Lithium Treatment Decreases Activities of Tau Kinases in a Murine Model of Senescence

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Abstract
Lithium modulates glycogen synthase kinase 3β (GSK-3β), a kinase involved in Alzheimer disease–related tau pathology. To investigate mechanisms of aging and the potential therapy of lithium in neurodegenerative disease, we treated senescence-accelerated mouse (SAMP8) mice, a murine model of senescence, and mice of the control SAMR1 strain with lithium. The treatment reduced hippocampal caspase 3 and calpain activation, indicating that it provides neuroprotection. Lithium also reduced both the levels and activity of GSK-3β and the activity of cyclin-dependent kinase 5 and reduced hyperphosphorylation of 3 different phosphoepitopes of tau: Ser199, Ser212, and Ser396. In lithium-treated primary cultures of SAMP8 and SAMR1 cerebellar neurons, there was a marked reduction in protease activity mediated by calpain and caspase 3. Both lithium and SB415286, a specific inhibitor of GSK-3β, reduced apoptosis in vitro. Taken together, these in vivo and in vitro findings of lithium-mediated reductions in GSK-3β and cyclin-dependent kinase 5 activities, tau phosphorylation, apoptotic activity, and cell death provide a strong rationale for the use of lithium as a potential treatment in neurodegenerative diseases.

Key Words: Aging, Brain, Lithium, Neurodegeneration, Senescence-accelerated mouse

INTRODUCTION
Aging may be defined as the sum of anatomic, histologic, and physiologic modifications (i.e. deteriorations) that occur over time in different cell types and in different organs and systems (1). It is characterized by a generalized loss in the complexity of dynamics regulating organ function and a uniform loss in the capacity of the organism to adapt to changing variables in its environment (2, 3). Recent studies suggest that cerebral changes are subtle and involve more than simple neuronal loss (4, 5). This loss is not a generalized process because it only affects specific parts of the brain (6). The incidence of neurodegenerative diseases such as Alzheimer disease (AD) increases significantly with age (7–10). Therefore, with the rapid increase in the aged segment of the population, it is crucial that mechanisms associated with senescence and the transition from benign aging to neurodegenerative disease are elucidated to prevent or forestall the development of diseases such as AD.

Rats and mice provide suitable experimental models for studying age-related degenerative diseases (11). The senescence-accelerated mouse (SAM) strains, for example, were developed by Takeda et al (12) in 1981 through phenotypic-selection sister-brother mating from ancestral AKR/J strains. There are at least 9 lines of these inbred strains that show accelerated senescence as an inherited phenotype and 3 lines of a senescence-resistant inbred strain. SAMP8 mice show a relatively strain-specific, age-associated phenotype with a shortened life span and early manifestations of senescence compared with long-lived senescence-resistant (SAMR1) mice (13, 14). The alterations in longevity and pathologic changes are similar to those in several geriatric disorders in humans (15). Furthermore, deficits in learning and memory observed in the SAMP8 strain mimic those observed in aged humans; therefore, the SAMP8 mouse may be an excellent model in which to study the pathophysiologic changes observed in early stages of AD. We and others have described various AD-related pathologic alterations in the brains of SAMP8 mice occurring as early as 5 months of age (16, 17).

The 2 histopathologic hallmarks in the brains of patients with AD are neuritic plaques with insoluble β-amyloid peptide aggregate cores and neurofibrillary tangles that consist of the hyperphosphorylation of the microtubule-binding tau protein. Hyperphosphorylation substantially reduces the affinity of tau for microtubules, leading to their...
instability and to increasing amounts of microtubule-unbound tau that is prone to self-aggregation (18, 19). Although intracellular neurofibrillary tangles attributed to abnormal hyperphosphorylation of tau are present in the brains of geriatric individuals who lack clinical evidence of AD (20), they nevertheless likely contribute to neuronal dysfunction.

Glycogen synthase kinase 3β (GSK-3β), a kinase implicated in the pathogenesis of AD-related tau pathology, is known to mediate neuron cell death in several ways (21). It is capable of catalyzing phosphorylation of tau in tandem with cyclin-dependent kinase 5 (cdk5), thereby impairing both tau binding to microtubules and promoting microtubule disassembly, which induces the formation of neurofibrillary tangles (22, 23). Consequently, the inhibition of tau kinases may help protect neurons from disruptions to the neuronal cytoskeleton and from abnormal tau fibril formation during aging, thereby favoring “healthy aging” (24, 25).

