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Proteolytic Stress Causes Heat Shock Protein Induction, Tau Ubiquitination, and the Recruitment of Ubiquitin to Tau-Positive Aggregates in Oligodendrocytes in Culture

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Molecular chaperones and the ubiquitin–proteasome system are participants in the defense against unfolded proteins and provide an effective protein quality control system that is essential for cellular functions and survival. Ubiquitinated tau-positive inclusion bodies containing the small heat shock protein αB-crystallin in oligodendrocytes are consistent features of a variety of neurodegenerative diseases, and defects in the proteasome system might contribute to the aggregation process. Oligodendrocytes, the myelin-forming cells of the CNS, are specifically sensitive to stress situations. Here we can show that in cultured rat brain oligodendrocytes proteasomal inhibition by MG-132 or lactacystin caused apoptotic cell death and the induction of heat shock proteins in a time- and concentration-dependent manner. Specifically, αB-crystallin was upregulated, and ubiquitinated proteins accumulated. After incubation with MG-132 the tau was dephosphorylated, which enhanced its microtubule-binding capacity. Proteasomal inhibition led to ubiquitination of tau and its association with αB-crystallin and to the occurrence of thioflavine S-positive aggregates in the oligodendroglial cytoplasm. These aggregates were positive for tau and also contained ubiquitin and αB-crystallin; hence they resembled the glial cytoplasmic inclusions observed in white matter disease and frontotemporal dementias with parkinsonism linked to chromosome 17 (FTDP-17). In summary, the data underscore the specific sensitivity of oligodendrocytes to stress situations and point to a causal relationship of proteasomal impairment and inclusion body formation.

Key words: **αB-crystallin; tau; ubiquitin; cytoskeleton; glial inclusions; proteasome; apoptosis**

Introduction

The abnormal accumulation and aggregation of cellular proteins are a characteristic feature of a variety of neurodegenerative diseases (Goedert et al., 1998). In Alzheimer's disease (AD) intracellular accumulations of the microtubule-associated (MT) protein tau are formed preferentially in nerve cells, while abundant neuronal and glial filamentous tau inclusions are prominent in familial multiple system tauopathy (MSTD), which belongs to a group of frontotemporal dementias with parkinsonism linked to chromosome 17 (FTDP-17) (Goedert et al., 1998; Lee et al., 2001). Tau-positive oligodendroglial inclusions are consistent features in the brains of patients with FTDP-17, progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD) (Chin and Goldman, 1996; Goedert et al., 1998; Komori, 1999; Berry et al., 2001). Glial fibrillary tangles (GFTs) or "coiled bodies" are observed specifically in Pick's disease, PSP, and CBD, and glial cytoplasmic inclusions (GCIs) in oligodendroglia are the histological hallmarks of multiple system atrophy (MSA). GCIs stain consistently and intensely with antibodies against ubiquitin, the small heat shock protein αB-crystallin, and α-synuclein and less intensely with antibodies against α- and β-tubulin; variable reports indicate that they are tau-positive (Murayama et al., 1992; Chin and Goldman, 1996; Lantos, 1998; Tu et al., 1998; Komori, 1999). They appear as loosely packed filaments.

We have shown previously that oligodendrocytes contain all six isoforms of tau (Gorath et al., 2001) and that stress-induced activation of protein phosphatase 2A (PP2A) and tau dephosphorylation constitute a major feature of the response to injury in these cells, which eventually undergo apoptotic cell death (Goldbaum and Richter-Landsberg, 2002). Tau under normal conditions is a highly soluble protein, and the molecular mechanisms leading to the formation of insoluble aggregates are not yet resolved. The accumulation of abnormally folded proteins, as a result of a variety of stress situations, triggers stress responses that lead to the induction of heat shock proteins (HSPs) (for review, see Richter-Landsberg and Goldbaum, 2003). HSPs act as molecular chaperones that facilitate protein repair and renaturation (Sherman and Goldberg, 2001). HSPs help to target nonrepairable proteins to the ubiquitin proteasomal pathway, i.e., misfolded proteins are ubiquitinated and degraded by the proteasomal machinery (Schwartz and Ciechanover, 1999). When the protein degradation machinery is exceeded or impaired, inclusion bodies or aggregates are formed (Alves-Rodrigues et al., 1998; Kopito, 2000). Whether the intracellular deposition of aggregated and often ubiquitinated proteins protects the cells from

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further damage or is toxic and the cause of severe cellular dysfunction remains to be clarified (for review, see Layfield et al., 2001; Taylor et al., 2002).

A loss of proteasomal function occurs in aging animals and might contribute to neurodegenerative disease (Keller et al., 2002). HSPs can be induced by direct impairment of the proteasomal degradation pathway by proteasome inhibitors (Lee and Goldberg, 1998; Ito et al., 2002; Goldbaum et al., 2003). The present study was undertaken to elucidate whether proteasomal inhibition in cultured oligodendrocytes causes the accumulation of HSPs and ubiquitin and the formation of cytoplasmic inclusions, as observed in neurodegenerative diseases with oligodendroglial pathology.

Materials and Methods

Materials and antibodies. Cell culture media were obtained from Invitrogen (Grand Island, NY). Okadaic acid, carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (MG-132), and lactacystin were purchased from Calbiochem (Bad Soden, Germany). Taxol and GTP were obtained from Sigma (St. Louis, MO).

For Western blot analysis the following tau antibodies were used as described previously (Vogelsberg-Ragaglia et al., 2000), and the working dilutions are given in parentheses: tau 17026 (1:1000), a phosphorylation-independent rabbit polyclonal antibody made against the largest human recombinant tau; monoclonal antibody (mAb) tau-1 (1:1000), specific for nonphosphorylated epitope located in amino acid residues 189–209; mAb paired helical filament-1 (PHF-1; 1:500), specific for phosphorylated serine 396/404; mAb 12E8 (1:250), specific for phosphorylated serine 262. The anti-α-tubulin mAb (1:1000) was obtained from Sigma (Taufkirchen, Germany). HRP-conjugated anti-mouse IgG was from Amersham Biosciences (Freiburg, Germany) and anti-rabbit IgG from Bio-Rad (Munich, Germany). The mAb anti-ubiquitin (SPA-203, 1:1000), mAb anti-αB-crystallin (SPA-222, 1:500), polyclonal anti-HSP25 (SPA-801, 1:1000), and mAb anti-HSP70 (SPA-810, 1:1000) were from StressGen (Victoria, BC, Canada). Polyclonal anti-poly(ADP-ribose) polymerase (anti-PARP; H-250, 1:500) was from Santa Cruz (Santa Cruz, CA).

Cell culture. Cells were kept at 37°C and 10% CO_2. Primary cultures of glial cells were prepared from the brains of 1- to 2-d-old Wistar rats, and oligodendrocytes were prepared from the flasks after 6–8 d as described previously (Goldbaum and Richter-Landsberg, 2002). Precursor cells were replated on poly-L-lysine-coated culture dishes (2.7×10^6 cells/10 cm dish) and kept for 5–7 d in serum-free DMEM to which insulin (5 μg/ml), transferrin (5 μg/ml), and sodium selenite (5 ng/ml) (Roche Diagnostics, Mannheim, Germany) were added. These cultures contain a highly enriched population of differentiated oligodendrocytes with a mature morphology.

Heat shock treatment. Culture dishes were sealed with Parafilm and immersed for 30 min in a water bath at 44°C, as described (Goldbaum and Richter-Landsberg, 2001). Thereafter, the cells were put into the incubator for 18 hr of recovery. Control cells were sealed for 30 min but remained in the incubator.

Oxidative stress. Oligodendrocytes were treated with hydrogen peroxide (50 μM) for 30 min, the medium was replaced, and the cells were put into the incubator for 18 hr of recovery.

Immunoblot analysis. Cellular monolayers of control and treated cells were washed with PBS once, scraped off in sample buffer (125 mM Tris, pH 6.7, 1 mM EDTA, 1% β-mercaptoethanol, 10% glycerol) containing 2% SDS, and boiled for 10 min. Protein contents in the samples were determined according to Neuhoff et al. (1979). For immunoblotting, total cellular extracts (5–30 μg of protein/lane) were separated by one-dimensional SDS-PAGE, using 7.5 or 12.5% polyacrylamide gels, and transferred to nitrocellulose membranes (0.45 μm; Schleicher & Schuell, Dassel, Germany). The blots were saturated with TBS-T (20 mM Tris, pH 7.5, 136.8 mM NaCl, 0.1% v/v Tween 20) containing 5% dry milk and incubated with the individual antibodies overnight at 4°C. After washing, incubation with HRP-conjugated anti-mouse (1:2000; Amersham Biosciences, Hercules, CA) or anti-rabbit IgG (1:5000; Bio-Rad, Munich, Germany) was performed for 1 hr, and the blots were visualized by the enhanced chemiluminescence procedure as described by the manufacturer (Amersham Biosciences, Braunschweig, Germany). All experiments were performed at least three times with similar results.

