000. Hyperphosphorylated tau and neurofilament and cytoskeletal disruptions in mice overexpressing human p25, an activator of cdk5. Proc. Natl. Acad. Sci. U. S. A. 97, 2910–2915.

Alonso, A.C., Grundke-Iqbal, I., Iqbal, K., 1996. Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. Nat. Med. 2, 783–787.

Alonso, A.D., Grundke-Iqbal, I., Barra, H.S., Iqbal, K., 1997. Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. Proc. Natl. Acad. Sci. U. S. A. 94, 298–303.

Bahr, B.A., 1995. Long-term hippocampal slices: a model system for investigating synaptic mechanisms and pathologic processes. J. Neurosci. Res. 42, 294–305.

Bahr, B.A., 2003. Dysfunction and activation of the lysosomal system: implications for and against Alzheimer's disease. In: Welsh, E.M. (Ed.), Focus on Alzheimer's Disease Research. Nova Science Publishers, Hauppauge, NY, pp. 115–150.

Bahr, B.A., Bendiske, J., 2002. The neuropathogenic contribution of lysosomal dysfunction. J. Neurochem. 83, 481–489.

Bahr, B.A., Vicente, J.S., 1998. Age-related phosphorylation and fragmentation events influence the distribution profiles of distinct tau isoforms in mouse brain. J. Neuropathol. Exp. Neurol. 57, 111–121.

Bahr, B.A., Godshall, A.C., Hall, R.A., Lynch, G., 1992. Mouse telencephalon exhibits an age-related decrease in glutamate (AMPA) receptors but no change in nerve terminal markers. Brain Res. 589, 320–326.

Bahr, B.A., Godshall, A.C., Murray, B.A., Lynch, G., 1993. Age-related changes in neural cell adhesion molecule (NCAM) isoforms in the mouse telencephalon. Brain Res. 628, 286–292.

Bahr, B.A., Abai, B., Gall, C.M., Vanderklish, P.W., Hoffman, K.B., Lynch, G., 1994. Induction of β-amyloid-containing polypeptides in hippocampus: evidence for a concomitant loss of synaptic proteins and interactions with an excitotoxin. Exp. Neurol. 129, 81–94.

Bahr, B.A., Hoffman, K.B., Kessler, M., Hennegriff, M., Park, G.Y., Yamamoto, R.S., Kawasaki, B.T., Vanderklish, P.W., Hall, R.A., Lynch, G., 1996. Distinct distributions of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor subunits and a related 53,000 M_R antigen (*GR53*) in brain tissue. Neuroscience 74, 707–721.

Bahr, B.A., Hoffman, K.B., Yang, A.J., Hess, U.S., Glabe, C.G., Lynch, G., 1998. Amyloid β protein is internalized selectively by hippocampal field CA1 and causes neurons to accumulate amyloidogenic carboxyterminal fragments of the amyloid precursor protein. J. Comp. Neurol. 397, 139–147.

Bendiske, J., Bahr, B.A., 2003. Lysosomal activation is a compensatory response against protein accumulation and associated synaptopathogenesis — an approach for slowing Alzheimer's disease? J. Neuropathol. Exp. Neurol. 62, 451–463.

Bendiske, J., Caba, E., Brown, Q.B., Bahr, B.A., 2002. Intracellular deposition, microtubule destabilization, and transport failure: an 'early' pathogenic cascade leading to synaptic decline. J. Neuropathol. Exp. Neurol. 61, 640–650.

Bevilaqua, L.R., Medina, J.H., Izquierdo, I., Cammarota, M., 2005. Memory consolidation induces *N*-methyl-D-aspartic acid-receptor and Ca^{2+}/calmodulin-dependent protein kinase II-dependent modifications in α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor properties. Neuroscience 136, 397–403.

Brown, D.F., Risser, R.C., Bigio, E.H., Tripp, P., Stiegler, A., Welch, E., Eagan, K.P., Hladik, C.L., White, C.L.I., 1998. Neocortical synapse density and Braak stage in the Lewy body variant of Alzheimer disease: a comparison with classic Alzheimer disease and normal aging. J. Neuropathol. Exp. Neurol. 57, 955–960.

