British Journal of Pharmacology (1997) 120, 807–812

Stimulation by extracellular ATP and UTP of the stress-activated protein kinase cascade in rat renal mesangial cells

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1 Extracellular adenosine 5′-triphosphate (ATP) and uridine 5′-triphosphate (UTP) have been shown to activate a nucleotide receptor (P_{2U} receptor) in rat mesangial cells that mediates phosphoinositide and phosphatidylcholine hydrolysis by phospholipases C and D, respectively. This is followed by an increased activity of the mitogen-activated protein kinase cascade and cell proliferation. Here we show that ATP and UTP potently stimulate the stress-activated protein kinase pathway and phosphorylation of the transcription factor c-Jun.

2 Both nucleotides stimulated a rapid (within 5 min) and concentration-dependent activation of stress-activated protein kinases as measured by the phosphorylation of c-Jun in a solid phase kinase assay.

3 When added at 100 μM the rank order of potency of a series of nucleotide analogues for stimulation of c-Jun phosphorylation was UTP > ATP = UDP = ATPγS > 2-methylthio-ATP > $\beta\gamma$-imido-ATP = ADP > AMP = UMP = adenosine = uridine. Activation of stress-activated protein kinase activity by ATP and UTP was dose-dependently attenuated by suramin.

4 Down-regulation of protein kinase C-α, -δ and -ε isoenzymes by 24 h treatment of the cells with 12-*O*-tetradecanoylphorbol 13-acetate did not inhibit ATP- and UTP-induced activation of c-Jun phosphorylation. Furthermore, the specific protein kinase C inhibitors, CGP 41251 and Ro 31-8220, did not inhibit nucleotide-stimulated c-Jun phosphorylation, suggesting that protein kinase C is not involved in ATP- and UTP-triggered stress-activated protein kinase activation.

5 Pretreatment of the cells with pertussis toxin or the tyrosine kinase inhibitor, genistein, strongly attenuated ATP- and UTP-induced c-Jun phosphorylation. Furthermore, N-acetyl-cysteine completely blocked the activation of stress-activated protein kinase in response to extracellular nucleotide stimulation.

6 In summary, these results suggest that ATP and UTP trigger the activation of the stress-activated protein kinase module in mesangial cells by a pathway independent of protein kinase C but requiring a pertussis toxin – sensitive G-protein and tyrosine kinase activation.

Keywords: Nucleotide receptor; stress-activated protein kinase; protein kinase C; pertussis toxin; renal mesangial cells

Introduction

Adenosine 5′-triphosphate (ATP) not only functions as the molecular unit of intracellular energy supply but also as an extracellular signalling molecule mediating cell-cell communication. The latter actions are initiated through binding of ATP to specific receptors. These purinoceptors either couple via G-proteins to intracellular effector enzymes (the P_{2Y} and P_{2U} subtypes) or are themselves part of ligand-gated ion channels (the P_{2X} and P_{2Z} subtypes) (Fredholm *et al*., 1994; Abbracchio & Burnstock, 1994).

In renal mesangial cells, ATP acts on a nucleotide receptor (P_{2U} receptor) that is equally well activated by uridine 5′-triphosphate (UTP) and mediates phosphoinositide hydrolysis and generation of 1,2-diacylglycerol and inositol 1,4,5-triphosphate (Pfeilschifter, 1990a,b) with a subsequent mobilization of intracellular calcium (Pavenstädt *et al*., 1993), activation of protein kinase C (PKC) (Pfeilschifter & Huwiler, 1996), and synthesis of prostaglandin E_2 (Pfeilschifter, 1990a; Schulze-Lohoff *et al*., 1992). Furthermore, we observed that extracellular ATP and UTP stimulate a phospholipase D-mediated phosphatidylcholine hydrolysis resulting in a sustained increase in cellular levels of 1,2-diacylglycerol (Pfeilschifter & Merriweather, 1993).