Sequential extraction. Sequential extraction of cellular proteins was performed by using buffers with increasing abilities to solubilize proteins: (1) high-salt buffer [HSB; containing (in mM): 10 Tris, 140 NaCl, 5 EDTA, 1 DTT, 1 PMSF plus 1.5 M KCl, pH 7.5]; (2) RIPA buffer [containing (in mM): 50 Tris, 150 NaCl, 2 EDTA plus 0.5% deoxycholate, 0.1% SDS, 1% Triton X-100, pH 7.5]; (3) SDS-sample buffer (SB). Buffers were supplemented with a mixture of protease inhibitors (Complete, Roche Diagnostics) and 1 mM phenylmethylsulfonyl fluoride (PMSF).

Cells were scraped off in PBS and centrifuged (15 min; 13,000 rpm at 4°C); the resulting pellet was resuspended in HSB (200 μl/2.7×10^6 cells), sonicated, and centrifuged (30 min; 22,000 rpm at 4°C). The HSB-insoluble pellet was resuspended in 100 μl of RIPA, sonicated, and centrifuged (30 min; 22,000 rpm at 4°C). The resulting fractions were supplemented with SB and the pellet taken up in SB for immunoblot procedure as described above. Equal amounts of control and MG-132-treated samples were loaded.

Immunoprecipitation. Oligodendrocytes (6×10^6 cells) were washed twice with PBS and scraped off. After centrifugation at 4000 rpm for 5 min, the cells were lysed (30 min at 4°C) in RIPA buffer supplemented with a mixture of protease inhibitors (Roche Diagnostics) and 1 mM PMSF. After centrifugation (13,000 rpm for 30 min) the protein concentrations of the resulting supernatants were determined. Equal amounts of proteins in the same volume were used for further immunoprecipitation. Cell lysates were centrifuged, and the remaining supernatants were incubated with polyclonal antibody tau 17026 (1:100; 18 hr at 4°C). Thereafter, protein A-Sepharose beads were added (5 mg/100 μl of cell lysate), and the mixture was incubated for an additional 2 hr at 4°C. After centrifugation the supernatant (SN) was removed for further analysis, and the beads were washed four times for 10 min with lysis buffer, centrifuged, and boiled in sample buffer for 5 min, yielding the immunoprecipitate (IP). The SN and IP samples were separated by SDS-PAGE and subjected to immunoblot procedure as described above.

Microtubule binding assay of tau and HSPs. To determine whether MG-132 alters the interaction of tau or HSPs with tubulin in the MTs of intact cells, we performed a MT binding assay as described (Goldbaum et al., 2003). Briefly, the cells were harvested in high-salt RAB buffer [containing (in mM): 0.5 $MgSO_4$, 1 EGTA, 2 dithiothreitol plus 0.1 M Tris and 0.75 M NaCl, pH 6.8] supplemented with 0.1% Triton X-100, 20 μM Taxol, 2 mM GTP, and a mixture of protease inhibitors (2 mM PMSF, TPCK, TLCK, leupeptin, pepstatin, and soy bean trypsin inhibitor, each at 1 μg/ml) at 37°C. Cell lysates were homogenized with 15 strokes in a warm Dounce homogenizer and then immediately centrifuged for 20 min at 50,000 × *g* at 25°C. The supernatant containing unbound tau and unbound HSPs was removed and the protein concentration determined. The remaining pellet was resuspended in a 2× volume of sample buffer corresponding to the total volume of supernatant after normalizing to total protein. The samples were subjected to immunoblot analysis as above. The ratio of tau and HSPs bound to MTs (P, pellet) versus soluble or unbound tau (S, soluble) was assessed by comparing the immunoreactivities in these two fractions.

Immunofluorescence. Cells were cultured on poly-L-lysine-coated glass coverslips (3.5×10^5 cells/35 mm dish) for 5 d in DMEM/supplement and then subjected to MG-132 as indicated. After being washed with PBS, the cells were fixed with methanol. The coverslips were washed three times and incubated for 1 hr with rabbit polyclonal anti-tau 17026 antibodies (1:1000), polyclonal anti-α-tubulin (1:100), mAb anti-ubiquitin (FK-2, 1:200; Affiniti, Exeter, UK), mAb HSP70 (1:100), or mAb αB-crystallin (SPA-222, 1:100). After being washed with PBS, the cells were incubated for 1 hr with TRITC-conjugated (1:100) and FITC-conjugated (1:100) secondary antibodies (Jackson ImmunoResearch, West Grove, PA), washed with PBS, and mounted. Nuclei were stained by 4′,6-diamidino-2-phenylindole (DAPI; 1.5 μg/ml) included in the mounting medium (Vectashield, Vector Laboratories, Burlingame, CA). Fluorescent labeling was studied with a Zeiss epifluorescence microscope

(Oberkochen, Germany) equipped with a digital camera and a Plan-Neofluar objective (100×).

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining. For terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) staining the cells were cultured as described for indirect immunofluorescence. After incubation with MG-132 the cells were fixed with 4% paraformaldehyde in PBS and permeabilized with 0.1% Triton X-100. Labeling was performed with an *in situ* cell death detection kit as described by the manufacturer (Roche Diagnostics). Briefly, enzyme solution was mixed with label solution (1:10), and the cells were incubated with the resulting TUNEL reaction solution for 60 min at 37°C in a humidified atmosphere in the dark. The coverslips were washed three times and incubated for 1 hr with mouse monoclonal anti-αB-crystallin antibodies (SPA-222, 1:100) and mAb anti-HSP70 (SPA-810, 1:100). After being washed with PBS, the cells were incubated for 1 hr with TRITC-conjugated (1:100) secondary antibodies, washed with PBS, mounted, and studied as described for indirect immunofluorescence.

Thioflavine S staining. Cells were fixed and subjected to indirect immunofluorescence staining as described above. Before mounting, the coverslips were immersed in thioflavine S solution (0.005%) for 5 min. Thereafter, the cells were washed three times in ethanol (70%), once in water, and then mounted.

MTT viability assay. To assess the cytotoxic potential of the compounds, we performed the MTT (tetrazolium) assay as described previously (Richter-Landsberg and Vollgraf, 1998). Briefly, oligodendrocyte precursor cells were prepared as described above, plated on poly-L-lysine-coated 96-microwell cell culture plates (2×10^4 cells/well), and grown for 7 d. Then the growth medium was removed, and fresh medium (100 μl/well) was added; the cells were stressed and incubated for the indicated times. Next 10 μl of MTT solution (5 mg/ml in PBS) was added to the wells, containing 100 μl of medium, and the plates were incubated for 4 hr. Thereafter, 100 μl of a solubilization solution (10% SDS in 0.01 M HCl) was added and incubated overnight to dissolve the water-insoluble formazan salt. Quantification was performed with an ELISA reader at 595 nm, using a 655 nm filter as a reference. Data are expressed as a percentage of the untreated controls, and values represent the means ± SD of eight microwells each of three independent experiments ($n = 24$).