Butler, D., Brown, Q.B., Chin, D.J., Batey, L., Karim, S., Mutneja, M.S., Karanian, D.A., Bahr, B.A., 2005. Cellular responses to protein accumulation involve autophagy and lysosomal enzyme activation. Rejuvenation Res. 8, 225–235.

Butler, D., Nixon, R.A., Bahr, B.A., 2006. Potential compensatory responses through autophagic/lysosomal pathways in neurodegenerative diseases. Autophagy 2, 234–237.

Callahan, L.M., Coleman, P.D., 1995. Neurons bearing neurofibrillary tangles are responsible for selected synaptic deficits in Alzheimer's disease. Neurobiol. Aging 16, 311–314.

Callahan, L.M., Vaules, W.A., Coleman, P.D., 1999. Quantitative decrease in synaptophysin message expression and increase in cathepsin D message expression in Alzheimer disease neurons containing neurofibrillary tangles. J. Neuropathol. Exp. Neurol. 58, 275–287.

Callahan, L.M., Vaules, W.A., Coleman, P.D., 2002. Progressive reduction of synaptophysic message in single neurons in Alzheimer disease. J. Neuropathol. Exp. Neurol. 61, 384–395.

Charrier, C., Ehrensperger, M.V., Dahan, M., Levi, S., Triller, A., 2006. Cytoskeleton regulation of glycine receptor number at synapses and diffusion in the plasma membrane. J. Neurosci. 26, 8502–8511.

Coleman, P., Federoff, H., Kurlan, R., 2004. A focus on the synapse for neuroprotection in Alzheimer disease and other dementias. Neurology 63, 1155–1162.

Davies, C.A., Mann, D.M., Sumpter, P.Q., Yates, P.O., 1987. A quantitative morphometric analysis of the neuronal and synaptic contents of the frontal and temporal cortex in patients with Alzheimer's disease. J. Neurol. Sci. 78, 151–164.

Divinski, I., Holtser-Cochav, M., Vulih-Schultzman, I., Steingart, R.A., Gozes, I., 2006. Peptide neuroprotection through specific interaction with brain tubulin. J. Neurochem. 98, 973–984.

Furukawa, K., Mattson, M.P., 1995. Taxol stabilizes [Ca^{2+}] and protects hippocampal neurons against excitotoxicity. Brain Res. 689, 141–146.

Furukawa, K., Wang, Y., Yao, P.J., Fu, W., Mattson, M.P., Itoyama, Y., Onodera, H., D'Souza, I., Poorkaj, P.H., Bird, T.D., Schellenberg, G.D., 2003. Alteration in calcium channel properties is responsible for the neurotoxic action of a familial frontotemporal dementia tau mutation. J. Neurochem. 87, 427–436.

Hall, G.F., Chu, B., Lee, G., Yao, J., 2000. Human tau filaments induce microtubule and synapse loss in an *in vivo* model of neurofibrillary degenerative disease. J. Cell Sci. 113, 1373–1387.

Heinonen, O., Soininen, H., Sorvari, H., Kosunen, O., Paljarvi, L., Koivisto, E., Riekkinen Sr., P.J., 1995. Loss of synaptophysin-like immunoreactivity in the hippocampal formation is an early phenomenon in Alzheimer's disease. Neuroscience 54, 375–384.

Hempen, B., Brion, J.P., 1996. Reduction of acetylated α-tubulin immunoreactivity in neurofibrillary tangle-bearing neurons in Alzheimer's disease. J. Neuropathol. Exp. Neurol. 55, 964–972.

Honer, W.G., Dickson, D.W., Gleeson, J., Davies, P., 1992. Regional synaptic pathology in Alzheimer's disease. Neurobiol. Aging 13, 375–382.