Recently we and others have observed that extracellular nucleotides stimulate growth of mesangial cells (Schulze-Lohoff *et al*., 1992; Huwiler & Pfeilschifter, 1994; Schulze-Lohoff *et al*., 1995a,b; Heidenreich *et al*., 1995), a hallmark of progressive glomerular disease. Moreover ATP and UTP were shown to trigger the mitogen-activated protein kinase (MAPK) cascade, also known as extracellular signal-regulated protein kinase (ERK) pathway (Huwiler & Pfeilschifter, 1994). The function of the MAPK cascade is to convert extracellular signals to intracellular mediators that regulate metabolism, secretion, gene expression and cell growth (Cano & Mahadevan, 1995).

Whereas the ERK pathway responds primarily to mitogenic agonists, two more recently characterized kinase cascades are responsive to cellular stresses such as interleukin 1, tumour necrosis factor-α, heat shock, uv-light, osmotic shock and metabolic poisons. These novel kinases were termed stress-activated protein kinases (SAPK, also termed c-Jun N-terminal kinase, JNK) and p38 kinase (Cano & Mahadevan, 1995; Woodgett *et al*., 1996).

This study demonstrates that in rat glomerular mesangial cells, the nucleotides ATP and UTP activate the SAPK module in a protein kinase C-independent way that utilizes a pertussis toxin-sensitive G-protein and protein tyrosine kinase activation.

Methods

Cell culture

Rat renal mesangial cells were cultivated as described previously (Pfeilschifter, 1990a,b). In a second step, single cells

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were cloned by limited dilution on 96-microwell plates. Clones with apparent mesangial-cell morphology were used for further processing. The cells were grown in RPMI 1640 supplemented with 10% (v/v) foetal-calf serum, penicillin (100 units ml^{-1}), streptomycin (100 $\mu g\ ml^{-1}$) and bovine insulin (0.66 units ml^{-1}). Mesangial cells exhibited the typical stellate morphology. Moreover, there was positive staining for the intermediate filaments desmin and vimentin, which is considered to be specific for myogenic cells, positive staining for Thy 1.1 antigen, and negative staining for factor VIII-related antigen and cytokeratin, which excludes endothelial and epithelial contaminations. For the experiments in this study passages 7–20 were used.

Peptide synthesis and generation of antibodies

For SAPKα and SAPKγ synthetic peptides based on the N-terminal sequences (MSDSKSDGQFYSVQVAC for SAPKα and MSRSKRDNNFYSVEIAC for SAPKγ), and for SAPKβ a peptide based on the C-terminal sequence (TDSSLEASAGPLGCCR), were synthesized, coupled to keyhole-limpet haemocyanin, and used to immunize rabbits (Kyriakis *et al*., 1994).

Western blot analysis

Cell extracts (100 μg) were subjected to SDS–PAGE (10% acrylamide gel) and proteins were transferred to nitrocellulose as previously described (Huwiler & Pfeilschifter, 1994).

After the transfer, nitrocellulose filters were washed extensively in distilled water and blocked in blocking buffer (50 mM Tris-HCl, pH 7.4, 200 mM NaCl, 0.2% (v/v) Triton X100 with 3% (w/v) horse serum) for 1 h at 25°C. Filters were then incubated for 4 h with specific antisera reactive with SAPKα, SAPKβ and SAPKγ, respectively (diluted in blocking buffer as indicated in the figure legend). After washing in buffer A (50 mM Tris-HCl, pH 7.4, 200 mM NaCl, 0.2% (v/v) Triton X100; 4×5 min), the filters were incubated for 1 h with horseradish peroxidase-conjugated anti-rabbit IgG antibodies in blocking buffer. Thereafter, filters were washed again (4×5 min) in buffer A and finally, for colour reaction the filters were incubated in PBS containing 0.5 mg of 3,3′-diaminobenzidine ml^{-1} and 0.03% H_2O_2 for 10 min and then washed extensively in distilled water.