Lithium chloride, an inhibitor of GSK-3β and a putative inhibitor of cdk5, is widely used as a mood stabilizer for manic-depressive illness (26). For the reasons previously described, it may be a promising therapeutic strategy to target AD-related neurodegeneration. Indeed, both in vitro and in vivo studies have addressed the potential of lithium for the treatment of acute brain injuries such as ischemia and chronic neurodegenerative diseases such as AD, Parkinson disease, tauopathies, and Huntington disease (25). For example, primary neuronal cultures treated with lithium are protected against phenytoin- and carbamazepine-induced apoptosis (27) and neurotoxicity induced by β-bungarotoxin (28). Similarly, dopaminergic neurotoxicity in mice using the N-methyl-D-aspartate antagonist kainic acid (24, 25).

Lithium or other GSK-3β inhibitors also suppress excitotoxicity in rat models of Huntington disease (31–34). Importantly, and of direct relevance to the present study, Pérez et al (35, 36) studied the ability of lithium to decrease tau aggregation in a transgenic mouse model of AD. Less is known, however, regarding the dynamics involved in tau phosphorylation during aging and related disorders and/or the ability of lithium to modulate tau phosphorylation in a senescent mouse model. The aim of the present study was to study effects of chronic lithium treatment in a SAM model and to elucidate mechanisms of lithium effects on tau phosphorylation.

MATERIALS AND METHODS

Animals and Treatments

One-month-old SAMP8 (n = 30) and SAMR1 (n = 30) mice were used. Control animals (n = 15 per group) were fed a standard diet (Harlam Ibe‘rica, Barcelona, Spain) with saline (NaCl, 450 mmol/L) provided ad libitum. The experimental groups (n = 15 per group) were provided free access to the control group diet (Harlam Ibe‘rica) supplemented with 0.3% lithium chloride. The mice were housed under pathogen-free conditions at a temperature of 24 ± 1.5°C and were exposed to a daily cycle of 14 hours of light and 10 hours of darkness. This diet has previously been determined to produce significantly elevated serum lithium levels. To ensure that lithium levels remained within a therapeutic range, blood samples were collected weekly, and serum lithium was measured by inductively coupled plasma mass spectrometry as described (36). Lithium concentrations in the serum of mice fed lithium-supplemented chow ranged between 0.8 and 1.2 mmol/L (therapeutic concentration in humans is 0.6–1.2 mmol/L). Animal weights were also monitored weekly to detect potential toxic effects of the lithium diet. After 8 weeks, the animals were killed by decapitation, and brains were carefully dissected. The brains were divided through the sagittal plane. Half of the brain was quick-frozen in dry ice for later Western blot and immunohistochemical analyses, and the other half was stored in RNA later (Ambion, Madrid, Spain) for gene transcription analysis by reverse-transcriptase-polymerase chain reaction (RT-PCR). All research protocols were approved by the Animal Research Committee of the University of Barcelona.

Western Blot Analysis

Aliquots of hippocampus homogenate containing 25 μg per sample (5 μg for α-spectrin analysis) were analyzed by Western blot. In brief, they were placed in sample buffer (0.5 mol/L of Tris-HCl, pH 6.8, 10% glycerol, 2% wt/vol sodium dodecyl sulfate, 5% vol/vol 2-mercaptoethanol, 0.05% bromophenol blue) and denatured by boiling at 95°C to 100°C for 90 seconds. Samples were separated by electrophoresis on 10% acrylamide gels. Thereafter, proteins were transferred to polyvinylidene fluoride sheets (ImmobilonTM-P; Millipore, Corp., Bedford, MA) using a transblot apparatus (Bio-Rad, Hercules, CA). Membranes were blocked for 1 hour at room temperature with 5% nonfat milk dissolved in Tris-buffered saline-T buffer (Tris, 50 mmol/L; NaCl, 1.5%; Tween 20, 0.05%; pH 7.5). They were then incubated with primary monoclonal antibodies against cdk5(sc-173), GSK-3β, pGSK-3β [Ser9], pGSK-3β [Tyr219] (1:1000; Affinity BioReagents, Golden, CO), and anti-pTau [Ser396], [Thr217], [Ser199], and [Thr181] (1:1000; Biosource, Carlsbad, CA); antibodies to α-spectrin were from Oncogene, and antibodies to β-actin (1:3000) were from Neomarkers (Fremont, CA). After 4 hours, blots were washed thoroughly in Tris-buffered saline-T buffer and incubated for 1 hour with a peroxidase-conjugated immunoglobulin G antibody (Amersham Corp., Arlington Heights, IL). Immunoreactive protein was viewed with a chemiluminescence-based detection kit following the manufacturer’s protocol (ECL kit; Amersham Corp.), whereas digital images were obtained using a Bio-Rad ChemiDoc system (BioRad). Semiquantitative analyses were carried out using the Quantity One software (Bio-Rad), and results were expressed as arbitrary units. The protein load was routinely monitored by phenol red staining of the blot membrane or immunodetection of β-actin.

