Hypertonic Stress Activates Glycogen Synthase Kinase 3β-mediated Apoptosis of Renal Medullary Interstitial Cells, Suppressing an NFκB-driven Cyclooxygenase-2-dependent Survival Pathway*  

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The survival of renal medullary interstitial cells (RMICs) requires their adaptation to rapid shifts in ambient tonicity normally occurring in the renal medulla. Previous studies determined that cyclooxygenase-2 (COX 2) activation is critical for this adaptation. The present studies find that these adaptive mechanisms are dampened by the simultaneous activation of an apoptotic pathway linked to a glycogen synthase kinase 3β (GSK 3β). Inhibition of GSK 3 by LiCl or specific small molecule GSK inhibitors increased RMIC survival following hypertonic stress, and transduction of RMICs with a constitutively active GSK 3β (AdGSK 3βA9) significantly increased apoptosis, consistent with a pro-apoptotic role of GSK 3β. Following GSK 3β inhibition, increased survival was accompanied by increased COX 2 expression and COX 2 reporter activity. In contrast, GSK 3β overexpression reduced COX 2 reporter activity. Importantly, enhanced RMIC survival produced by GSK 3β inhibition was completely dependent on COX 2 because it was abolished by a COX 2-specific inhibitor, SC58236. The signaling pathway by which GSK 3β suppresses COX 2 expression was then explored. GSK 3β inhibition increased both NFκB and β-catenin activity associated with increased IκB and increased β-catenin levels. The increase in COX 2 following GSK 3β inhibition was entirely blocked by NFκB inhibition using mutant IκB adenovirus. However, adenoviral overexpression of β-catenin did not increase COX 2 levels. These findings suggest that GSK 3β negatively regulates COX 2 expression and that GSK 3β inhibitors protect RMICs from hypertonic stress via induction of NFκB-COX 2-dependent pathway.

To survive and function normally, renal medullary cells rely on a unique ability to withstand the rapidly shifting osmotic environment present in the renal medulla (2). Failure to adapt to the harsh environment in the renal medulla contributes to the development of papillary necrosis, observed following non-steroidal anti-inflammatory drug use, pregnancy, and diabetes mellitus (3). Hypertonic stress activates two opposing cellular signaling cascades that either lead to cell death or promote cell survival. The balance between these two pathways determines cell fate. Several mechanisms have been proposed to contribute to the ability of renal medullary cells to survive hypertonic stress, including accumulation of organic osmoles (4, 5) and induction of heat shock proteins (6, 7). In addition, recent studies demonstrate that renal medullary interstitial cells (RMICs) depend on robust COX 2 activity to adapt to hypertonic stress, both in vitro and in vivo (2, 8, 9). Conversely, the mechanisms contributing to hypertonic stress-induced RMIC cell death are less clearly defined.

GSK 3β signaling has been implicated in a variety of biological processes associated with altered cell survival and differentiation, including Wnt-associated developmental patterning and β-catenin-driven tumorigenesis (10, 11). Recent studies demonstrate that the GSK 3β inhibitor lithium protects neurons from stress-induced cell death (12–17). A link between GSK 3β activity and neuronal cell survival following hypertonic stress has also been suggested, though the downstream mechanisms of these effects are uncharacterized (18). Some data suggest that COX 2 is a downstream target of Wnt/GSK 3β/β-catenin signaling, promoting proliferation and tumorigenesis (19), and previous studies have shown that COX 2 is critical for renal interstitial cell survival. The aim of the present study was to examine whether GSK 3β modulates renal medullary interstitial cell survival through a COX 2-dependent mechanism.

MATERIALS AND METHODS

Cell Culture—Rabbit medullary interstitial cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin (20). Cultures were incubated at 37 °C in 95% air/5% CO2. Osmolality of the control medium was 330 mosmol/kg. Hyperosmotic medium was prepared by adding NaCl to achieve the indicated tonicity.

Cell Viability Analysis—Cell viability was assessed using crystal violet (21, 22). Following hyperosmotic stress for predetermined time periods, culture medium was removed and plates were washed with phosphate-buffered saline. The remaining viable attached cells were stained with 0.5% crystal violet in 50% methanol for 15 min. The plates were then gently rinsed with water and dried. A solution containing 0.1 M citrate sodium in 20% methanol, pH 5.4, was added; 30 min later, the absorbance at 570 nm was read using a spectrophotometer. The percentage of cell survival was defined as the relative crystal violet absorbance of treated versus untreated cells. Remaining unattached cells in the medium were confirmed to be dead by a trypan blue exclusion assay.