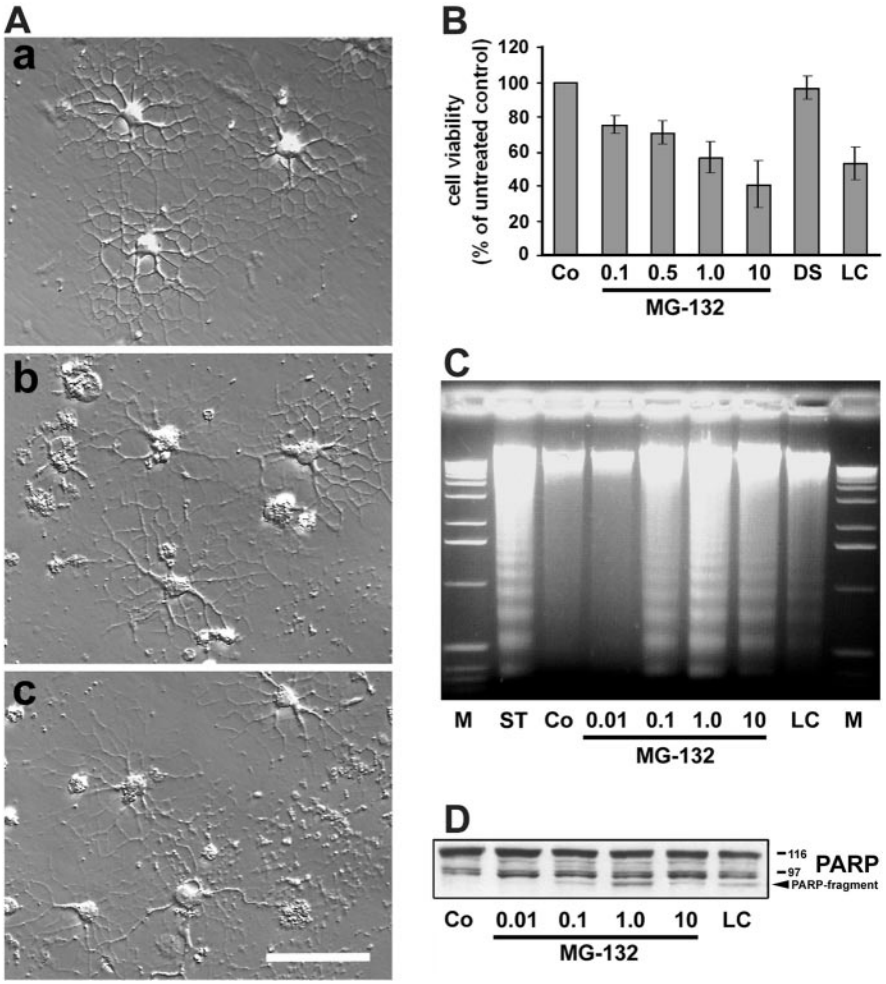

Figure 1. Proteasomal inhibition causes apoptotic cell death in oligodendrocytes. *A*, Effect of MG-132 and lactacystin on cell morphology. Oligodendroctyes (7 d *in vitro*, 7 DIV) were untreated (*a*) or incubated with 10 μM MG-132 (*b*) or 10 μM lactacystin (*c*) for 18 hr. Hoffmann modulation contrast images are shown. Scale bar, 50 μm. *B*, MTT survival assay of oligodendrocytes (7 DIV) exposed to proteasomal inhibitors. Cells were treated with MG-132 (1–10 μM), lactacystin (LC; 10 μM), or DMSO (DS; 0.1%), which was used to solubilize both inhibitors. An MTT assay was performed after 18 hr of incubation. Co, Untreated control. Values represent the means ± SD of eight microwells each of three independent experiments ($n = 24$). *C*, DNA fragmentation after proteasomal stress in oligodendrocytes. Oligodendrocytes (7 DIV) were treated with MG-132 (0.01–10 μM, 18 hr) or lactacystin (LC; 10 μM, 18 hr). DNA was isolated and separated on 1.5% agarose gels, stained with ethidium bromide, and visualized under UV light. M, 1 kb DNA ladder; ST, cells incubated in the presence of staurosporine (200 nM) for 18 hr; Co, untreated control. The experiment was repeated several times with nearly identical results. *D*, Activation of caspase 3 as demonstrated by PARP cleavage. Cells were treated with MG-132 (0.01–10 μM, 18 hr) or lactacystin (LC; 10 μM, 18 hr). Cell lysates were prepared and subjected to immunoblot analysis by using antibodies against PARP. Arrow on the right indicates the 85 kDa PARP cleavage product. Co, Untreated control.

Assay for DNA fragmentation. Total genomic DNA was isolated from cell pellets of oligodendrocytes (2.7×10^6 cells for each experimental condition). DNA samples (5 μg) and a 1 kb DNA ladder standard (Invitrogen, Karlsruhe, Germany) were separated on 1.5% agarose gels, visualized by ethidium bromide staining, and photographed.

RNA extraction and reverse transcription. RNA from oligodendrocytes (2.7×10^6 cells) was isolated with RNeasy (Qiagen, Hilden, Germany) as described by the manufacturer for animal cells. RNA was dissolved in diethylpyrocarbonate-treated (DEPC) water and quantified by spectrophotometry. RNA (1 μg) was used for reverse transcription in a final volume of 20 μl. First-strand synthesis was performed with 2 mM of each dNTP, 12.5 pmol each of oligo-dT_{15} and random hexamer primer, 4 μl of 5× reaction buffer, 1 μl of dimethylsulfoxide (DMSO), and 200 U of M-MLV reverse transcriptase (Promega, Madison, WI). After a denaturation step and incubation at 37°C for 1 hr, the reaction mixture was diluted to 80 μl with DEPC-treated water and stored at −80°C. Subsequently, 1–2 μl was used for PCR analysis.

PCR and primers. Primers were synthesized by Pharmacia (Freiburg, Germany) and were designed by using the PrimerSelect software (DNASTAR, Madison, WI). For the analysis of HSPs the following oligonucleotides were used: αB-crystallin, 5′-TGCAGTGACAGCAGGCTTCT-3′ and 5′-GAGAGCACCTGTTGGAGTCT-3′ (Van Stipdonk et al., 2000); HSP32/HO-1, 5′-AAGGAGGTGCACATCCGTGCA-3′ and 5′-ATGTTGAGCAGGAAGGCGGTC-3′ (Kutty et al., 1995); HSP70, 5′-AGCTGCTGCAGGACTTCTTC-3′ (nt 1220–1239) and 5′-GCTGATCTTGCCCTTGAGAC-3′ (nt 1847–1866); HSP25, 5′-GTTAAGACCAAGGAAGGCGTGG-3′ and 5′-CTACTTGGCTCCAGACTGTTCC-3′ (Suzuki et al., 2001).

For the analysis of tau the following oligonucleotides (Müller et al., 1997) were used: 5′-CGCCAGGAGTTTGACACAATG-3′ (nt 52–72)

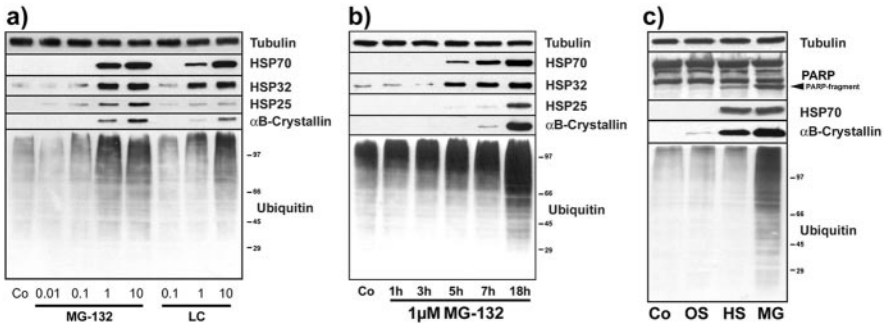

Figure 2. Induction of heat shock proteins by proteasomal stress. *a*, Oligodendrocytes (7 DIV) were exposed to MG-132 (0.01–10 μM) or lactacystin (LC; 0.1–10 μM) as indicated for 18 hr. Cell lysates were prepared and subjected to immunoblot analysis by using antibodies against the following heat shock proteins: HSP70, HSP32, HSP25, αB-crystallin, and ubiquitin and antibodies against α-tubulin, as indicated on the right. *b*, Cells were treated with 1 μM MG-132 for different time periods (1–18 hr) and analyzed by immunoblot with a panel of antibodies against the following heat shock proteins: HSP70, HSP32, HSP25, αB-crystallin, ubiquitin, and α-tubulin. *c*, Comparison of proteasomal stress, heat shock, and oxidative stress. Oligodendrocytes (7 DIV) were subjected to oxidative stress (OS; 50 μM H_2O_2, 30 min, 18 hr recovery), heat stress (HS; 44°C, 30 min, 18 hr recovery), or MG-132 (MG; 1 μM, 18 hr). Cell lysates were analyzed by immunoblot procedure with antibodies against tubulin, PARP, HSP70, αB-crystallin, and ubiquitin, as indicated on the right. Arrow on the right indicates the 85 kDa PARP cleavage product. Note that only MG-132 leads to the accumulation of ubiquitinated proteins. Co, Untreated control.

and 5′-GAGATGTGTCCCCAGACACCA-3′ (nt 1967–1987). Numbering is according to the rat tau sequence from peripheral nervous system (Goedert et al., 1992).