pGSK-3β and pTau Immunohistochemistry

Immunohistochemistry was carried out following the avidin-biotin-peroxidase complex method. After blocking endogenous peroxidase, the sections were incubated with normal serum and then incubated at 4°C overnight with one of the primary antibodies. Phospho-specific tau rabbit
polyclonal antibodies against [Ser202] and [Ser396] (all of them from Calbiochem, San Diego, CA) were used at a dilution of 1:100. The anti-pGSK-3β [Ser217] monoclonal antibody (StressGen, San Diego, CA) was used at a dilution of 1:500. After incubation with primary antibody, the sections were incubated for 1 hour with biotinylated anti-mouse or anti-rabbit immunoglobulin G diluted 1:100, followed by avidin-biotin-peroxidase complex at a dilution of 1:100 for 1 hour at room temperature. The peroxidase reaction was visualized with 0.05% dianisidine and 0.01% hydrogen peroxide. Some sections were counterstained with hematoxylin.

Reverse-Transcriptase-Polymerase Chain Reaction

Total RNA was extracted from hippocampus tissue samples using Trizol reagent according to the manufacturer’s instructions. Isolated RNA was treated with amplification Grade DNase I to remove contaminant genomic DNA for 7 minutes at room temperature. First-strand cDNA was reverse-transcribed from 2 μg of total RNA using a First-Strand synthesis system kit from Invitrogen (Carlsbad, CA). β-Actin primers were designed to cross a large expanse of intronic sequence between Exons 2 and 3 of the mouse gene (Table). Primer nonreactivity with contaminating genomic DNA was tested by including controls that omitted the RT enzyme from the cDNA synthesis reaction (RT-negative controls). The lack of primer dimerization or nonspecific PCR product bands was also tested.

RT-PCR Quantification Using SYBR Green

Quantification of relative gene expression by RT-PCR was performed using the ABI PRISM 7700 Sequence Detection System. Using a SYBR Green PCR kit, RT-PCR was carried out. Primer concentration and PCR melting temperature were adjusted to avoid nonspecific PCR products because SYBR Green binds nonspecifically to each double-strand DNA product formed during amplification. The optimum temperature was that which gave the maximum reading for the specific product when the nonspecific product could no longer be detected.

Once the optimum temperature had been determined, quantitative PCR was carried out using the following thermal cycling program. Stage 1 was undertaken at 95°C for 15 minutes. Stage 2 consisted of 3 steps: 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Stage 2 was repeated for 40 cycles. The relative mRNA expression was calculated by the standard curve method. In brief, both β-actin and target gene amplifications were run in separate tubes. Standard curves were obtained for all genes using decreasing amounts of cDNA template. Polymerase chain reactions were performed in duplicate for standard curves, whereas samples were tested in triplicate at a final volume of 25 μl in all cases. For each cDNA template, the cycle threshold (Ct) necessary to detect the amplified product was determined, and semilogarithmic standard plots were drawn (Ct vs cDNA amount). The calculations were performed based on the comparative Ct method (37). This method uses the formula \( 2^{-\Delta\Delta Ct} \) to calculate the expression of target genes normalized to a calibrator (Nβ-fold). The Ct indicates the cycle number in which the amount of amplified target reaches a fixed threshold. The Ct data for all target and housekeeping genes in each sample were used to create ΔCt values \( \Delta Ct = Ct \) (target gene) – Ct (housekeeping gene). We used β-actin as a reference (housekeeping) gene. Therefore, \( \Delta\Delta Ct \) values were calculated by subtracting the calibrator from the ΔCt value of each target. The relative quantity (RQ), or Nβ-fold, was calculated using the equation RQ = \( 2^{-\Delta\Delta Ct} \) (38, 39). Real-time RT-PCR data were quantified using the SDS 2.2 software package. Nonreactivity of the primers was tested by including controls that omitted the cDNA template, which was replaced by buffer. Genomic DNA contamination was tested by including those total RNA samples from RT-PCR reactions that lacked an RT enzyme. The absence of nonspecific PCR product was tested in all samples by analyzing the melting temperature profile via a 7700 Sequence 8 detector. The program consisted of the following: Stage 1, 95°C for 1 minute; Stage 2, 60°C for 1 minute; and then, an increase in temperature up to a final temperature of 95°C at Stage 3, with a 19-minute ramp time. Fluorescence data were collected for each PCR reaction, and melting graphs were drawn to confirm the presence of a single specific product.