Caspase-3/7 Activity—Fluorometric assay of caspase-3/7 activity was conducted using an Apo-ONE™ homogeneous caspase-3/7 assay kit (Promega). This assay uses rhodamine 110, bis(N-CBZ-t-aspartyl-t-glu-tamyl-t-valyl-t-aspartic acid amide) (Z-DEVD-R110) as substrate. Cells were cultured in 12-well plates with 10% fetal bovine serum, 1% (v/v) penicillin, and 0.1% (v/v) streptomycin. The cells were treated with various compounds and then incubated for 24 h. The plates were washed twice with PBS, and the cells were lysed with 50 µL of cell lysis buffer (Promega). The absorbance of each well was measured at 550 nm using a spectrophotometer.

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which was substituted with serum-free medium for 2 h prior to experiment. Cells were subjected to hypertonic stress of varying tonicity for indicated periods of time and lysed using lysis buffer (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 100 μM phenylmethylsulfonyl fluoride, 10 μM of aprotinin/ml, 10 μM of leupeptin/ml, 5100 μM phenylmercaptan, 5 μg/ml of pepstatin, and 0.2% Nonidet P-40). The protein concentration was measured by the bicinonic acid method. All measurements of caspase activity were carried out in triplicate in 96-well clear-bottom plate. Buffer and substrate were mixed at a proportion of 100:1 and incubated with cell lysate for indicated periods of time and lysed using lysis buffer (20 μg of protein/lane) were analyzed via immunoblot for GSK 3β and pGSK 3β Ser-9.

Fluorescence-activated Cell Sorter Analysis of Apoptosis—The level of apoptosis was analyzed in living cultures using a combined annexin V-propidium iodide staining using a Vybrant™ apoptosis assay kit (Molecular Probes, Eugene, OR). Briefly, cells were subjected to hypertonic stress in the presence or absence of GSK 3β inhibitor LiCl and SB 216763 for 8 h. Cells, including dead and floating cells, were harvested and washed in cold phosphate-buffered saline. 100 μl of 1 × 10⁶/ml cells were taken for each assay and stained with PI and annexin V. Negative and positive controls were maintained individually for annexin V and PI. Stained cells were analyzed by flow cytometry (BD FACScan; BD Biosciences) (23, 24).

Immunoblotting—Cells were washed with phosphate-buffered saline and lysed in SDS Lamelli buffer. Protein concentration was determined using bicinchoninic acid protein assay (Sigma). Protein extract (20 μg) was loaded in each lane of a 10% SDS-PAGE mini-gel and run at 120 V. Protein was transferred to a nitrocellulose membrane at 22 V overnight at 4 °C. The membrane was washed three times with TBST (50 mM Tris, pH 7.5, 150 mM NaCl, 0.05% Tween 20) and then incubated with blocking buffer (150 mM NaCl, 50 mM Tris, 0.05% Tween 20, and 5% Carnation™ nonfat dry milk, pH 7.5) for 1 h at room temperature. The membrane was then incubated with anti-mouse antibodies for GSK-3β and β-catenin (1:2500 and 1:500, respectively; BD Transduction Laboratories) or anti-rabbit antibodies for pGSK (Ser-9) (1:1000; Cell Signaling), LeBα (1:1000; Santa Cruz Biotechnology), and COX 2 (1:1000; Cayman) in blocking buffer overnight at 4 °C. After being washed three times, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:5000 for anti-mouse and 1:10,000 for anti-rabbit) for 1 h at room temperature, followed by three 15-min washings. Antibody labeling was visualized by addition of chemiluminescence reagent (Renaissance; PerkinElmer Life Sciences), and the membrane was exposed to Kodak XAR-5 film.