For the analysis of myelin basic protein (MBP) and α-tubulin genes the following primers were used: MBP, 5′-GACCCTCACAGCGACACG-GAT-3′ (nt 41–61) and 5′-CTGCTGAGGGACAGGCCTCTC-3′ (nt 336–356) (Roach et al., 1983); α-tubulin, 5′-AGCTCTACTGCCTGG-AACATG-3′ (nt 131–151) and 5′-TAAGTTAGTGTAGGTTGGGCG-3′ (nt 727–747) (Lemischka et al., 1981). Control experiments were performed with the following primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5′-CCCACGGCAAGTTCAACGGCA-3′ (nt 220–240) and 5′-TGGCAGGTTTCTCCAGGCGGC-3′ (nt 805–825) (Fort et al., 1985).

PCR reactions were performed as previously described (Richter-Landsberg and Gorath, 1999) in a volume of 23 μl containing (in mM): 10 Tris-HCl, pH 9.0, 50 KCl, 2 $MgCl_2$, 0.2 of each dNTP plus 0.8 μM of each primer. After an initial denaturation for 2 min at 95°C, 2 μl of *Taq*-polymerase (0.5 U/μl) was added. Then 25–32 cycles were performed in a Biometra (Goettingen, Germany) Thermocycler, each consisting of 1 min denaturation at 95°C, 1 min primer annealing at 56–70°C, depending on the primer pair used, and 2 min elongation at 72°C. After a terminal extension for 5 min at 72°C the reactions were stored at 4°C. Then 6 μl was used for analysis by agarose gel electrophoresis.

Results

Proteasome inhibitors cause apoptotic cell death in oligodendrocytes

To inhibit proteasomal activity, we used MG-132, which belongs to the class of peptide proteasome inhibitors. Additionally, in some experiments we have applied lactacystin, which has been described as a more specific inhibitor (Lee and Goldberg, 1998). Both inhibitors at a concentration of 10 μM within 18 hr caused morphological damage in oligodendrocytes; cellular processes disintegrated and membranous blebs, indicating apoptotic cell death, were observed (Fig. 1*A*). Cell viability was determined by using the MTT assay. Figure 1*B* shows that half-maximal cytotoxicity was exerted in the range of 10 μM. DNA fragmentation assay further confirms that MG-132 and lactacystin induced programmed cell death in oligodendrocytes (Fig. 1*C*). This effect was observed after treatment with MG-132 with a concentration as low as 0.1 μM (Fig. 1*C*). MG-132- and lactacystin-induced apoptotic cell death was accompanied by the activation of caspase 3, which is a major player in the regulation of apoptosis. Caspase 3 activity could be demonstrated by the appearance of the 85 kDa cleavage product of poly(ADP-ribose) polymerase (PARP; Fig. 1*D*). PARP is a polypeptide of 116 kDa and a substrate of caspase 3. Within 18 hr after the treatment the 85 kDa PARP fragment was observable.

Proteasome inhibition induces the accumulation of HSPs and ubiquitinated proteins

To investigate the effect of proteasome inhibition on the presence of HSPs, we applied antibodies against the following stress proteins: HSP70, HSP32, HSP25, and αB-crystallin and antibodies against ubiquitin. MG-132 and lactacystin induced the accumulation of HSPs and ubiquitin in a concentration-dependent manner. After 18 hr of incubation maximal induction was observed after treatment with MG-132 or lactacystin at a concentration of 1.0 or 10 μM, respectively (Fig. 2*a*). Time course analysis further indicates that the effects of MG-132 (1.0 μM) on HSP and ubiquitin accumulation were maximal after 18 hr (Fig. 2*b*). When different stress situations were compared, e.g., oxidative stress (OS) exerted by hydrogen peroxide (50 μM; 30 min, 18 hr recovery) or heat shock (HS; 44°C; 30 min, 18 hr recovery), immunoblot analysis revealed that only the inhibition of proteasome activity by MG-132, but not OS or HS, led to the accumulation of ubiquitin in oligodendrocytes (Fig. 2*c*). MG-132 and HS also caused PARP cleavage, which was observed after OS only to a very small extent (Fig. 2*c*). In contrast to MG-132 and HS, OS did not lead to the induction of HSP70, as we have described previously (Goldbaum and Richter-Landsberg, 2001).

MG-132 stimulates HSP mRNA synthesis

To test whether HSP induction by proteasomal inhibition is caused by the accumulation of nondegradable protein or is also a result of an increase in mRNA synthesis indicating the induction of protein synthesis, we extracted total RNA from oligodendrocytes after various times of treatment with MG-132 (1 μM). After reverse transcription the resulting cDNA was subjected to semiquantitative PCR (RT-PCR). Reaction conditions were chosen such that amplification was in a linear range, and amplification of GAPDH was performed from each cDNA to control for equal loading of cDNA samples. Figure 3 demonstrates that MG-132 caused an increase in mRNA encoding HSP70, HSP32, HSP25, and αB-crystallin, whereas the levels of MBP mRNA and of α-tubulin mRNA remained at the control level. Similarly, the level of tau mRNA did not change (Fig. 3). Quantitative evaluation indicates that αB-crystallin mRNA was increased maximally approximately ninefold after 18 hr of treatment, whereas HSP70 mRNA was induced maximally (eightfold) after 8 hr (Fig. 3*c*).

MG-132 effects on cellular distribution of HSP70, αB-crystallin, and ubiquitin

Indirect immunofluorescence was performed to determine the cellular localization of HSP70 and αB-crystallin after proteasome inhibition. Figure 4*A* demonstrates that MG132 (1 μM, 18 hr) caused the accumulation of HSP70 and αB-crystallin in the cell body. Although the cell nuclei in cells with αB-crystallin upregulation appeared normal (Fig. 4*Ae*), HSP70 induction was accom-