In vitro *kinase assays*

The solid-phase Jun kinase assay was performed as described previously (Hibi *et al*., 1993) with a glutathione S-transferase (GST)-c-Jun (5-89) fusion protein coupled to glutathione-sepharose beads as substrate. In brief, 10 μg of GST-c-Jun was coupled to 10 μl of glutathione-sepharose in 0.5 ml of TLB buffer (20 mM Tris-HCl, pH 7.4, 137 mM NaCl, 2 mM EDTA, 1% Triton X100, 1 mM Na_3VO_4, 2 mM sodiumpyrophosphate, 1 mM PMSF, 10 $\mu g\ ml^{-1}$ leupeptin, 25 mM β-glycerophosphate) for 30 min at 4°C. The beads were then centrifuged for 20 s at 14000 *g*, washed twice with TLB buffer and incubated for 2 h at 4°C with cell extracts in TLB buffer containing 200 μg of protein. Thereafter, the complexes were washed twice with TLB buffer and once with 20 mM HEPES, pH 7.4, 20 mM $MgCl_2$ before the kinase reaction was started by addition of 30 μl of kinase buffer (20 mM HEPES, pH 7.4, 20 mM $MgCl_2$, 2 mM dithiothreitol (DTT), 20 mM β-glycerophosphate, 0.1 mM Na_3VO_4 20 mM p-nitrophenylphosphate, 10 μM ATP and 2 μCi [^{32}P-γ-ATP) to the complexes and incubated for 20 min at 30°C. To stop the reaction, 30 μl of $2\times$ Laemmli sample buffer was added and the samples were heated for 5 min at 95°C. Proteins were separated by SDS–PAGE (13% acrylamide gel) and phosphorylated GST c-Jun was detected by autoradiography and quantitated by a phosphorimager (Molecular Dynamics).

Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA). For multiple comparisons with the same control group, the limit of significance was divided by the number of comparisons according to Bonferroni.

Chemicals

ATP, ADP, AMP, ATPγS, UTP, UDP, UMP, adenosine and uridine were from Boehringer Mannheim (Rotkreuz, Switzerland); $\beta\gamma$-imido-ATP was from Fluka Chemie (Buchs, Switzerland); 2-methylthio-ATP was from ANAWA (Wangen, Switzerland); suramin was from Bayer AG (Leverkusen, Germany); TPA and pertussis toxin were from Calbiochem (Lucerne, Switzerland); [^{32}P-γ]-ATP (specific activity: >5000 Ci $mmol^{-1}$), anti-rabbit horseradish peroxidase-linked IgG and hyperfilm MP were from Amersham; glutathione-sepharose 4B was from Pharmacia Fine Chemicals, (Uppsala, Sweden); GST-c-Jun and CGP 41251 ((8R*,9S*,10S*,12S*)-(−)-10-N-methyl-aminobenzoyl-9-methoxy-8-methyl-2,3,9,10,11,12-hexahydro-8,18-epoxy-1H,8H,12H-2,7b,12a-triazodibenzo[a,g]cyclonona(cde)trinden-1-one) were produced by Ciba-Geigy Ltd. (Basel, Switzerland); Ro 31-8220 {3-[1-[3-(Amidinothio)propyl-1H-indoyl-3-yl]-3-(1-methyl-1H-indoyl-3-yl)maleimide methane sulfonate} was a kind gift of F. Hoffmann-La Roche (Basel, Switzerland); all cell culture nutrients were from Gibco BRL (Basel, Switzerland); all other chemicals were from Fluka (Buchs, Switzerland).