SAMR1 and SAMP8 Neuronal Cultures: Viability and Apoptotic Measurements

Primary cultures of neurons were prepared from 7-day-old SAMR1 or SAMP8 mouse pups following the method of Verdaguer et al (39). In brief, after cerebella had been quickly removed and freed of meninges, they were manually sliced with a sterile blade. Tissue samples were then trypsinized and treated with DNAase. Cells were adjusted to a concentration of 8 x 10⁵ cells/ml and plated on poly-L-lysine-coated plates at a density 320,000 cells/cm². Cultures were grown in complete media (BME; containing 10% fetal calf serum, 2 mmol/L of L-glutamine, 0.1 mg/ml of gentamicin, and 25 mmol/L of L-glutamine, 0.1 mg/ml of gentamicin, and 25
mmol/L of KCl). C-Arabinoside (10 μmol/L) was added 16 to 18 hours after plating to inhibit the growth of nonneuronal cells. Cultures prepared by this method were enriched in granule neurons to more than 95% purity. After 4 to 5 days in vitro, cells were incubated in complete medium containing lithium chloride (ranging from 1 to 5 mmol/L). Neuroprotective effects were measured after 1, 7, or 15 days of lithium exposure, that is, after 20 days in vitro. Anti-apoptotic effects after 15 days to the selective GSK3 inhibitor SB415286 were tested in a concentration range of 5 to 30 μmol/L.

To assess the loss in cell viability, we used the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium) method (40). At a final concentration of 250 μmol/L, MTT was added to the cells and incubated for 1 hour, thereby allowing the reduction in MTT to produce a dark blue formazan product. Media were removed, and cell content was dissolved in dimethylsulfoxide. The formation of formazan was measured by the absorbance change at 595 nm using a microplate reader (Bio-Rad). Viability results are expressed in percentages. The absorbance measured from nontreated cells was taken to be 100%.

Apoptosis was assessed as follows: culture medium was removed, and the cells were collected from culture plates by pipetting with phosphate-buffered saline solution (PBS). Flow cytometry analysis was carried out using Epics XL flow cytometer adding propidium iodide (PI) 30 minutes before analysis. The instrument was calibrated using the standard configuration, that is, excitation of the sample was carried out with a 488-nm cooled argon-ion laser at 15 mW power. Forward scatter, side scatter, and red (620 nm) fluorescence for PI were acquired. Optical alignment was based on optimized signal from 10-nm fluorescent beads (Immunotech, Epics Division, Miami, FL). Time was used as a control of the instrument stability. Red fluorescence was projected on a 1,024-monoparametric histogram. Aggregates were excluded by gating single cells of their area versus peak fluorescence signal. Apoptosis was evaluated using appropriate software (WinMIDI). To detect morphologic evidence of apoptosis, PI staining was used. After treatment, cells were fixed in 4% paraformaldehyde/PBS, pH 7.4, for 1 hour at room temperature, washed with PBS, and incubated for 3 minutes with a solution of PI in PBS (10 μg/ml). Stained cells were visualized under UV illumination using a 20× objective (Nikon Eclipse; Tokyo, Japan), and their digitized images were captured. Apoptotic cells showed shrunken, brightly fluorescent, apoptotic nuclei and exhibited high fluorescence and condensed chromatin compared with non-apoptotic cells. Apoptotic cells were scored by counting at least 500 cells for each sample in 3 different experiments.

**Statistical Analysis**

Data are expressed as the mean ± standard error of mean from at least 3 different samples (from animal tissue or in vitro experiments) for both protein determination and gene expression analyses. In all cases, data were analyzed by analysis of variance, followed by post hoc Tukey Kramer multiple-comparison tests. p values lower than 0.05 were
considered significant. N°-Fold values in gene expression up to 0.5 and less than 2 were assumed to be nonsignificant according to values obtained from negative control genes (data not shown).

