Uric acid inhibits renal proximal tubule cell proliferation via at least two signaling pathways involving PKC, MAPK, cPLA₂, and NF-κB

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by at least two distinct signaling pathways, one that involves the activation of NF-κB and another that involves the activation of cPLA₂, which occurs via the initial activation of PKC, and subsequent activation of p38 MAPK.

MATERIALS AND METHODS

Materials. Adult New Zealand White male rabbits (1.5–2.0 kg) were from Dae Han Experimental Animal Co., (Chungju, Korea). All procedures for animal management followed the standard operation protocols of Chonnam National University. An Institutional Review Board at Chonnam National University approved our research proposal and relevant experimental procedures, including animal care. Appropriate management of experimental samples and quality control of the laboratory facility and equipment were maintained. Class IV surgical gloves and soybean trypsin inhibitor were from Life Technologies (Grand Island, NY). Uric acid, mepacrine, arachidonyl trifluoromethyl ketone (AACOCF₃), SP 600125, and SB 203580 were from Calbiochem (La Jolla, CA). [³H]thymidine and [γ-³²P]ATP were from DuPont/NEN (Boston, MA), and Liquiscint was from National Diagnostics ( Parsippany, NJ). Antibody to p44/42, p38, SAPK/JNK, PKC, cPLA₂, and IkB-α were from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were of the highest purity commercially available.

Isolation of rabbit renal proximal tubules and culture conditions. Primary rabbit kidney proximal tubule cell cultures were prepared by a modification of the method of Chung et al. (7). Kidneys were perfused via the renal artery, first with PBS, and then with DMEM/F-12 containing 0.5% iron oxide (wt/vol) until the kidney turned gray-black. Renal cortical slices were disrupted (4 strokes; Dounce homogenizer), and the homogenate was passed sequentially through a 253-μm filter and an 83-μm mesh filter. The tubules andglomeruli on the top of the 83-μm filter were transferred into sterile DMEM/F-12, and glomeruli containing iron oxide were removed using a magnetic stirring bar. The remaining proximal tubules were briefly incubated in DMEM/F-12 containing 60 μg/ml collagenase (class IV) and 0.025% soybean trypsin inhibitor, washed by centrifugation, and resuspended in DMEM/F-12 containing three growth supplements (5 μg/ml insulin, 5 μg/ml transferrin, and 5 × 10⁻⁶ M hydrocortisone). After plating, the PTCs were maintained at 37°C in a 5% CO₂ humidified environment in DMEM/F-12 medium containing the three supplements. The medium was changed once a day after plating and then every 3 days. PTC cultures possess a number of characteristics typical of proximal tubules, including Na⁺-dependent α-methylglucoside uptake, PTH-sensitive cAMP synthesis, and the brush-border enzymes alkaline phosphatase. These characteristics differ from those of primary cell cultures derived from unpurified rabbit kidney preparations.

[³H]thymidine incorporation. The rate of DNA synthesis was determined by [³H]thymidine incorporation studies (6). To summarize, PTCs were incubated in either the presence or absence of uric acid for 24 h, followed by a 24-h incubation with 1 μCi of [methyl-³H]thymidine (37°C). The cultures were washed with PBS, fixed (10% trichloracetic acid, 23°C, 15 min), and then washed twice (5% TCA). The TCA-precipitable material was solubilized (2 N NaOH, 23°C), and the radioactivity was counted in a liquid scintillation counter. (LS 6500, Beckman Instruments, Fullerton, CA), using Liquiscint scintillation fluid. All experiments were performed in triplicate, and values were compared percentagewise to the control (mean counts/min in the presence of uric acid divided by mean counts/min in the absence of uric acid and multiplied by 100).

Cell viability and LDH assay. The number of cells and viability were counted using the following methodology. The cells were washed twice with PBS and trypsinized from the culture dishes; then, the cell suspension was mixed with a 0.4% (wt/vol) trypsin blue solution and the number of live cells was determined using a hemocytometer. Cells failing to exclude the dye were considered nonviable. Cell injury was assessed by LDH activity. The level of LDH activity in the medium was measured by using a LDH assay kit. For measurement of LDH activity, PTCs were treated with different concentration of uric acid for 8 h. LDH activity was expressed as the percentage of control.