Results

Extracellular ATP and UTP stimulate SAPK activation

Western blot analysis of mesangial cell lysates with polyclonal antisera specific for SAPK-α, -β and -γ confirmed that SAPKα and -γ but not SAPK-β are expressed in mesangial cells (Figure 1). In whole cell extracts from mesangial cells, anti SAPK-α IgG labelled a prominent band at 54 kDa, a weak band at 46 kDa and a weak 70 kDa doublet (Figure 1). Anti SAPK-γ detected a single band at 54 kDa whereas anti SAPK-β did not give any signal (Figure 1). The 46 kDa and 54 kDa SAPK isoforms corresponded to alternatively spliced variants of the corresponding SAPK-α, -β and -γ genes and ten isoforms were recently identified in human brain by molecular cloning (Gupta *et al*., 1996). The 70 kDa bands are unknown proteins that have also been observed in rat fibroblasts and lack kinase activity toward GST-c-Jun (Miller *et al*., 1996).

The ERK and SAPK families phosphorylate Ser-63 and Ser-73 of N-terminal transactivation domain of c-Jun *in vitro* (Alvarez *et al*., 1991). In contrast to ERKs which do not bind

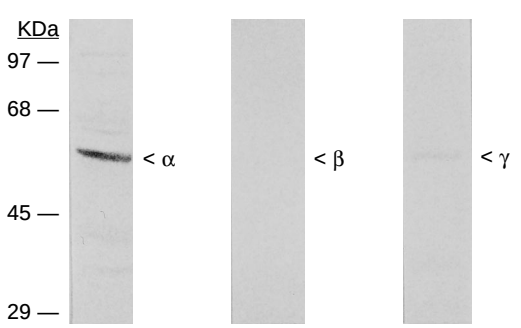

Figure 1 Immunodetection of SAPK-α, -β and -γ isoforms in mesangial cells. Mesangial cell homogenate was separated on SDS–PAGE (10% acrylamide gel), transferred to nitrocellulose membrane and Western blot analysis was performed with antisera reactive with SAPKα, SAPKβ and SAPKγ as indicated at a dilution of 1 : 300 each. Bands were visualized by horseradish peroxidase.

c-Jun with high affinity (Hibi *et al*., 1993; Gupta & Davis, 1994), the SAPKs bind to c-Jun and may be responsible for phosphorylation of c-Jun *in vivo* (Kallukini *et al*., 1994). We used a solid-phase kinase assay with c-Jun (5-89) coupled to glutathione S-transferase as a substrate that binds all SAPK isoforms and after precipitation with glutathione-Sepharose the N-terminal domain of c-Jun is phosphorylated by activated SAPKs (Hibi *et al*., 1993).

The data in Figure 2 show that ATP and UTP augmented SAPK activity within 5 min. Maximal activation of SAPK was obtained after 10 to 20 min and was 16–24 fold more than basal levels. Whereas the response to ATP was transient and was back to control levels within 60 min, the response to UTP was more sustained and a marked activation of SAPK was still seen after 60 min (Figure 2).

Figure 3 shows the concentration-dependency of SAPK activation by ATP (a) and UTP (b). In this experiment again UTP seemed to be more potent than ATP and confirms previous observations where UTP always tended to be more potent than ATP in terms of phosphoinositide and phosphatidylcholine hydrolysis and MAPK stimulation (Pfeilschifter, 1990b; Pfeilschifter & Merriweather, 1993; Huwiler & Pfeilschifter, 1994).

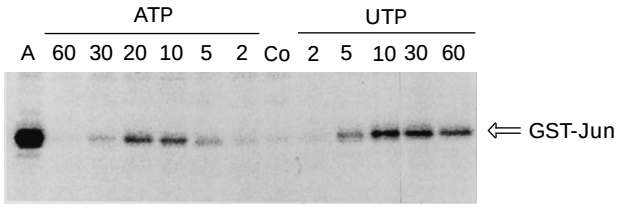

Figure 2 Time course of ATP- and UTP-stimulated SAPK activity in mesangial cells. Quiescent mesangial cells were treated with vehicle (Co), ATP (100 μM) or UTP (100 μM) for the indicated time periods (in min). Thereafter cells were harvested and SAPK activity was measured as described in the Methods section. Similar results were obtained in two independent experiments.