RESULTS

Lithium Blood Levels and Influence on Weight in Mice

There were no significant differences in weight gain between control and treated animals of both strains (SAMP8 and SAMR1; Fig. 1A). Serum lithium levels of SAMR1 and SAMP8 mice fed a lithium-supplemented diet throughout the treatment period measured approximately 0.5 meq/L (Fig. 1B).

Effect of Lithium on Caspase/Calpain Activity

Caspase and calpain activation apoptosis pathways are implicated in neuronal demise. We studied these pathways using the experimental tool of breakdown of α-spectrin, a 210-kd structural protein that is specifically cleaved by calpain to a 145-kd fragment (α-spectrin breakdown product 145) or by caspase 3 to a 120-kd fragment (α-spectrin breakdown product 120). When we analyzed the effect of lithium treatment in hippocampal extracts from treated and control mice, we

![Graph showing GSK3β activity](image)

**FIGURE 3.** (A) Lithium treatment reduces hippocampal p-glycogen synthase kinase (GSK) 3 [Tyr219]. Representative Western blot analysis of pGSK-3β [Ser9] (inactive enzyme) and of pGSK-3β [Tyr219] (active enzyme) in hippocampi of control SAMPR1 and SAMP8 mice and treated with lithium. Bar chart shows the semiquantitative analysis of both proteins evaluated under the different experimental conditions. Open bars, control mice; solid bars, treated mice. Each point represents the mean ± SEM of 4 to 5 animals, carried out in duplicate. (B) Immunohistochemical analysis of pGSK-3β [Tyr219] (active enzyme) in hippocampus of untreated (a) and 8-week-treated (b) SAMP8 mice; arrows indicate positively immunostained cells. Bar, 25 μm. All experiments were carried out at least in duplicate using 4 to 5 different animals.

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observed a reduction in the activation of both enzymatic activities in lithium-treated mice (Figs. 2A, B).

**Effects of Lithium on Expression of Tau-Related Kinases**

Western blot using an antibody against the phosphorylated form of GSK-3\(\alpha\) in [Ser9] (inactive enzyme) and in [Ser 217] (active form) demonstrated a reduction in GSK-3\(\alpha\) activation. As expected, there was a significant increase in levels of pGSK-3\(\alpha\)[Ser9] in lithium-treated mice compared with controls and a concomitant decrease in pGSK-3\(\alpha\)[Ser217] levels (Fig. 3A). Immunohistochemical staining revealed similar findings when the active form of GSK-3\(\alpha\)(pGSK-3\(\alpha\)[Tyr219]) was examined. Photomicrographs show a reduction in the active form of this kinase in a lithium-treated SAMP8 mouse compared with a control SAMP8 mouse (Fig. 3B).

No significant differences were detected in cdk5 expression in hippocampal samples from lithium-treated and control SAMR1 and SAMP8 mice (Fig. 4; left side). Experiments carried out to evaluate changes in the proteolytic fragment of p35, p25, which may be responsible for cdk5 activation, however, showed that in both strains, the transformation of p35 to p25 was prevented by lithium treatment (lower p25/p35 ratios) (Fig. 4; right side). Therefore, lithium is able to prevent activation of cdk5/p35 to cdk5/p25.

To determine whether the changes observed in both kinases after lithium treatment are related to transcriptional activity, we measured the transcriptional activity of these 2 genes via RT-PCR, analyzing the results using the comparative Ct method. We found no significant differences in the transcriptional activity of GSK-3\(\beta\) and cdk5 genes in SAMP8 lithium-treated compared with nontreated animals (not shown).

**Effects of Lithium on Tau Phosphorylation**

Cyclin-dependent kinase 5 and GSK-3\(\beta\) can catalyze the phosphorylation of different substrates, including tau. Western blot analysis of Tau Ser199 (specific phosphorylation site for GSK-3\(\beta\)), Tau Thr217 (specific phosphorylation site for cdk5), Tau Thr181, and Tau Ser396 (GSK-3\(\beta\) and cdk5) expression indicated that lithium treatment resulted in significant reduction in the expression of these epitopes compared with animals fed with control diet in both SAMR1 and SAMP8 strains (Fig. 5A). No differences were detected between the strains. Results on tau phosphorylation in SAMP8 animals were corroborated by immunohistochemical analysis. There was less Tau Ser396 and Ser212 hippocampal neuronal immunostaining in SAMP8 mice treated with lithium than in control SAMP8 mice (Fig. 5B). No modification in tau gene translation was observed in any condition (data not shown).