GSK 3β Kinase Activity—Kinase activity of GSK 3β immunoprecipitated from cell lysate was analyzed in vitro. After being subjected to hypertonic stress for the indicated periods of time, cells were lysed on ice in freshly prepared buffer (20 mM Tris-HCl, pH 7.5, 137 mM NaCl, 1 mM EDTA, 20 mM NaF, 1% Triton X-100, 10% glycerol, 1 mM dithiothreitol, 1 mM sodium vanadate, 0.5 μg of aprotinin/ml, 0.5 g of leupeptin/ml, 1 mM phenylmethylsulfonyl fluoride, 1 μM microcystin LR). Lysates were clarified by centrifugation for 10 min at 13,000 rpm in a microcentrifuge and precleared with protein G-Sepharose. 100 μg of total cellular protein diluted in lysis buffer was immunoprecipitated with 1 μg of anti-GSK-3β antibody (mouse monoclonal anti-rat GSK 3β) overnight by rocking incubation at 4 °C followed by addition of protein G-Sepharose and incubation for another 1 h. Immunoprecipitates were washed twice with lysis buffer, twice with wash buffer (10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA, 0.2 mM sodium vanadate, 1 μM microcystin LR), and twice with kinase reaction buffer (20 mM HEPES, pH 7.4, 10 mM MgCl₂, 1 mM dithiothreitol, 0.2 mM EGTA). Activity of the immunoprecipitated GSK-3β was assayed in a total volume of 40 μl of kinase buffer containing 3.75 μM of phospho-glycogen synthase peptide 2, 15 μM cold ATP, and 10 μCi of [³²P]ATP. After 20 min of incubation at 30 °C, 20 μl of SDS lysis buffer was added and incubated at 70 °C to stop the reaction. Reaction mixtures were centrifuged, and 15 μl of the supernatant was spotted onto Whatman P81 phosphoellobulose paper. Filters were washed in three changes of 0.75% phosphoric acid, rinsed in acetone, dried, and [³²P] incorporation measured in a liquid scintillation counter. Non-phosphorylated glycogen synthase peptide was used as negative control, and nonspecific [³²P] incorporation was subtracted from values obtained using the phospho-glycogen synthase peptide.
RESULTS

Hypertonic Stress Increases GSK 3β Activity in Cultured RMICs—Measurement of GSK 3β enzymatic activity using primed glycogen synthase as a substrate showed that hypertonic stress significantly increased GSK 3β activity in a time-dependent manner to 150% of that present in non-stressed cells within 10 min (150 ± 13 versus 100 ± 9; p < 0.005, n = 4) and 171% by 30 min (171 ± 9.5 versus 100 ± 9; p < 0.005, n = 4) (Fig. 1A). Acutely increasing the ambient tonicity from 300 to 550 mosM (addition of 125 mM NaCl) also reduced the immunoreactive inactive form of GSK 3β, phospho-GSK 3β, in cultured RMICs (Fig. 1B). Because total GSK 3β protein expression did not change, these findings are also consistent with increased GSK 3β activity following hypertonic stress.

Fig. 3. A, specific inhibitors of GSK decrease hypertonic stress-induced caspase activity. Cultured RMICs were treated with GSK 3β inhibitors, SB216763 (3 or 5 μM), SB415286 (20 or 30 μM), or LiCl (30 mM) and simultaneously subjected to hypertonic stress. Caspase activity was measured fluorometrically. *, p < 0.05; **, p < 0.001 versus control (vehicle). B, annexin-V and PI staining. Cultured RMICs were treated with GSK 3β inhibitors SB216763 (5 μM) or LiCl (30 mM) and simultaneously subjected to hypertonic stress for 9 h. 1 × 10⁶ cells were stained with annexin and PI as described, followed by flow cytometry. Cell population in the lower left quadrant represents viable cells; those in the lower right, upper right, and upper left quadrants represent early and late apoptotic and necrotic cells, respectively. C, effect of constitutively active GSK 3β on caspase 3/7 activity. Cultured RMICs were transduced with GFP or GSK 3β-A9 via adenoviral vector. 48 h later, caspase activity in whole-cell lysates was fluorometrically assayed as described. *, p < 0.05; **, p < 0.0001 versus AdGFP-infected cells.

Reporter Gene Assay: β-Catenin-TCF/LEF, NFκB, and COX-2—β-Catenin-TCF/LEF reporter plasmid activity (generously provided by Drs. Kinzler and Vogelstein) (25) was determined using 12-well culture plates seeded at a density of 2 × 10⁵ RMICs/well. Following 24 h of growth, cells were transfected with β-catenin-TCF/LEF reporter and a plasmid containing Renilla luciferase driven by TK promoter using SuperFect (QIAGEN). Forty-eight h post-transfection, cells were subjected to hypertonic stress in the presence or absence of specific inhibitors SB216763 (3 or 5 μM) and simultaneously subjected to hypertonic stress. Caspase activity in whole-cell lysates was fluorometrically assayed as described. *, p < 0.05; **, p < 0.001 versus control (vehicle).
Increased GSK 3β Activity Is Associated with Apoptosis—

Shifting tonicity from 300 to either 550 or 600 mosm reduced RMIC viability by 46 ± 9% and 76 ± 5.3%, respectively (Fig. 2A). GSK 3β inhibitor LiCl prevented hypertonic stress-induced cell death. LiCl (30 mM) potently inhibited GSK 3β, demonstrated by increased levels of inactive phospho-GSK 3β (Ser-9) (Fig. 2B). LiCl also increased RMIC viability following hypertonic stress to 80% of that observed in unstressed cells (Fig. 2A).