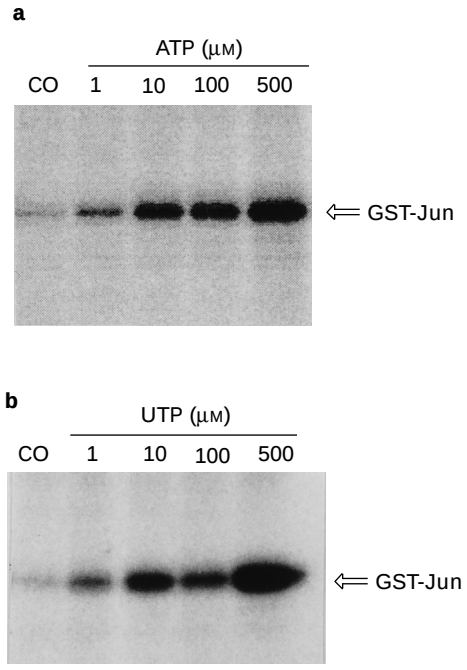

Figure 3 Concentration-dependence of (a) ATP- and (b) UTP-stimulated SAPK activity in mesangial cells. Quiescent mesangial cells were treated with either vehicle (co) or the indicated concentrations of ATP (a) and UTP (b) for 10 min. Cells were harvested and 200 μg of cell protein was taken for SAPK activity measurement as described in the Methods section. Similar results were obtained in three independent experiments.

Preincubation of the cells with the putative P_2-receptor antagonist, suramin, dose-dependently attenuated both ATP- and UTP-stimulated SAPK activity (Figure 4).

Figure 5 displays the specificity for different nucleotides in triggering SAPK activation in mesangial cells. ATP, UTP, UDP and ATPγS potentially stimulated SAPK activity whereas 2-methylthio-ATP, $\beta\gamma$-imido-ATP and ADP were somewhat less effective. In contrast, AMP, UMP, adenosine and uridine had only a weak effect on c-Jun phosphorylation (Figure 5). The relative potencies of UTP, ATP and ATPγS and the fact that the specific P_{2Y} purinoceptor agonist, 2-methylthio-ATP is less effective in activating SAPK in mesangial cells argues for the exclusive involvement of a nucleotide receptor (P_{2U} receptor) in SAPK stimulation (Pfeilschifter, 1990b; O'Connor, 1992). This receptor obviously also recognizes UDP, whereas UMP and uridine showed only minor activity.

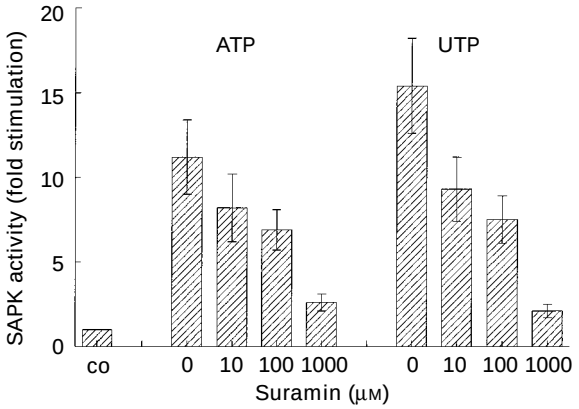

Figure 4 Inhibition of ATP- and UTP-stimulated SAPK activity by suramin. Mesangial cells were treated with vehicle (Co), ATP (100 μM) or UTP (100 μM) in the absence or presence of the indicated concentrations of suramin (μM). Cells were then harvested and SAPK activity was measured as described in the Methods section. Results are means ± s.d. of two independent experiments.