Ikonomovic, M.D., Mizukami, K., Davies, P., Hamilton, R., Sheffield, R., Armstrong, D.M., 1997. The loss of GluR2(3) immunoreactivity precedes neurofibrillary tangle formation in the enterohinal cortex and hippocampus of Alzheimer brains. J. Neuropathol. Exp. Neurol. 56, 1018–1027.

Ivy, G.O., Schottler, F., Wenzel, J., Baudry, M., Lynch, G., 1984. Inhibitors of lysosomal enzymes: accumulation of lipofuscin-like dense bodies in the brain. Science 226, 985–987.

Kushner, S.A., Elgersma, Y., Murphy, G.G., Jaarsma, D., van Woerden, G.M., Hojjati, M.R., Cui, Y., LeBoutillier, J.C., Marrone, D.F., Choi, E.S., De Zeeuw, C.I., Petit, T.L., Pozzo-Miller, L., Silva, A.J., 2005. Modulation of presynaptic plasticity and learning by the H-ras/extracellular signal-regulated kinase/synapsin I signaling pathway. J. Neurosci. 25, 9721–9734.

Lee, V.M., 1995. Disruption of the cytoskeleton in Alzheimer's disease. Curr. Opin. Neurobiol. 5, 663–668.

Li, G., Faibushevich, A., Turunen, B.J., Yoon, S.O., Georg, G., Michaelis, M.L., Dobrowsky, R.T., 2003. Stabilization of the cyclin-dependent kinase 5 activator, p35, by paclitaxel decreases beta-amyloid toxicity in cortical neurons. J. Neurochem. 84, 347–362.

Lipton, A.M., Cullum, C.M., Satumtira, S., Sontag, E., Hynan, L.S., White III, C.L., Bigio, E.H., 2001. Contribution of asymmetric synapse loss to lateralizing clinical deficits in frontotemporal dementias. Arch. Neurol. 58, 1233–1239.

Mandelkow, E.M., Stamer, K., Vogel, R., Thies, E., Mandelkow, E., 2003. Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. Neurobiol. Aging 24, 1079–1085.

Masliah, E., Terry, R.D., DeTeresa, R.M., Hansen, L.A., 1989. Immunohistochemical quantification of the synapse-related protein synaptophysin in Alzheimer disease. Neurosci. Lett. 103, 234–239.

Masliah, E., Mallory, M., Hansen, L., DeTeresa, R., Terry, R.D., 1993. Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43, 192–197.

Masliah, E., Mallory, M., Alford, M., DeTersesa, T., Hansen, L.A., McKeel Jr., D.W., Morris, J.C., 2001. Altered expression of synaptic proteins

occurs early during progression of Alzheimer's disease. Neurology 56, 127–129.

Michaelis, M.L., Ranciat, N., Chen, Y., Bechtel, M., Ragan, R., Hepperle, M., Liu, Y., Georg, G., 1998. Protection against β-amyloid toxicity in primary neurons by paclitaxel [Taxol]. J. Neurochem. 70, 1623–1627.

Michaelis, M.L., Ansar, S., Chen, Y., Reiff, E.R., Seyb, K.I., Himes, R.H., Audus, K.L., Georg, G.I., 2005a. β-amyloid-induced neurodegeneration and protection by structurally diverse microtubule-stabilizing agents. J. Pharmacol. Exp. Ther. 312, 659–668.

Michaelis, M.L., Seyb, K.I., Ansar, S., 2005b. Cytoskeletal integrity as a drug target. Curr. Alzheimer Res. 2, 227–229.

Parness, J., Horwitz, S.B., 1981. Taxol binds to polymerized tubulin in vitro. J. Cell Biol. 91, 479–487.

Rice, A., Yanbin, L., Michaelis, M.L., Himes, R.H., Georg, G.I., Audus, K.L., 2005. Chemical modification of paclitaxel (taxol) reduces P-glycoprotein interactions and increases permeation across the blood–brain barrier in vitro and in situ. J. Med. Chem. 48, 832–838.

Samuel, W., Masliah, E., Hill, L.R., Butters, N., Terry, R., 1994. Hippocampal connectivity and Alzheimer's dementia: effects of synapse loss and tangle frequency in a two-component model. Neurology 44, 2081–2088.