**Viability and Apoptotic Determinations in Neuronal Primary Cultures**

Inspection of 20-day cerebellar neuronal cultures indicated that, although non-lithium-treated cells showed evidence of neurite disintegration, cytoplasmic shrinkage, loss of membrane integrity, and neuritic processes, cultures exposed to lithium or SB415286 for 7 or 14 days seemed more healthy and normal (Fig. 6A).

**Cell Viability**

Lithium (0.1–5 mmol/L) treatment of SAMR1 and SAMP8 primary cultures for 1, 7, and 15 days increased viability, as assessed by MTT values. Cell viability was significantly higher at 1 (up to 125%) and 2 weeks (up to 135%) compared with control (set as 100%; see Materials
Features of Apoptosis

Flow cytometry analysis indicated a neuroprotective role of lithium. Specifically, chronic treatment with lithium for 1, 7, or 15 days after 20 days in vitro significantly reduced the extent of nuclear condensation (Fig. 7A). These results were corroborated by quantifying the numbers of condensed nuclei in neurons from both the SAMR1 and SAMP8 strains under the same experimental conditions. The results showed markedly reduced nuclear condensation in cultures pretreated with lithium (Fig. 7B). By fluorescence microscopy, control cells have condensed and shrunken nuclei, whereas lithium-treated cells have more normal-sized nuclei (Fig. 7C). The addition of SB415286 (5–30 μmol/L), a highly specific inhibitor of GSK-3β, for 15 days in culture also demonstrated antiapoptotic effects both with respect to numbers of condensed nuclei and aneuploid cells as measured by flow cytometry (Fig. 7B) and morphology (Fig. 7C). Importantly, and consistent with the in vivo results, the in vitro anti-apoptotic effects correlate with an increase in the inactive pGSK-3β Ser9 (Fig. 6C), suggesting

FIGURE 5. (A) Representative Western blots showing the increase in pTau [pSer199] and pTau [pSer396] and pTau [pThr217] in hippocampi of SAMR1 and SAMP8 mice untreated or administered lithium. Bar chart quantifies optical densities of Western blot bands. Open bars, control mice; solid bars, those treated with lithium. Each point represents the mean ± SEM of 4 to 5 animals, carried out in duplicate. The statistical analysis used was 1-way analysis of variance, followed by Tukey test *, p < 0.05, ** p < 0.01 vs control. (B) Immunohistochemical studies for tau [pSer212] (a, b) and tau [pSer396] (c, d) in the hippocampi of SAMP8 mice (both of control [a and c] and those treated with lithium [b and d]). Bar, 25 μm. All experiments were carried out at least in duplicate using 4 to 5 samples from different animals.
that lithium exerts its neuroprotective effects via an inhibitory action on this kinase.

**DISCUSSION**

The purpose of the present study was to examine whether lithium can potentially be effective in preventing neurodegeneration, a process frequently associated with aging. In addition, we sought to determine whether the neuroprotective mechanism of lithium was through the inhibition of tau kinases (6, 41, 42). We used the murine senescence model SAMP8 to study some of the molecular pathways involved in neuronal changes that accompany aging (43–48).

Lithium is a well-characterized inhibitor of GSK-3β, and as expected, we found that in treated animals of both the SAMP8 and SAMR1 strains, it reduced the activity of this kinase. Although conflicting studies on the neuroprotective role of lithium can be found in the literature (e.g. some reporting pro-oxidant effects and others reporting anti-oxidant effects), most studies report inhibition of GSK-3β (41, 42, 49–54). Therefore, we investigated other possible targets of this ion, that is, calpain/caspase activity and cysteine proteases related to neuronal alterations in AD (55). In this regard, we previously reported that activation of these proteases occurs in 5-month-old SAMP8 mice (17). Notably, our present study demonstrates that calpain and caspase 3 activation are present even at early ages in these animals, and, importantly, that chronic lithium treatment reduces both calpain and caspase 3 activation in the 2 strains. These data are in accordance with previous reports that show that lithium exhibited indirect effects on caspase and calpain activation both in vitro and in vivo (36, 56). It is well established that calpain participates in the activation of the cdk5 pathway (57) and in fact is capable of truncating p35 to p25, the specific coactivator of cdk5 (58, 59). Our present results indicate that lithium is able to reduce calpain activity, causing a decrease in p35 truncation to p25 and leading to a reduction in cdk5 activity in vivo, as we previously demonstrated in vitro (56). Because tau phosphorylation has been shown to occur after cdk5 activation via the truncation of p35 (57–59), it is reasonable to postulate that its inhibition by lithium would reduce severe tau phosphorylation as occurs in the SAM model.