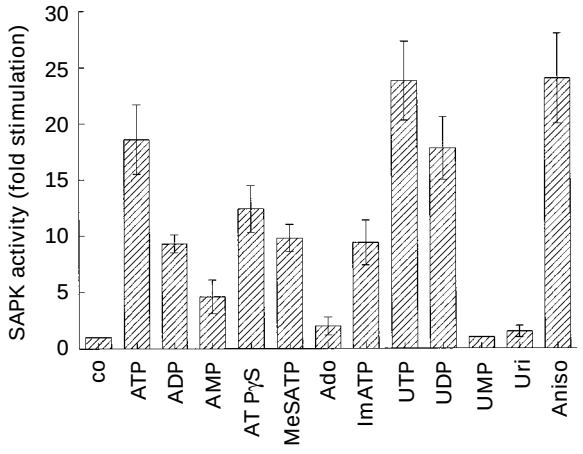

Figure 5 Effects of different nucleotide analogues and nucleosides on SAPK activity in mesangial cells. Quiescent mesangial cells were treated for 10 min with either vehicle (co), anisomycin (50 ng ml^{-1}; Aniso), ATP, AMP, ATPγS, 2-methylthio-ATP(MeS-ATP), $\beta\gamma$-imido-ATP (Im-ATP), adenosine (Ado), UTP, UDP, UMP or uridine (Uri) at a concentration of 100 μM each. Cells were then harvested and SAPK activity was measured as described in the Methods section. Anisomycin was used as positive control; its activation of the SAPK pathway is well documented (Woodgett *et al*., 1996). Results are means ± s.d. of two independent experiments.

Protein kinase C is not involved in ATP- and UTP-stimulated SAPK activation

We have previously shown that mesangial cells express four PKC isoenzymes, PKC-α, -δ, -ε and -ζ. No PKC-β, -γ, -η, -θ or -μ isoforms were detected (Huwiler *et al*., 1991a,b; 1992; 1993 and unpublished observations).

We were therefore interested to investigate what role, if any, PKC activation plays in nucleotide-induced SAPK activation. The possible involvement of PKC in ATP- and UTP-stimulated SAPK activity was examined with phorbol esters, PKC down regulation experiments, as well as inhibitors of PKC. Addition of PKC-activating 12-*O*-tetradecanoylphorbol 13-acetate (TPA) did not increase SAPK activity (Table 1), suggesting that phorbol ester-sensitive PKC isoenzymes are unable to stimulate SAPK activity themselves. Moreover, PKC down-regulation by prolonged TPA treatment did not inhibit ATP- or UTP-stimulated SAPK activity. A 24 h treatment with TPA (500 nM) that causes complete depletion of α-, δ- and ε-isoenzymes of PKC in mesangial cells (Huwiler *et al*., 1991a,b; 1992; 1993) did not significantly affect c-Jun phosphorylation in response to ATP or UTP (Table 1).

In line with these findings, the specific PKC inhibitors CGP41251 (Meyer *et al*., 1989) and Ro 31-8220 (Davis *et al*., 1989) did not or only marginally reduced ATP- and UTP-stimulated SAPK activity as shown in Table 1. In summary, these data clearly demonstrate that nucleotide stimulation of the SAPK cascade does not involve a phorbol ester-sensitive PKC isoenzyme in mesangial cells.

Inhibition of ATP- and UTP-stimulated SAPK activation by genistein, pertussis toxin and N-acetyl-cysteine

In order to study the possible mechanisms controlling the activation of SAPKs by ATP and UTP in mesangial cells, we used a number of specific inhibitors. We have shown previously that pertussis toxin attenuates nucleotide-induced production of inositol 1,4,5-trisphosphate and 1,2-diacylglycerol (Pfeilschifter, 1990a). Preincubation of mesangial cells with pertussis toxin (50 ng ml^{-1}) strongly inhibited ATP- and UTP-stimulated SAPK activity by approximately 80% and 75%, respectively (Table 1). These data suggest that a pertussis toxin-sensitive heterotrimeric G-protein (G_i or G_o) is a key regulator of nucleotide-induced activation of the SAPK signalling pathway.