Studies using more specific small molecule inhibitors of GSK 3β, including SB216763 and SB415286 (32), yielded similar results. When the ambient tonicity was increased from 300 to 600 mosm, apoptosis as measured by caspase 3/7 activity increased 7-fold. Treatment with GSK 3β inhibitors significantly reduced caspase activity by 2–3-fold following hypertonic stress (600 mosm) (305 ± 63 n = 6, at 3 µM SB216763, and 214 ± 57 n = 6 at 5 µM SB216763 versus 692 ± 93 in vehicle-treated cells, p < 0.005 and p < 0.001, respectively). Similar results were obtained with another GSK 3β inhibitor, SB415286, which at the concentrations of 20 and 30 µM reduced hypertonic stress-induced caspase activity in RMICs by 33 and 46%, respectively (Fig. 3A).

Flow cytometric analysis of relative levels of annexin V-PI staining of cells showed that within 8 h of hypertonic stress, 49% of cells become apoptotic compared with control (18%), with 32% cells in the early apoptotic stage and 10 and 7%, respectively, in the late apoptotic and necrotic stages. However, the apoptotic effects could be considerably decreased by simultaneously treating the cells with GSK 3β inhibitors (LiCl, 24%, and SB 216763, 19%) (Fig. 3B). These studies are consistent with a role for GSK 3β activity in promoting cell death following hypertonic stress.

To further confirm the proapoptotic role of GSK 3β, caspase 3/7 activity was examined in unstressed RMICs transduced with a constitutively active GSK 3β adenosinovirus (AdGSK 3βA9). Transduction with AdGSK 3βA9 (30 and 100 multiplicity of infection) increased caspase activity above control AdGFP-transduced RMICs (368% ± 77 and 1043% ± 121 versus 100 ± 43, p < 0.005 and p < 0.0001, respectively) (Fig. 3C).

Inhibition of GSK 3β Up-regulates COX 2 and Promotes Survival of RMICs following Hypertonic Stress—Previous studies suggest that COX 2-derived prostanooids play an important role in promoting RMIC survival following hypertonic stress (2, 9, 20). The effect of GSK 3β inhibitors on COX 2 expression was determined using SB216763, SB415286, or LiCl. All three GSK inhibitors increased COX 2 expression in cultured RMICs (Fig. 4A), suggesting GSK 3β activity tonically suppresses COX2 levels in RMICs. GSK 3β inhibitors also significantly increased COX 2 reporter activity in LiCl-treated (3-fold) and SB216763-treated (3.8-fold) RMICs (Fig. 4B). Conversely, RMICs transfected with a plasmid for wild type GSK 3β reduced COX 2 reporter activity to less than half of control (Fig. 4B).

To examine whether protective effects of GSK 3β inhibitors on RMIC survival depend on COX 2 activity, the effect of a COX 2-selective inhibitor (SC58236, 10 µM) (20) was examined. Treatment of cells with SC58236 completely abolished the protective effects of GSK 3β inhibitors Li or the SB compounds (Fig. 5) following hypertonic stress. This concentration of COX 2 inhibitor did not have any effect on RMIC survival in nonstressed cells.

GSK 3β Activity Modulates COX 2 Expression via an NFκB-dependent Pathway—Activation of NFκB contributes to increased COX 2 expression following stress (2, 33). Recent studies indicate that NFκB activity may be regulated by GSK 3β (34, 35). To test whether COX 2 induction by GSK 3β inhibitors required NFκB, RMICs were transduced with an adenosivirus encoding mutant dominant negative inhibitor of NFκB (IκB). The IκB(mut) reduced COX 2 expression in cells treated with the GSK 3β inhibitor SB216763 (Fig. 6A). These findings support a role for NFκB in the COX 2 induction by GSK 3 inhibitors.