Arachidonic acid release. To quantitate arachidonic acid (AA) release by modification of the method of Xing et al. (44), confluent PTCs were incubated for 24 h (DMEM/F-12+0.5 μCi/ml [³H]AA and the 3 growth supplements). The monolayers were then washed and incubated for 1 h (37°C; DMEM/F-12 with specified factors). At the end of the incubation, the medium was transferred to ice-cold tubes containing 55 mM EGTA and 5 mM EDTA, centrifuged (12,000 g), and soluble material was counted in a liquid scintillation counter. Both the [³H]AA released and cell-associated [³H]AA were standardized with respect to protein. Then, released [³H]AA was compared percentagewise to cell-associated [³H]AA (present at the beginning of the incubation).

Membrane preparation for cPLA₂ and PKC blotting. The day before the experiment, the medium was changed and after appropriate treatments, the medium was removed. The cells were washed in PBS and removed by scraping into PBS. After microcentrifugation, the cells were resuspended in buffer A (in mM: 137 NaCl, 8.1 Na₂HPO₄, 2.7 KCl, 1.5 KH₂PO₄, 2.5 EDTA, 1 dithiothreitol, and 0.1 PMSF, as well as 10 μg/ml leupeptin, pH 7.5) and lysed by trituration with a 21.1-gauge needle. The lysates were first centrifuged (1,000 g, 10 min, 4°C), followed by centrifugation (100,000 g, 1 h, 4°C). The particular fractions were suspended in buffer A, washed by centrifugation, and finally resuspended in buffer A containing 0.05% (vol/vol) Triton X-100. The protein content of each fraction was quantified by the Bradford procedure (5).

Western blot analysis. PTCs were solubilized in sample buffer (10% SDS, 20% glycerol, 2% β-mercaptoethanol, 2.9 mM Tris, pH 6.8). Samples (20 μg) subjected to electrophoresis through 10% SDS-polyacrylamide gels were transferred to nitrocellulose. The nitrocellulose blots were blocked with 5% skim milk in TBST (10 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20) for 1 h and incubated with primary antibody at dilutions recommended by the supplier. The blots were then washed and then incubated with goat anti-rabbit-IgG conjugated to horseradish peroxidase. The bands were visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech).

EMSA. EMSAs were performed as previously described by Jeon et al. (25) with modification. PTCs were lysed in hypotonic buffer (10 mM HEPES, 1.5 mM MgCl₂, 1% Nonidet P-40, pH 7.5) and centrifuged (3,000 g, 5 min). The nuclear pellet was solubilized in hyper tonic buffer (in mM: 30 HEPES, 1.5 MgCl₂, 450 KCl, 0.3 EDTA, 1 DTT, and 1 PMSF, as well as 10% glycerol, 1 μg/ml of aprotinin, and 1 μg/ml of leupeptin), and, after lysis, was centrifuged (14,500 g, 15 min). DNA binding assays were then conducted (36) by incubating nuclear extracts (5 μg) with poly (dI-dC) and a [γ-³²P]-labeled DNA probe (5'-GAT-CTC-AGA-GGG-GAC-CTT-CG-AGA-GA-3') in binding buffer (in mM: 100 KCl, 30 HEPES, 1.5 MgCl₂, 0.3 EDTA, 1 DTT, and 1 PMSF, as well as 10% glycerol, 1 μg/ml of aprotinin, and 1 μg/ml of leupeptin) for 10 min. Nuclear protein/DNA complexes were separated from free probe by electrophoresis through a 4.8% polyacrylamide gel in 0.5x TBE buffer. Following electrophoresis, the gel was dried and subjected to autoradiography.

Statistical analysis. Results were expressed as means ± SE. The difference between two mean values was analyzed by means of ANOVA. Differences were considered statistically significant when P < 0.05.