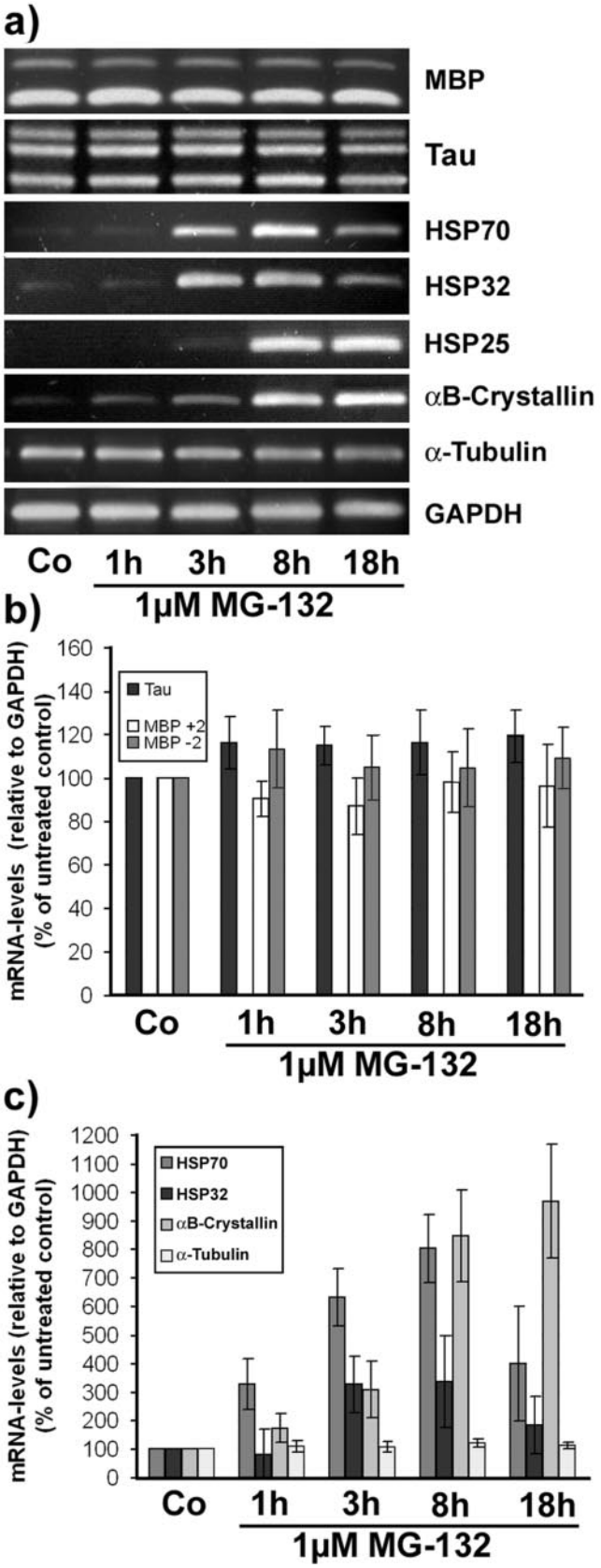

Figure 3. MG-132 causes the induction of HSP mRNA. Oligodendrocytes (7 DIV) were incubated with MG-132 (1 μM) for the indicated times, and mRNA was extracted and subjected to PCR after reverse transcription (RT-PCR). RT-PCR analysis was performed in relation to GAPDH mRNA and MBP mRNA. *a*, Photographs of agarose gels. The PCR products representing the different mRNAs are marked on the right. *b*, *c*, Quantitative evaluation. Experimental conditions were the same as in *a*. Agarose gels were photographed and scanned; the expression levels of the individual mRNAs were determined and normalized to GAPDH expression by densitometric evaluation. The levels of untreated control (Co) were set as 100%. For tau mRNA, primers were used to determine total tau mRNA representing all six isoforms (Gorath et al., 2001). For MBP mRNA, primers were used yielding two amplification products, either with exon 2 (+2; top band) or without exon 2 (−2; bottom band). Data represent the mean ± SD of three independent experiments, and each three PCRs were performed with the resulting cDNA ($n = 9$).

panied by the occurrence of deformed nuclei, indicative of apoptotic processes (Fig. 4*Af*). This was observable in all experiments and all cells that were positive for HSP70. TUNEL staining further corroborated this finding and demonstrates that cells expressing HSP70 also were labeled by the TUNEL method, indicative of extensive DNA degradation, whereas cells expressing αB-crystallin were negative (Fig. 4*B*). MG-132 did not affect all cells equally, as previously shown by MTT assay (Fig. 1*B*); this also is demonstrated by indirect immunofluorescence, showing intact cells in the vicinity of affected cells upregulating HSPs (Fig. 4*A*). Affected cells were characterized by the presence of fewer cell processes as compared with unaffected cells. Often only one major cellular process was seen. MG-132 under the same conditions caused the translocation of ubiquitin from the cell nuclei and its upregulation and aggregate-like assembly (Fig. 4*C*). In MG-132-treated cells ubiquitin-positive protein deposits accumulated in the cell body and also were seen in the larger primary cell extensions (Fig. 4*C*).

MG-132 causes tau dephosphorylation and the association of αB-crystallin and ubiquitin with microtubules and tau

Because tau is the major component of inclusion bodies in tauopathies and often colocalizes with HSPs, we have investigated whether proteasome inhibition alters tau phosphorylation and its interaction with microtubules and leads to an association with HSPs. The effect of MG-132 on tau phosphorylation was examined by using a panel of phosphorylation-dependent antibodies against tau. Figure 5 demonstrates that MG-132 in a concentration- and time-dependent manner caused the dephosphorylation of tau. This was detected prominently in the AT-8, PHF-1, and 12E8 epitopes, which previously have been shown to be the major stress-affected phosphorylation sites in oligodendroglial tau (Goldbaum and Richter-Landsberg, 2002). In contrast to HS and OS, this effect was not transient but was observable even 18 hr after the treatment (Fig. 5*b*). Similarly, lactacystin caused the dephosphorylation of tau (Fig. 5*a*).

To investigate whether MG-132 caused the recruitment of HSPs to the MTs, we isolated MTs from control and treated cells after stabilization with Taxol. The presence of HSPs was determined in the pellet (MT fraction) and the supernatant (soluble fraction) by Western blot procedure. Figure 6 shows that specifically αB-crystallin and ubiquitin were detectable mainly in the MT fraction, and HSP70, HSP32, and HSP25 were detected in both. Also, MG-132-induced tau dephosphorylation was accompanied by an induction in tau-binding capacity (Fig. 6*a*), similar to the effects observed after treatment with LiCl, an inhibitor of glycogen synthase kinase (GSK3β) (Goldbaum et al., 2003). Hence proteasomal inhibition caused the association of αB-crystallin and ubiquitin with the cytoskeleton. This effect was not observed when cells were treated with okadaic acid (OA; 50 nM, 18 hr) (Fig. 6*a*), leading to tau hyperphosphorylation and its detachment from the MTs (Goldbaum et al., 2003; this study).

To assess whether αB-crystallin and/or ubiquitin directly interact with tau and whether the phosphorylation state of tau af-

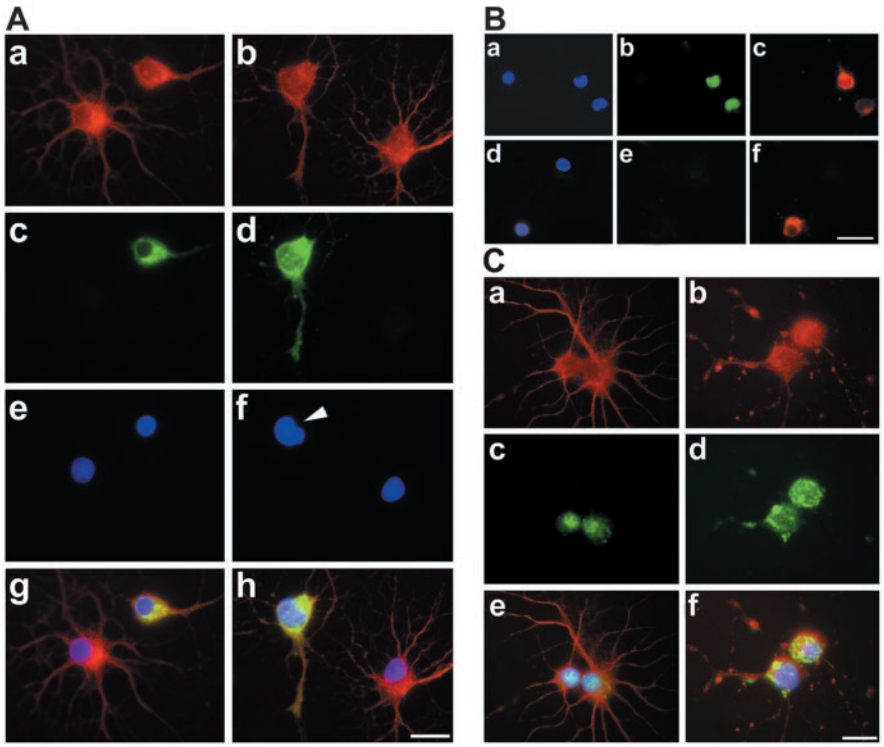

Figure 4. Distribution of HSPs in oligodendrocytes after proteasomal inhibition. *A*, Effect of MG-132 on αB-crystallin and HSP70. Oligodendrocytes were subjected to MG-132 (1 μM) for 18 hr. Indirect immunofluorescence staining was performed with mAb anti-α-tubulin (red; *a, b*), mAb anti-αB-crystallin (green; *c*), or mAb HSP70 (green; *d*). *e, f*, Staining with DAPI (blue). *g, h*, Overlay with DAPI. Scale bar, 20 μm. *B*, TUNEL staining. Oligodendrocytes were treated with MG-132 (1 μM, 18 hr); TUNEL staining (*b, e*) was performed, followed by indirect immunofluorescence with antibodies against HSP70 (*c*) or αB-crystallin (*f*). Nuclei were stained with DAPI (*a, d*). Scale bar, 25 μm. *C*, Effect of MG-132 on ubiquitin. Oligodendrocytes either were untreated (*a, c, e*) or treated with MG-132 (1 μM, 18 hr; *b, d, f*). Indirect immunofluorescence was performed with polyclonal anti-α-tubulin (red; *a, b*) and mAb anti-ubiquitin (green; *c, d*). *e, f*, Overlay with DAPI. Scale bar, 20 μm. Note that ubiquitin immunoreactivity translocates from the cell nucleus to the cell body after proteasomal inhibition.