Activation of GSK 3β also suppressed NFκB-driven gene expression. When GSK 3β was overexpressed (transfection) in RMICs, NFκB reporter activity significantly decreased by 32% (100 ± 8 versus 69 ± 6, p < 0.001). Conversely, the GSK inhibitors LiCl or SB216763 increased NFκB reporter activity by 31 and 60%, respectively, compared with controls (131 ± 14, 160 ± 8 versus 100 ± 8, p < 0.01 and 0.001, respectively) (Fig. 6B). GSK 3 inhibitors also completely blocked the ability of trans-
normalcy occurring in the renal medulla. Studies in cultured RMICs show that abruptly doubling tonicity (from 300 to 600 mosM) overwhelms these adaptive mechanisms, leading to cell death (9). The present studies suggest that GSK 3β activity is an important signaling pathway contributing to RMIC death following hypertonic stress and that GSK 3β antagonizes the COX 2-dependent cell survival pathway (Fig. 8). This conclusion is based on several lines of evidence: (a) hypertonicity increased GSK 3β activity; (b) GSK 3β activation caused cell death, and its inhibition promoted RMIC survival; (c) COX 2 inhibition abolished the cytoprotective effects of GSK 3 inhibitors; and (d) the activation of GSK 3β reduced COX 2 expression, whereas its inhibition increased COX 2.

GSK 3β is a highly conserved protein kinase thought to be constitutively active in differentiated cells (1). It is an important component of the Wnt signaling pathway, and its inhibition by Wnt signaling is believed to play a critical role in cell proliferation during embryogenesis. Constitutive GSK 3β activity can be suppressed by a variety of stimuli, including Wnt ligands, insulin, epidermal growth factors, and fibroblast growth factor (10), mainly via phosphorylation of the NH2-terminal serine 9 residue (38). Conversely, GSK 3β may be activated by stress, including hypoxia in A7r5 cells (39) or potassium deprivation of sensory neurons (40). Altered GSK 3β activity has been implicated in several human diseases, including cancer, diabetes mellitus, and Alzheimer’s (1).

The present studies provide evidence suggesting a role for GSK 3β activation regulating renal medullary interstitial cell survival following hypertonic stress in renal medullary cells. Hypertonic stress increased GSK 3β kinase activity and decreased levels of the inactive phospho-GSK 3β (Ser-9) in a time-dependent manner. Whether reduced activity of inhibitory kinases (e.g., protein kinase B), which normally phosphorylate and inactivate GSK 3β, or higher phosphatase activity, or both contribute to this activation of GSK 3β remains unexplored.

Three distinct GSK 3 inhibitors, LiCl, SB216763, and SB415286, reduced RMIC apoptosis following hypertonic stress as indicated by caspase 3/7 activity and annexin V-PI staining. The concordance of results obtained with LiCl and selective small molecule ATP-competitive inhibitors of GSK 3 activity, including SB415286 and SB216763, is important because the latter compounds are structurally distinct and highly selective for GSK 3 (32). Furthermore, the potency of these GSK 3β-selective inhibitors in preventing RMIC death correlated well with their relative in vitro potency of inhibiting GSK 3β, with SB216763 being more potent than SB415286 (40). Pro-apoptotic effects of GSK 3β in RMICs agree with previous observations, showing GSK 3β activation to be involved in neuronal cell death following hypoxia and serum deprivation (17, 39).

The mechanism by which activated GSK 3β promotes cell death is poorly understood. Previous studies have established that COX 2-derived prostanoids play a critical role in RMIC survival (2, 43). Of relevance to the present study are previous observations that LiCl induces COX 2 expression in mouse mammary epithelial cells (44). The present studies demonstrate that GSK 3 inhibition not only induces COX 2 expression but also protects RMICs from death following hypertonic stress. COX 2 activity is crucial for RMIC survival because the COX 2-selective inhibitor SC58236 completely abolished the protective effect of GSK 3β inhibitors. These findings provide a functional correlate to the induction of COX 2 by GSK 3β inhibitors and link COX 2 function to cytoprotection afforded by GSK 3β inhibitors.