RESULTS

Effect of uric acid on [³H]thymidine incorporation. The possibility that uric acid affects DNA synthesis was evaluated
thymidine incorporation was significantly reduced (20% inhibition at 10⁻⁴ M, and up to 70% inhibition at 10⁻³ M), although there is no cytotoxic effect within these concentration ranges (Table 1). In humans, normal uric acid levels are higher than in the rabbit (up to 350 μM), and hyperuricemia is observed at uric acid levels >420 μM. For this reason, all subsequent experiments were performed using 5 × 10⁻³ M uric acid for 8 h.

Involvement of PKC and MAPKs in the uric acid-induced inhibition of [³H]thymidine incorporation. Previously, PKC was proposed as being a possible mediator of the effects of uric acid on vascular smooth muscle cell proliferation (38). For this reason, the involvement of PKC as a mediator of the inhibitory effect of uric acid on [³H]thymidine incorporation was examined. Three different PKC inhibitors, bisindolylmaleimide I (10⁻⁷ M), staurosporine (10⁻⁹ M), and H-7 (10⁻⁷ M), were employed. Figure 2A shows that each of the three PKC inhibitors individually blocked the uric acid-induced inhibition of [³H]thymidine incorporation. This result suggests that PKC activation is required for the effect of uric acid on [³H]thymidine incorporation to be elicited. Indeed, Fig. 2B shows evidence of the translocation of PKC from the cytosol to the particulate fraction following treatment with uric acid.

Previously, uric acid was observed to activate MAPK in vascular smooth muscle cells, an event that was associated with the stimulatory effect of uric acid on vascular smooth muscle cell growth (30). Thus the possibility was evaluated that MAPKs are similarly involved in mediating the uric acid-induced inhibition of [³H]thymidine incorporation in PTCs. Figure 3A shows the effect of uric acid on the activation of p38 MAPK, SAPK/JNK, and p44/42 MAPK over a 4-h incubation period. In uric acid-treated cultures, the level of phosphorylation of p38 MAPK and SAPK/JNK increased ∼3.5-fold between a 10- and 15-min incubation period and then decreased to the control level (the level observed at time 0). As observed with p38 MAPK, p44/42 MAPK phosphorylation also increased transiently, starting from 10 min, but returning to control levels following a 4-h incubation with uric acid (Fig. 3A). Possibly, the activation of either p38 MAPK, SAPK/JNK, or p44/42 MAPK is required to observe the uric acid-mediated inhibition of [³H]thymidine incorporation. The effects of the MAPK inhibitors SB 203580 (p38 MAPK), SP 600125 (SAPK/JNK), and PD 98059 (p44/42 MAPK) on the uric acid-mediated inhibition of [³H]thymidine incorporation was examined. Figure 3B shows that both SB 203580 and SP 600125 prevented the uric acid-induced inhibition of [³H]thymidine incorporation, unlike the case with PD 98059.

Relationship among cPLA₂, PKC, and MAPKs in the uric acid-induced inhibition of [³H]thymidine incorporation. The relationship between PKC and MAPK in mediating the uric acid-induced inhibition of [³H]thymidine incorporation was further examined. The involvement of p38 MAPK, SAPK/JNK, and p44/42 MAPK in the uric acid-induced inhibition of [³H]thymidine incorporation was examined. The effects of the MAPK inhibitors SB 203580 (p38 MAPK), SP 600125 (SAPK/JNK), and PD 98059 (p44/42 MAPK) on the uric acid-mediated inhibition of [³H]thymidine incorporation was examined. Figure 3B shows that both SB 203580 and SP 600125 prevented the uric acid-induced inhibition of [³H]thymidine incorporation, unlike the case with PD 98059.