Sasaki, S., Iwata, M., 1996. Impairment of fast axonal transport in the proximal axons of anterior horn neurons in amyotrophic lateral sclerosis. Neurology 47, 535–540.

Schiff, P.B., Fant, J., Horwitz, S.B., 1979. Promotion of microtubule assembly *in vitro* by Taxol. Nature 277, 665–667.

Schmitt, W.B., Arianpour, R., Deacon, R.M., Seeburg, P.H., Sprengel, R., Rawlins, J.N., Bannerman, D.M., 2004. The role of hippocampal glutamate receptor-A-dependent synaptic plasticity in conditional learning: the importance of spatiotemporal discontiguity. J. Neurosci. 33, 7277–7282.

Sponne, I., Fifre, A., Drouet, B., Klein, C., Koziel, V., Pincon-Raymond, M., Olivier, J.L., Chambaz, J., Pillot, T., 2003. Apoptotic neuronal cell death induced by the non-fibrillar amyloid-beta peptide proceeds through an early reactive oxygen species-dependent cytoskeleton perturbation. J. Biol. Chem. 278, 3437–3445.

Stokin, G.B., Lillo, C., Falzone, T.L., Brusch, R.G., Rockenstein, E., Mount, S.L., Raman, R., Davies, P., Masliah, E., Williams, D.S., Goldstein, L.S., 2005. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science 307, 1282–1288.

Sze, C.I., Troncoso, J.C., Kawas, C., Mouton, P., Price, D.L., Martin, L.J., 1997. Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. J. Neuropathol. Exp. Neurol. 56, 933–944.

Tamaru, M., Yoneda, Y., Ogita, K., Shimizu, J., Nagata, Y., 1991. Age-related decreases of the *N*-methyl-D-aspartate receptor complex in the rat cerebral cortex and hippocampus. Brain Res. 542, 83–90.

Tandon, A., Bachoo, M., Weldon, P., Polosa, C., Collier, B., 1996. Effects of colchicine application to preganglionic axons on choline acetyltransferase activity and acetylcholine content and release in the superior cervical ganglion. J. Neurochem. 66, 1033–1041.

Terry, R.D., Masliah, E., Salmon, D.P., Butters, N., DeTeresa, R., Hill, R., Hansen, L.A., Katzman, R., 1991. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann. Neurol. 30, 572–580.

Trushina, E., Heldebrant, M.P., Perez-Terzic, C.M., Bortolon, R., Kovtun, I.V., Badger II, J.D., Terzic, A., Estevez, A., Windebank, A.J., Dyer, R.B., Yao, J., McMurray, C.T., 2003. Microtubule destabilization and nuclear entry are sequential steps leading to toxicity in Huntington's disease. Proc. Natl. Acad. Sci. U. S. A. 100, 12171–12176.

Van Zundert, B., Alvarez, F.J., Tapia, J.C., Yeh, H.H., Diaz, E., Aguayo, L.G., 2004. Developmental-dependent action of microtubule depolymerization on the function and structure of synaptic glycine receptor clusters in spinal neurons. J. Neurophysiol. 91, 1036–1049.

Wakabayashi, K., Narisawa-Saito, M., Iwakura, Y., Arai, T., Ikeda, K., Takahashi, H., Nawa, H., 1999. Phenotypic down-regulation of glutamate receptor subunit GluR1 in Alzheimer's disease. Neurobiol. Aging 20, 287–295.

Whitlock, J.R., Heynen, A.J., Shuler, M.G., Bear, M.F., 2006. Learning induces long-term potentiation in the hippocampus. Science 313, 1093–1097.

Zhang, B., Maiti, A., Shively, S., Lakhani, F., McDonald-Jones, G., Bruce, J., Lee, E.B., Xie, S.X., Joyce, S., Li, C., Toleikis, P.M., Lee, V.M., Trojanowski, J.Q., 2005. Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. Proc. Natl. Acad. Sci. U. S. A. 102, 227–231.