COX 2 expression can be induced via multiple mechanisms, including increased mRNA transcriptional rates, mRNA stabi-
Fig. 7. A, β-catenin protein levels. Cultured RMICs were subjected to hypertonic stress (550 mosM) for 12 h. Total cell lysates (20 μg of protein/lane) were immunoblotted for β-catenin. B, β-catenin protein levels in the presence of GSK 3β inhibitors. RMICs were treated with SB216763 or SB415286 for 8 h. Total cell lysates (20 μg of protein/lane) were immunoblotted for β-catenin. C, TCF/LEF activity in the presence of LiCl. RMICs were co-transfected with TCF/LEF-driven luciferase vector and TK driving Renilla luciferase plasmid. 24 h later, cells were subjected to hypertonic stress (500 and 550 mosM). 12 h later, luciferase activities were tested as described. β-Catenin reporter activity was normalized by Renilla luciferase activity. *, p < 0.005 versus control (300 mosM). D, TCF/LEF activity in the presence of LiCl. Cultured RMICs were co-transfected with β-catenin-TCF/LEF-driven luciferase vector and TK driving Renilla luciferase plasmid. 24 h later, cells were treated with LiCl (30 μM). 12 h later, luciferase activities were tested as described. β-Catenin reporter activity was normalized by Renilla luciferase activity. *, p < 0.01 versus control. E, COX 2 protein levels in cells overexpressing β-catenin. Cultured RMICs were transduced with Adβ-catenin (75 multiplicity of infection) as described. After 72 h, cells were lyzed and immunoblotted for COX 2. F, TCF/LEF activity in cells overexpressing β-catenin. Cultured RMICs were transduced with TCF/LEF-driven luciferase vector and TK driving Renilla luciferase plasmid. 24 h later, they were transduced with Adβ-catenin. 32 h later, luciferase activities were tested as described. TCF/LEF reporter activity was normalized to Renilla luciferase activity. *, p < 0.001 versus control.

Fig. 8. Biochemical pathways leading cell survival versus apoptosis following hypertonic stress.

GSK 3 Inhibition Promotes Renal Interstitial Cell Survival

GSK 3β/β-catenin pathway is unlikely to play a significant role in modulating RMIC COX 2 expression following osmotic stress. In contrast, the present evidence supports a major role for NFκB in mediating COX 2 up-regulation by GSK. NFκB activation occurs in response to a variety of cellular stresses, and inhibition of NFκB can lead to apoptosis (47, 48). Importantly, previous studies demonstrated that hypertonic stress activates an NFκB-COX 2-linked survival mechanism in RMICs (2). In its inactive form, NFκB exists as a cytosolic complex bound to the inhibitory protein IκB (49). Phosphorylation of IκB by a specific kinase (IKK) leads to ubiquitin/ATP-dependent proteasomal degradation of IκB, releasing p65/RelA heterodimer to translocate to the nucleus and transactivate target genes (50). The functional relationship between NFκB and GSK 3β remains controversial, with some reports suggesting that GSK 3β activity is necessary for NFκB activation (35, 51) and others showing that GSK 3β activity inhibits NFκB (34, 52). Genetic disruption of GSK 3β did not affect the early steps of NFκB activation (degradation of IκB and nuclear translocation of NFκB) in embryonic fibroblasts (35). Nevertheless, GSK 3β was recently found to phosphorylate the COOH terminus of p65 subunit of NFκB in vitro, which could increase NFκB-mediated gene transactivation (51). Conversely, an inhibitory effect of GSK 3β on NFκB was shown in PC12 cells where Wnt-1-induced activation of NFκB could be mimicked by inhibition of GSK 3β (34). Similar observations were made in astrocytes in which overexpression of constitutively active GSK 3β stabilized IκB and reduced IKK activity (52). The present studies demonstrate that inhibition of GSK 3β activates NFκB.
and COX 2 expression, consistent with findings that GSK 3β decreases NFkB activity.

The use of GSK 3 inhibitors, including lithium, has been proposed as a therapeutic intervention in a variety of diseases, including diabetes mellitus, neurodegeneration, cancer, and inflammation (53). Lithium has been widely used for the treatment of bipolar disorders and Alzheimer’s disease (54). Its use in this setting is associated with reproducible renal side effects, including diabetes insipidus and chronic renal tubulointerstitial nephropathy (41). Though the cellular basis of these renal effects are uncertain, it is of interest that urinary prosta
glandin E2 excretion is increased in rats receiving Li (42), consistent with up-regulation of renal cyclooxygenase by lithium. Whether increased renal PGE2 production occurs as a result of increased COX 2 expression following Li treatment remains to be determined. The present findings are consistent with this possibility. Furthermore, increased renal interstitial cell num-
ber because of enhanced cell survival could contribute to renal tubulo-interstitial cell infiltrates observed in the setting of chronic lithium exposure (41). The concordance between the effects of lithium and other GSK 3 inhibitors on renal interstitial cell function suggests that newer pharmacologic inhibitors of GSK 3β could have similar renal effects as lithium.

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