Table 1. Effects of uric acid on proximal tubule cell growth and cytotoxicity

<table>
<thead>
<tr>
<th>Uric acid (M)</th>
<th>Control</th>
<th>10⁻³ M</th>
<th>5 × 10⁻⁵ M</th>
<th>10⁻⁴ M</th>
<th>10⁻³ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>370.0±8.9</td>
<td>334.5±19.2</td>
<td>279.5±14.1</td>
<td>247.0±18.5</td>
<td>185.4±14.2</td>
</tr>
<tr>
<td>Cell Number, ×10⁴/dish</td>
<td>100.0±1.1</td>
<td>98.8±0.9</td>
<td>96.3±0.1</td>
<td>99.6±1.1</td>
<td>97.4±0.5</td>
</tr>
<tr>
<td>Cell Viability, % of control</td>
<td>1.0</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
</tr>
</tbody>
</table>

Values are the means ± SE of 3 independent experiments with triplicate dishes. Primary cultured renal proximal tubule cells were treated with 10⁻³, 5 × 10⁻⁵, 5 × 10⁻⁴, or 10⁻³ M uric acid, respectively. Then, cell count, viability, and lactate dehydrogenase (LDH) assay were conducted. *P < 0.05 vs. control.
acid-induced inhibition of $[^3]$H$\text{thymidine}$ incorporation was examined. The possible involvement of cPLA$_2$ was also examined. Figure 4 shows that the uric acid-induced activation of p38 MAPK was blocked by H-7 (a PKC inhibitor, $10^{-6}$ M) as well as by SB 203580 (a p38 MAPK inhibitor, $10^{-7}$ M), but not by AACOCF$_3$ (a cPLA$_2$ inhibitor, $10^{-6}$ M). These observations are consistent with the hypothesis that MAPK activation that occurs in response to uric acid is dependent on the activation of PKC, rather than cPLA$_2$.

However, these latter observations do not exclude the hypothesis that PLA$_2$ activation is a part of the response of PTCs to elevated uric acid. To evaluate whether PLA$_2$ is involved in the uric acid response, the effect of uric acid on $[^3]$H$\text{AA}$ release was examined. Figure 5A shows that $[^3]$H$\text{AA}$ release increased significantly after a 1-h incubation with uric acid at a concentration of $10^{-4}$ M. Also consistent with the involvement of PLA$_2$ were our observations indicating that following uric acid treatment, cPLA$_2$ translocated from the cytosol to the particulate fraction (Fig. 5B). The observation (Fig. 5C) that the uric acid-induced inhibition of $[^3]$H$\text{thymidine}$ incorporation was

![Graph](image_url)

**Fig. 2.** Effects of PKC inhibitors on uric acid-induced inhibition of $[^3]$H$\text{thymidine}$ incorporation and PKC translocation. A: PTCs were treated with bisindolylmaleimide I ($10^{-6}$ M), staurosporine ($10^{-8}$ M), or H-7 ($10^{-6}$ M) for 30 min before treatment with uric acid ($5 \times 10^{-4}$ M) for 8 h. Values are means ± SE of 3 independent experiments with triplicate dishes. Open bars, control; filled bars, uric acid. *$P < 0.05$ vs. control. **$P < 0.05$ vs. uric acid alone. B: PKC protein that was present in either the cytosolic or membrane fraction was then detected by means of Western blotting, as described in MATERIALS AND METHODS. The arrow indicates the 80-kDa band corresponding to PKC. The example shown is a representative of 4 experiments. The data are expressed as a percentage of basal value in each fraction and are the means ± SE of 4 independent experiments. *$P < 0.05$ vs. each control.

![Graph](image_url)