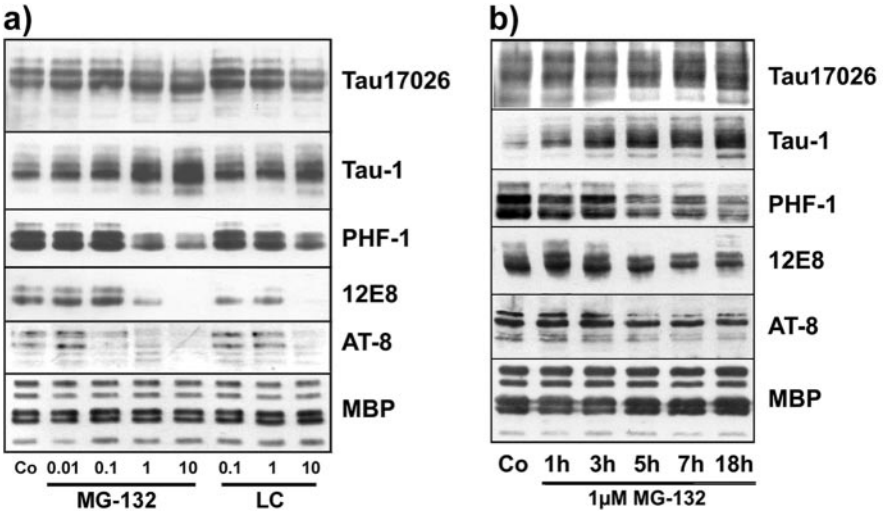

Figure 5. Proteasomal inhibition leads to dephosphorylation of tau proteins. *a*, Oligodendrocytes were treated with MG-132 (0.01–10 μM) or with lactacystin (LC; 0.1–10 μM) for 18 hr. Co, Untreated control. Cell lysates were analyzed by immunoblot procedure with phosphorylation-independent antibody tau 17026, reacting with total tau, and a panel of phosphorylation-dependent antibodies (tau-1, PHF-1, 12E8, AT-8) and antibodies against MBP, as indicated on the right. *b*, Oligodendrocytes either were untreated (Co) or treated with 1 μM MG-132 for the indicated times (1–18 hr). Cell lysates were prepared and subjected to immunoblot analysis by using tau antibodies and antibodies against MBP, as in *a*.

fects this interaction, we performed coimmunoprecipitation analysis. Cells were treated with OA (25 nM, 6 hr), MG-132 (1 μM, 18 hr), or both in sequence. OA caused hyperphosphorylation of tau specifically detectable with 12E8 antibodies, the induction of HSP32, and a slight induction of HSP70, HSP25, αB-crystallin, and of ubiquitinated proteins (Fig. 6*b*). Treatment with OA followed by MG-132 led to further accumulation of all HSPs studied, and hyperphosphorylated tau remained prominently expressed (Fig. 6*b*). Cell lysates of control and treated cells were precipitated with polyclonal tau 17026 antibodies. The immunoprecipitates were separated by SDS-PAGE and then subjected to immunoblot procedure, using antibodies against ubiquitin, αB-crystallin, HSP25, HSP32, and HSP70. Figure 6*c* depicts that tau was precipitated successfully from the cell lysate. Immunoprecipitated tau from MG-132-treated cells was ubiquitinated (Fig. 6*c*), and tau coimmunoprecipitated with αB-crystallin (Fig. 6*c*), whereas HSP32 and tubulin remained in the supernatant. Ubiquitinated tau was separated in the molecular weight range of 66–45 kDa; additionally, a high-molecular-weight species was observed, possibly representing poly-ubiquitinated and/or aggregated tau. Small amounts of HSP70 and HSP25 also were detectable in the immunoprecipitates under these conditions. In the immunoprecipitates of cell lysates derived from control cells, the association of ubiquitin or αB-crystallin with tau was not detectable. Similarly, tau hyperphosphorylation by OA did not cause the association of ubiquitin or αB-crystallin with tau (Fig. 6*c*). Also, tau dephosphorylation by LiCl (20 mM, 24 hr) did not lead to the presence of either ubiquitin or αB-crystallin in the immunoprecipitates (data not shown). Hence proteasomal inhibition caused tau ubiquitination and its association with αB-crystallin, and this effect was not a consequence of the phosphorylation status of tau. Immunoprecipitation performed with antibodies against αB-crystallin followed by Western blot procedure with antibodies against ubiquitin did not reveal the association of ubiquitin with αB-crystallin (data not shown); hence ubiquitin that is associated with the tau immunoprecipitate is not attributable to ubiquitinated αB-crystallin.

MG-132 leads to an increase of ubiquitin and tau in the RIPA buffer insoluble fraction

Cells were treated with MG-132 (1 μM, 18 hr) and extracted with buffers with increasing protein solubilization abilities,

i.e., HSB, RIPA, and SDS-sample buffer (see Materials and Methods). Western blot analysis revealed that MG-132 increased the insolubility of tau. The majority of tau is extractable with HSB. After MG-132 treatment an increased amount (10–15% more than in the control) remained insoluble and was found in the pellet remaining after extraction with RIPA; it could be solubilized only in SDS-sample buffer (Fig. 7). αB-Crystallin was not detectable in the HSB and RIPA fractions but only in the SDS-sample buffer extract, whereas HSP70 was present in all three. Ubiquitin was not detectable in HSB and mainly present in the SDS-sample buffer extract. Hence tau, which was insoluble in high salt and RIPA buffer containing Triton X-100 and soluble only in SDS-sample buffer, was found together with ubiquitin and αB-crystallin in the least soluble fraction (Fig. 7).

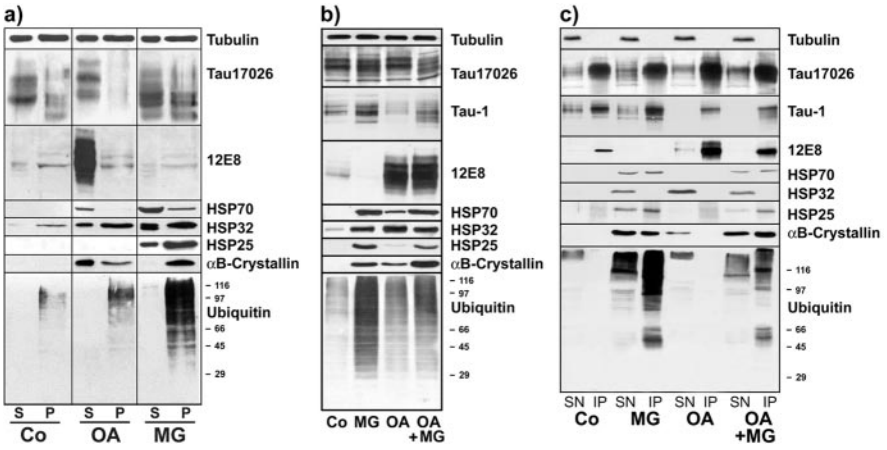

Figure 6. Proteasomal stress leads to the association of αB-crystallin and ubiquitin with microtubules and tau. *A*, Microtubule binding assay. Oligodendrocytes were untreated (Co) or treated with the protein phosphatase inhibitor okadaic acid (OA; 50 nM, 18 hr) or with MG-132 (MG; 1 μM, 18 hr). Cell lysates were separated into cytoskeletal (P) and soluble fractions (S) after MT assembly and were subjected to immunoblot analysis by using polyclonal tau 17026, mAb 12E8, mAb anti-α-tubulin, and a panel of antibodies to the following heat shock proteins: HSP70, HSP32, HSP25, and αB-crystallin. OA leads to the hyperphosphorylation of tau proteins and to its detachment from the MTs, as indicated by an increase of tau in the soluble (S) and a decrease in the cytoskeletal (P) fraction. MG-132 increases the binding affinity of tau to MTs, as indicated by an increase of tau in the cytoskeletal fraction (P). HSP25, αB-crystallin, and ubiquitin are found predominantly to be associated with the cytoskeleton, whereas HSP70 is present mainly in the soluble fraction; HSP32 is present in both. *b, c*, MG-132 leads to the ubiquitination of tau and to its association with αB-crystallin. Oligodendrocytes were untreated (Co), treated with okadaic acid (OA; 25 nM, 6 hr), with MG-132 (MG; 1 μM, 18 hr), or with OA (25 nM, 6 hr), followed by 18 hr of incubation with MG-132 (OA + MG). Cell lysates were subjected to immunoblot procedure by using a panel of different antibodies as indicated on the right (*b*). Immunoprecipitation of cell lysates was performed with anti-tau 17026 antibodies (*c*). The immunoprecipitate (IP) and the remaining supernatant (SN) were subjected to immunoblot analysis by using antibodies against ubiquitin and against α-tubulin and a panel of antibodies against HSPs, as indicated on the right (*c*). Note that tau is ubiquitinated and associated with αB-crystallin only after treatment with MG-132.