In tandem with GSK-3β, cdk5 regulates tau phosphorylation (60, 61), and several reports have described cross talk between these 2 kinases. Here, we demonstrate that lithium treatment leads to a reduction in GSK-3β activity and a significant reduction in different tau phosphoepitopes: Ser199 (phosphorylated specifically by GSK-3β), Thr 217 (cdk5-specific epitope), and Thr181, Ser 212, and Ser396 (phosphorylated by cdk5 and GSK-3β) in the hippocampi of both strains. This led us to focus the study on targets of these kinases. Similar results have been obtained by Caccamo et al (62) in a transgenic model with β-amyloid plaques and tangles. Likewise, recent studies have shown that lithium may inhibit the hyperphosphorylation of tau in the brains of mice via inhibition of GSK-3 by blocking the accumulation of β-amyloid (53). Others have also demonstrated neuroprotective roles for lithium in a transgenic model of AD (29, 48) and in cellular models of neurotoxicity (27, 51).

The preclinical results may translate to humans because there is some evidence that lithium administration can constitute a preventive treatment for AD (63). Moreover, a pilot biomarker study to see whether lithium can lower tau and amyloid beta levels in cerebrospinal fluid and be safely tolerated in older AD patients will be undertaken by the Alzheimer Disease Cooperative Study by a National Institute of Aging nationwide consortium. A principal unresolved issue is whether lithium treatment increases cellular life span.
FIGURE 6. (A) Representative phase-contrast photomicrographs of SAMR1 and SAMP8 neurons under different experimental conditions. (B) Concentration-response curve showing the effect of lithium exposure on neurons from SAMR1 and SAMP8 mice 20 days in vitro. The dotted line marks control (100% viability) and maximal recovery. (C) Western blot showing the increase in GSK-3β [Ser9] (inactive enzyme) after lithium treatment is also showed. Bar chart quantifies optical densities of Western blot bands. Open bars, control mice; solid bars, treated mice. Each point represents the mean ± SEM of 3 to 5 cultures, carried out in quadruplicate. *, p < 0.05.
through its neuroprotective activity. To this end, we sought to answer this question by using neuronal cultures from SAMR1 and SAMP8 mice. The addition of lithium to the medium increased the viability of cultures of neurons taken from both strains. To confirm the in vivo experimental results in which we observed a reduction in the activity of apoptotic enzymes such as caspase 3, we found a reduction in the apoptotic features, increased viability, amelioration of morphologic abnormalities, and an increase in the inactive form of GSK-3β in primary neuronal cultures. Moreover, these results were obtained using both lithium and SB415286, a specific inhibitor of GSK-3β, such as effective in vitro pharmacologic tools (54).

In conclusion, based on the in vivo and in vitro results in the SAMP8 mouse senescence model, we suggest that lithium treatment offers new possibilities for slowing the various neurodegenerative processes associated with cerebral aging. This hypothesis should be further examined using behavioral assays and longevity studies. Nevertheless, the ability of lithium to reduce the kinds of neurodegenerative processes that occur in AD and other neurodegenerative diseases is now clearly established.

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REFERENCES

27. Nonaka S, Katsube N, Chuang DM. Lithium protects rat cerebellar granule cells against apoptosis induced by anticonvulsants, phenytoin and carbamazepine. J Pharmacol Exp Ther 1998;286:539–47
41. Shaffer M, Goodenough S, Moosmann B, Behl C. Inhibition of glycogen synthase kinase 3 beta is involved in the resistance to oxidative stress in neuronal HT22 cells. Brain Res 2004;1005:84–89

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62. Caccamo A, Oddo S, Tran LX, LaFerla FM. Lithium reduces tau phosphorylation but not A beta or working memory deficits in a transgenic model with both plaques and tangles. Am J Pathol 2007;170:1669–75