**Fig. 3.** Time course of mitogen-activated protein kinase (MAPK) activity by uric acid and effects of SP 600125, SB 203580, or PD 98059 on uric acid-induced inhibition of $[^3]$H$\text{thymidine}$ incorporation. A: PTCs were treated for different time intervals (0–240 min) with uric acid. Then, phosphorylated p38, SAPK/JNK, and p44/42 MAPKs were detected, as described in MATERIALS AND METHODS. The experiment shown is representative of 4 experiments. Bottom: data are expressed as a relative increase in basal value and the means ± SE of 4 independent experiments. Open bars, phospho p38; filled bars, phospho SAPK/JNK; hatched bars, phospho p44/42. B: PTCs were treated with SP 203580 (a p38 MAPK inhibitor), SB 600125 (a SAPK/JNK inhibitor), or PD 98059 (a p44/42 MAPK inhibitor; $10^{-6}$ M) for 30 min before treatment with uric acid or were incubated with uric acid alone for 8 h. Values are means ± SE of 3 or 5 independent experiments with triplicate dishes. Open bars, control; filled bars, uric acid, *$P < 0.05$ vs. control. **$P < 0.05$ vs. uric acid alone.
blocked by AACOCF<sub>3</sub> (a cPLA<sub>2</sub> inhibitor) and mepacrine (a nonspecific PLA<sub>2</sub> inhibitor) was also consistent with the involvement of PLA<sub>2</sub> as well as PKC and MAPK.

Possibly, PLA<sub>2</sub> activation is dependent on the activation of both PKC and p38 MAPK by uric acid. Consistent with this hypothesis, H-7 (10<sup>-7</sup> M), SB 203580 (10<sup>-6</sup> M), and AACOCF<sub>3</sub> (10<sup>-6</sup> M) all blocked the uric acid-induced increase in [H]<sup>3</sup>AA release (Fig. 6). Nuclear factor-κB is a transcription factor that has been observed to regulate proteins such as cPLA<sub>2</sub> in inflammatory disease (33). However, SN 50 (a NF-κB inhibitor, 500 ng/ml) did not significantly prevent the effect of uric acid on [H]<sup>3</sup>AA release by the PTCs.

**Involvement of NF-κB in the uric acid-induced inhibition of [H]<sup>3</sup>thymidine incorporation.** Because uric acid has been reported to activate NF-κB in other cell types (20), the possible involvement of NF-κB in uric action in PTCs was nonetheless further examined. The activated form of NF-κB is composed of two proteins, p65 (Rel A) and a p50 subunit. Cytoplasmic...
Fig. 7. Time course of uric acid on NF-κB activation and DNA binding activity. PTCs were treated for different time intervals (0–90 min) with uric acid. The levels of nuclear NF-κB p65 and cytosolic phospho IκB-α and NF-κB activity were determined by Western blotting (A) and EMSA (B), respectively. Band represents 65 kDa of NF-κB p65 and 41 kDa of phospho IκB-α. Bottom: data are expressed as a relative increase in basal value in each fraction and are the means ± SE of 4 independent experiments. *P < 0.05 vs. control.

Previously, the effects of uric acid on renal proximal tubule cells have not been extensively studied. However, an understanding of the effects of uric acid on renal PTCs is important, as the renal proximal tubule is the major site for uric acid reabsorption and secretion in the kidney (16). After initial filtration by the glomerulus, uric acid is subsequently reabsorbed by URAT1, an apical urate/anion exchange system in the renal proximal tubule (22). Reabsorbed uric acid may also be secreted in the renal proximal tubule via basolateral organic anion transporters OAT1 and OAT3 (in exchange for dicarboxylate), as well as by apical voltage-driven organic anion efflux transporters (OATv1) and apical MRP4, a member of the ATP-binding cassette transporter family (22).