MG-132 induces the occurrence of thioflavine S-positive cytoplasmic inclusions that colocalize with ubiquitin, αB-crystallin, and tau

To investigate whether proteasomal inhibition leads to aggregate formations similar to those observed in neurodegenerative diseases, we performed indirect immunofluorescence with mAb antibodies to ubiquitin, followed by thioflavine S staining. Thioflavine S is a histochemical dye specifically binding to crossed β-pleated sheet structures that is used to identify fibrillary protein aggregates. Figure 8*A* demonstrates that MG-132-induced ubiquitin accumulations in oligodendrocytes also were labeled by thioflavine S, indicating the presence of filamentous, misfolded proteins. These thioflavine S-positive aggregates also contained tau, as indicated by indirect immunofluorescence staining with polyclonal tau antibodies (Fig. 8*B*). Intense staining was observed mainly in one of the major cell processes and the adjacent cytoplasm; the staining pattern resembled that observed in coiled bodies (Goldbaum et al., 2003). Indirect immunofluorescent double staining performed with anti-tau and anti-ubiquitin antibodies, furthermore, indicated that tau and ubiquitin were colocalized in the aggregates (Fig. 8*C*). Also, αB-crystallin immunoreactivity was present in thioflavine S-positive protein deposits (Fig. 8*D*). Hence in oligodendrocytes the thioflavine S-positive protein aggregates can be induced by MG-132, and these inclusions contain tau protein, αB-crystallin, and ubiquitin (Fig. 8).

Discussion

Inclusion body pathology in a variety of neurodegenerative disorders originating in nerve cells or glia is demonstrated in many studies by immunohistochemical detection of ubiquitin and HSPs. The presence of HSPs, which initially may serve a protective role, points to the involvement of stress situations during pathogenesis (for review, see Layfield et al., 2001; Richter-Landsberg and Goldbaum, 2003). Inclusion bodies, furthermore, are characterized by the presence of specific misfolded cellular proteins. Although α-synuclein is the major constituent of Lewy bodies in Parkinson's Disease, in AD and other tauopathies deposits of fibrillary tau are prominent in neuronal and glial cells (Goedert et al., 1998; Lee et al., 2001). Inhibition of the proteasomal pathway contributes to the accumulation of ubiquitinated proteins and aggregates, caused by their impaired clearance, and also may lead to HSP induction (Lee and Goldberg, 1998).

Here we show that proteasomal inhibition by MG-132 and lactacystin in oligodendrocytes leads to apoptotic cell death and to the induction of HSPs in a time- and concentration-dependent manner. Specifically, αB-crystallin, which is a consistent constituent of glial cell inclusions (Chin and Goldman, 1996; Goldbaum et al., 2003), and ubiquitin were upregulated. MG-132 caused the dephosphorylation of tau and a decrease in its solubility. Furthermore, proteasomal inhibition led to ubiquitination of tau, its association with αB-crystallin, and the occurrence of thioflavine S-positive aggregates in the oligodendroglial cytoplasm. These aggregates were positive for tau and also contained ubiquitin and αB-crystallin; hence they resembled glial cytoplasmic inclusions observed in white matter diseases and FTDP-17.

Proteolytic stress-induced tau dephosphorylation in oligodendrocytes was detected by tau-1, 12E8, and PHF-1 antibodies and thus occurred at similar epitopes as previously described for the effects of HS and OS in oligodendrocytes (Goldbaum and Richter-Landsberg, 2002). However, in contrast to HS and OS, the dephosphorylation of tau was not transient, but prominent, even after 18 hr of treatment. The biological activity of tau is regulated by phosphorylation. Dephosphorylation, specifically in the flanking domains of the MT binding repeats, stabilizes the MT network (Johnson and Hartigan, 1998; Buée et al., 2000). Hyperphosphorylation of tau has been connected with its pathological aggregation. Phosphorylation at Ser^{262}, which is recognized by the 12E8 antibody, strongly reduces tau affinity to MTs and leads to its detachment. On the other hand, tau phosphory-

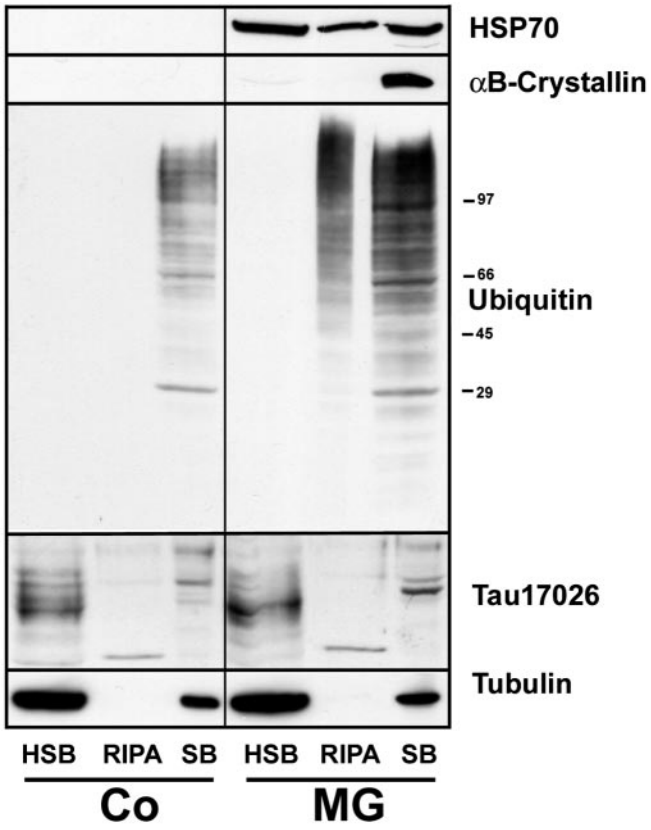

Figure 7. MG-132 alters the solubility of tau. Oligodendrocytes were treated with MG-132 (MG; 1 μM, 18 hr) and extracted with buffers of increasing protein solubilization ability, namely HSB, RIPA, and SDS-sample buffer (see Materials and Methods). Co, Untreated control. Note that after treatment with MG-132 the amount of tau and ubiquitin is increased in the least soluble fraction (SB). Also, αB-crystallin is detectable only in the SB fraction.

lation at the same site might protect tau against aggregation and may stabilize tau against proteolytic degradation (Schneider et al., 1999; Krishnamurthy et al., 2000). It might be speculated that tau dephosphorylation leading to an increased MT binding activity, which occurs in oligodendrocytes after thermal and oxidative stress (Goldbaum and Richter-Landsberg, 2002) and, as shown in this study, also after proteolytic stress, initially is an attempt to stabilize the MT network.

Microtubule binding assay further demonstrated that αB-crystallin and ubiquitin bind to the MTs and were detectable only barely in the cytoplasmic fraction. Thus proteasomal inhibition caused the recruitment of ubiquitin and the small HSP αB-crystallin to the cytoskeleton and induced its specific interaction with tau, as shown by coimmunoprecipitation. Proteasome-mediated degradation of tau has been observed in several studies (Canu et al., 2000; Cardozo and Michaud, 2002; David et al., 2002; Goldbaum et al., 2003); however, this has been attributed to degradation by the 20S proteasomes without the requirement of ubiquitination (David et al., 2002). Our study demonstrates for the first time that proteasomal impairment leads to tau ubiquitination. This effect most likely was caused by the accumulation of misfolded tau protein and was not related merely to the altered phosphorylation state of tau, because it was not observed after treatment with okadaic acid, leading to tau hyperphosphorylation, or treatment with LiCl, leading to tau dephosphorylation. This assumption is sustained further by the present finding that MG-132 led to an increase in the proportion of Triton X-100-insoluble tau.

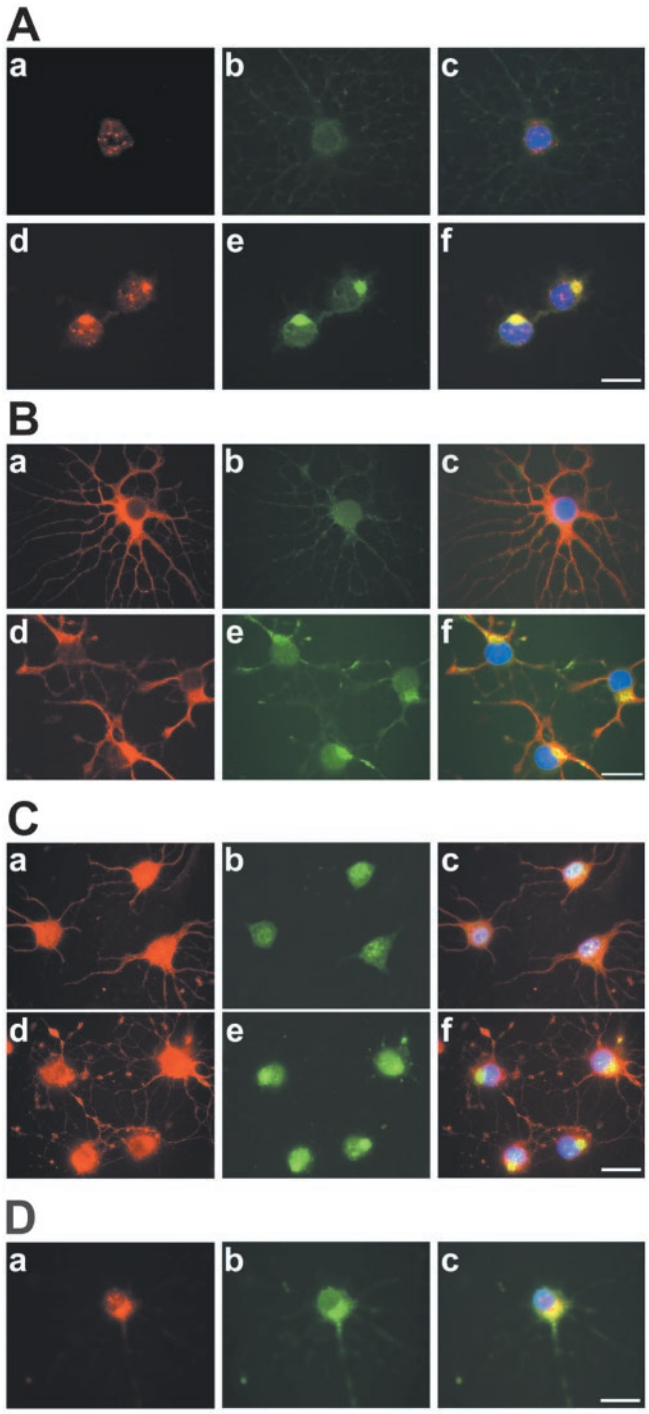

Figure 8. Proteasomal stress leads to the formation of ubiquitin- and thioflavine S-positive inclusions containing αB-crystallin. Oligodendrocytes either were untreated (Co) or subjected to MG-132 (1 μM) for 18 hr. Scale bars, 20 μm. *A*, Presence of ubiquitin in inclusions bodies. Indirect immunofluorescence was performed with monoclonal anti-ubiquitin (red; *a, d*), followed by thioflavine S staining (green; *b, e*). *c, f*, Overlay with nuclear DAPI staining (blue). *a–c*, Untreated control; *d–f*, MG-132-treated cells. *B*, Presence of tau in inclusion bodies. Indirect immunofluorescence with polyclonal anti-tau 17026 (*a, d*), followed by thioflavine S staining (green; *b, e*). *c, f*, Overlay with nuclear DAPI staining (blue). *a–c*, Untreated control; *d–f*, MG-132-treated cells. *C*, Colocalization of tau and ubiquitin in the inclusion bodies. Indirect immunofluorescence double staining was performed with polyclonal anti-tau 17026 (red; *a, d*) and monoclonal anti-ubiquitin (green; *b, e*). *c, f*, Overlay with nuclear DAPI staining (blue). *a–c*, Untreated control; *d–f*, MG-132-treated cells. *D*, Presence of αB-crystallin in inclusion bodies. Indirect immunofluorescence was performed with mAb αB-crystallin (red; *a*), followed by thioflavine S staining (green; *b*). *c*, Overlay with nuclear DAPI staining (blue).

The accumulation of misfolded proteins leads to the activation of the ubiquitin–proteasome pathway (Glickman and Ciechanover, 2002). Proteasomal inhibitors and other stressors, which impair this system, cause the upregulation of HSPs and ubiquitin. HSPs constitute the major cellular defense against unfolded proteins (for review, see Sherman and Goldberg, 2001), and their expression is connected to the protection of neural cells (for review, see Richter-Landsberg and Goldbaum, 2003). HSPs and ubiquitinated proteins detected in inclusion bodies, on the one hand, point to an unsuccessful attempt by the ubiquitin–proteasomal machinery to remove abnormal proteins. On the other hand, ubiquitin might be involved in protective mechanisms by surface-coating hydrophobic domains of protein aggregates, thereby preventing further growth of the aggregates or interactions with other cell constituents such as the cytoskeleton, as has been suggested by Gray (2001). Multi-ubiquitin chains of at least four subunits are required for substrate recognition of the 26S proteasome (Pickart, 2000), whereas modification of proteins by single or short ubiquitin chains might be a regulator of the location and cellular activity (Hicke, 2001). In PHFs tau mono-ubiquitination seems to be predominant (Morishima-Kawashima et al., 1993). It remains to be established whether proteasome inhibition leads to mono- or poly-ubiquitination of tau in oligodendrocytes.

αB-Crystallin belongs to the group of small HSPs that have physiological roles as modulators of the cytoskeleton (Liang and MacRae, 1997; Head and Goldman, 2000). In the CNS αB-crystallin is primarily inducible in oligodendrocytes (Goldbaum and Richter-Landsberg, 2001) and has been connected to inclusion body formation (Head and Goldman, 2000). It is the major component of Rosenthal fibers, the characteristic inclusion bodies found in astrocytes of patients with Alexander's disease, and is involved in the modulation of intermediate filament organization under conditions of physiological stress and neurodegenerative disease (Head and Goldman, 2000). Oligodendrocytes do not have intermediate filaments but are rich in MTs that maintain the cellular morphology and provide the basis for intracellular transport processes (Richter-Landsberg, 2001). Here we show that αB-crystallin is recruited to the cytoskeleton and directly interacts with tau, possibly to prevent MT disorganization and tau aggregation. Hence αB-crystallin, which has chaperone activity, might be involved in the protection of the MT network, as has been suggested previously (Arai and Atomi, 1997; Goldbaum et al., 2003), and inclusion body formation is a later stage of the pathogenic response.

Our data further show that proteasomal inhibition by MG-132 or lactacystin in oligodendrocytes caused the onset of programmed cell death, which was accompanied by activation of the caspase pathway. Oligodendrocytes are metabolically most active cells (Pfeiffer et al., 1993); the maintenance of the myelin sheath makes the energy requirement two- to threefold higher than other brain cells (Connor and Menzies, 1996), and an effective protein quality control system appears to be essential for oligodendroglial functions and survival. As indicated by the present study, the chronic impairment of the ubiquitin–proteasome pathway cannot be counteracted by the induction of HSPs. Specifically, the increase and association of the chaperone αB-crystallin with tau and the MTs fail to rescue the cells. Also, oligodendrocytes are not protected by upregulation of HSP70, which in other cellular models and transgenic mice has been shown to prevent tau aggregation and promoted tau binding to MTs (Dou et al., 2003). It might be speculated that the long-term inhibition of the proteasome system, leading to the continuous presence of misfolded proteins and upregulation of HSPs, depletes the cells of chaperone activity and contributes to cell death. Furthermore, although the neurotoxic mechanisms of aggregate formation remain controversial, intracellular fibrillary protein deposits can impair intracellular trafficking and disturb cell morphology, which eventually has deleterious consequences.

To summarize, the present data indicate that proteasomal inhibition in oligodendrocytes causes the upregulation of HSPs and the accumulation of ubiquitinated proteins, including tau. Tau dephosphorylation and the specific stress-induced interaction of αB-crystallin with the cytoskeleton possibly represent a protective means; however, this is unsuccessful, and continuous proteasomal stress leads to a severe disturbance of the MT network and tau-positive inclusion bodies are formed. Hence the impairment of the ubiquitin–proteasome pathway is linked to oligodendroglial cell death and inclusion body formation and might contribute to neurodegenerative diseases with glial cell pathology.

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