The renal PTC culture system utilized in these studies has previously been shown to possess transport systems typical of the renal proximal tubule, including a high-affinity Na⁺-glucose cotransport system typical of the straight portion of the renal proximal tubule, rather than the proximal convoluted tubule, which possesses a low-affinity, high-capacity Na⁺-glucose cotransport system (40). Thus we cannot exclude the possibility that PTCs are also a model system for studying urate reabsorption, although our primary PTC culture system models are of urate-secreting renal PTCs. Although we have not directly measured intracellular urate accumulation or secretion in our studies, we have previously shown that our primary PTC cell culture system possesses a p-aminohippurate (PAH) transport system (45). Indeed, such a PAH transport system (OAT1 and OAT3) has been proposed to be responsible for the secretion of uric acid as well as organic anions such as PAH (16). Thus the results of our studies can be interpreted as indicating that under conditions of hyperuricemia, the transported uric acid inhibits the proliferation of urate-secreting renal PTCs.
In these studies, uric acid was observed to inhibit the incorporation of \(^{3}\text{H}\)thymidine into DNA by up to 70\% following a 24-h incubation period with 1 mM uric acid. The uric acid concentration utilized in the majority of the experiments in this report was 500 \(\mu\text{M}\), a concentration within the range observed in hyperuricemia. The level of inhibition of \(^{3}\text{H}\)thymidine incorporation obtained at 500 \(\mu\text{M}\) uric acid was \(\approx 40\%\) following a 24-h incubation with \(^{3}\text{H}\)thymidine. These observations do not exclude the possibility of a more complete inhibition of DNA synthesis following more prolonged exposure to uric acid at this concentration. Alternatively, a complete inhibition of \(^{3}\text{H}\)thymidine incorporation may be obtained immediately under the conditions employed, but affecting only a subset of the cells present in the primary cultures.

Uric acid reportedly stimulates rat vascular smooth muscle cell proliferation in vitro (28, 34). The observed effects of uric acid on vascular smooth muscle cells cannot be explained by the activation of receptors for uric acid. Vascular smooth muscle cells are very likely responding to the effects of intracellular rather than extracellular uric acid. Vascular smooth muscle cells possess organic anion transporters responsible for urate uptake. In vascular smooth muscle cells, uric acid has been observed to activate two classes of MAP kinases (p44/42 MAPK and p38 MAPK), resulting in the activation of NF-\(\kappa\)B and ultimately an increase in cell proliferation (26). The MAPK activation observed in vascular smooth muscle cells has been reported to result in the activation of NF-\(\kappa\)B, which results in increased production of growth factors (including PDGF) and increased cell growth.

Unlike the case with vascular smooth muscle cells, we have observed here that uric acid inhibits the proliferation of renal PTCs. Nevertheless, similarities in the mechanism of uric acid action were observed in the PTCs. As shown in Fig. 9, uric acid activates PKC in PTCs, a necessary event for the activation of MAPK. However, unlike the case with vascular smooth muscle cells, in PTCs only p38 MAPK and SAPK/JNK are activated in response to uric acid, rather than p44/42 MAPK (which may...
explain the absence of a growth-stimulatory effect of uric acid in PTCs). NF-κB activation is observed in the uric acid-treated PTCs, as was observed in the vascular smooth muscle cells. NF-κB activation is essential for eliciting the inhibitory effect of uric acid on cell proliferation, rather than on growth. The activation of cPLA2 was observed in PTCs in response to uric acid. Although PLA2 activation was dependent on the activation of PKC and p38 MAPK by uric acid, our results indicated that NF-κB was not involved. Thus our results indicate that the uric acid-mediated inhibition of PTC proliferation is dependent on two distinct pathways, one involving PKC, p38 MAPK, and cPLA2, and the other involving NF-κB.

These differences in responsiveness may be due to differences in the concentrations of uric acid employed in the studies. The present report of an inhibitory effect of uric acid on renal PTC proliferation is consistent with the observed development of renal insufficiency in subjects with elevated uric acid (24). In the present study, uric acid (at concentrations over 10^{-4} M, or >2 mg/dl) were observed to inhibit thymidine incorporation into acid-precipitable material. This uric acid concentration (500 μM) is within the range known to have deleterious physiological effects. Hyperuricemia is usually defined as >7 mg/dl (0.4 mM) in men and >6.5 mg/dl (>0.39 mM) in women (26). Normal uric acid concentrations in humans are normally much higher than in other mammals due to the absence of uricase activity in humans and other primates. Indeed the uricase mutation has been proposed to have provided an evolutionary advantage because of the antioxidant action of uric acid (26). However, this does not explain the observation that patients with higher uric acid levels with cardiovascular disease generally have worse outcomes (26). Nevertheless, despite the lower levels of uric acid in other mammalian species, these other species have been successfully shown to model human hyperuricemia. An excellent example is the hyperuricemia, gout, and urate nephropathy observed in urate oxidase-deficient mice (44).

To our knowledge, this is the first demonstration that uric acid can promote the translocation of PKC from the cytosol to the membrane fraction of renal PTCs. The role of PKC as a mediator of the inhibitory effect of uric acid on [3H]thymidine incorporation was also indicated by our results with three PKC inhibitors, including bisindolylmaleimide I, staurosporine, and H-7. Each of these PKC inhibitors individually blocked the inhibitory effect of uric acid on [3H]thymidine incorporation. Although both bisindolylmaleimide I and staurosporine can also inhibit cAMP-dependent protein kinase, at the concentrations of bisindolylmaleimide I and staurosporine utilized in this report (10^{-7} and 10^{-9} M, respectively), both of these inhibitors selectively inhibit PKC rather than PKA. Unlike the case with either bisindolylmaleimide I or staurosporine, H-7 is a relatively less-specific inhibitor. Nevertheless, the observation that PKC is translocated from the cytosol to the particulate fraction following treatment with uric acid, in combination with the observed effects of all three inhibitors, strongly suggests that PKC plays a role in mediating the inhibitory effect of uric acid on the proliferation of renal PTCs.

MAPKs also play critical roles in mediating the response of renal cells to stress and in promoting renal cell growth and survival (3, 15, 37). Like most cells, three classes of MAPKs are expressed in renal PTCs, including extracellular-signal regulated kinases 1 and 2 (ERK 1/2), c-jun NH2-terminal kinase (JNK), and p38 MAPK (15). However, little is known about the effect of uric acid on MAPKs in renal PTCs. The results of our investigations indicate that uric acid stimulates the phosphorylation and activation of both p38 MAPK and SAPK/JNK, whereas p44/42 MAPK was unaffected. In addition, our studies with the p38 MAPK inhibitor SB 203580, the SAPK/JNK inhibitor SP 600125, and the MEK1 inhibitor PD 98059 are also consistent with a role of p38 MAPK as well as SAPK/JNK in mediating the inhibitory effect of uric acid on [3H]thymidine incorporation. At the concentration utilized in this report, SP200125 is a highly selective inhibitor of SAPK/JNK as opposed to either p38 MAPK, p44/42 MAPK, or PKA (11).

To our knowledge, our observation that uric acid causes the activation of JNK is novel and suggestive of a new mechanism that underlies the inhibitory effect of uric acid on the proliferation of PTCs. This result is consistent with a previous report that an increase in uric acid on the inhibition of JNK was associated with improved renal function following injury, and an accelerated rate of renal repair as normal renal function is once again obtained (9).

Our results suggest that the activation of both PKC and p38 MAPK by uric acid is a contributing factor, which results in the phosphorylation of cPLA2 and an increased release of AA from the PTCs, as observed in other experimental systems (4, 10, 23, 30). The activation of PLA2 has been associated with processes that lead to renal cell proliferation (1, 39). However, our present results indicate that the activation of cPLA2 is a part of the process that leads to the uric acid-induced inhibition of renal PTC proliferation.

Our observation of the activation of NF-κB in response to uric acid is in agreement with previous reports with mononuclear phagocytes and smooth muscle cells (27, 31). In these previous reports, NF-κB activation by uric acid resulted in a stimulation of cell proliferation, unlike the case with our PTCs. However, the activation of NF-κB is not necessarily associated with an increase in cell growth. Indeed, previous reports with cultured endometrial cells and monocytes show an association between NF-κB activation and growth inhibition (2, 20). Differences in response may be explained by cell type differences, as well as differences in the signaling pathways involved. We have obtained evidence indicating that cPLA2 activation does not involve NF-κB, although the PKC and p38 MAPK pathways are involved. However, we cannot exclude the possibility that PKC and p38 MAPK are also involved in the activation of NF-κB in the PTCs, which also plays a role in the events which occur in response to hyperuricemia in renal PTCs. Further studies will be necessary to determine the relevance of these findings to the onset of renal disease. (43)

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REFERENCES