Table 1 Effects of various compounds on ATP- and UTP-stimulated SAPK activity in mesangial cells

Addition	*SAPK activity* (fold stimulation)
Control	1.0
ATP	3.85±0.47*
UTP	5.62±1.88*
TPA (100 nM)	1.05±0.08
TPA (500 nM, 24h)	1.01±0.05
TPA (500 nM, 24h)+ATP	3.70±0.28*
TPA (500 nM, 24h)+UTP	5.07±0.68*
CGP 41251 (1 μM)+ATP	4.20±0.53**
CGP 41251 (1 μM)+UTP	6.42±0.44**
Ro 31-8220 (1 μM)+ATP	4.08±0.62**
Ro 31-8220 (1 μM)+UTP	5.72±0.48**
Genistein (10 μM)+ATP	1.18±0.20^{+}
Genistein (10 μM)+UTP	1.48±0.26^{+}
Pertussis toxin+ATP	1.38±0.32^{+}
Pertussis toxin+UTP	1.72±0.51^{+}
N-acetyl-cysteine (20 mM)+ATP	0.67±0.25^{++}
N-acetyl-cysteine (20 mM)+UTP	1.02±0.18^{+}

Quiescent mesangial cells were pretreated for 24 h with TPA (500 nM), or for 30 min with CGP 41251 (1 μM), Ro 31-8220 (1 μM), genistein (10 μM), N-acetyl-cystein (20 mM) and then stimulated with either vehicle (control), 100 μM ATP, 100 μM UTP or 100 nM TPA for 10 min as indicated. Thereafter cells were harvested and SAPK activity was measured as described in the Methods section. The data are means±s.d. of three independent experiments. Significant differences from control: $^{*}P<0.05$; $^{**}P<0.01$; ANOVA. Significant differences from corresponding ATP and UTP stimulation (without inhibitors): $^{+}P<0.05$; $^{++}P<0.01$; ANOVA.

In many cell types, G_i-coupled receptors trigger MAPK activation via a βγ-subunits-dependent activation of Ras protein (Faure *et al*., 1994; Crespo *et al*., 1994) which includes the activation of a Src-related tyrosine kinase (Daub *et al*., 1996; Wan *et al*., 1996). We assessed the role of tyrosine kinases in nucleotide-stimulated SAPK activation by use of a specific inhibitor of protein tyrosine kinases, genistein, which is an isoflavone compound from fermentation broth of *Pseudomonas spp*. (Akiyama *et al*., 1987). As shown in Table 1, genistein markedly reduced ATP- and UTP-stimulated SAPK activity, thus suggesting that tyrosine phosphorylation is probably associated with the activation of the SAPK cascade by extracellular nucleotides.

In a next step we evaluated the effect of the free radical scavenger and antioxidant N-acetyl-cysteine (NAC) on nucleotide-induced c-Jun phosphorylation. NAC has been shown to block SAPK and nuclear factor κB activation in response to uv light and other cellular stresses (Devary *et al*., 1993; Adler *et al*., 1995). Coincubation of mesangial cells with extracellular nucleotides and NAC resulted in a complete inhibition of c-Jun phosphorylation (Table 1). NAC itself had no significant effect on SAPK activity. These data emphasize the role of redox regulation in SAPK activation by extracellular nucleotides.

Discussion

There is considerable evidence suggesting that extracellular ATP and UTP can function as signalling molecules in a wide range of physiological responses of cells (Gordon, 1986; O'Connor, 1992; El-Moatassim *et al*., 1992; Burnstock, 1993). More recently, the role of extracellular nucleotides as possible mediators of inflammation, cell growth and cell death has generated considerable interest (Burnstock, 1993; Di Virgilio, 1995; Brake *et al*., 1996).

Stimulation of DNA synthesis by extracellular nucleotides has been found for a variety of cultured cells including mouse fibroblasts, A431 epidermoid carcinoma cells and DDT_1-MF-2 vas deferens cells (Huang *et al*., 1989), endothelial cells (Van Daele *et al*., 1992), aortic smooth muscle cells (Malam-Souley *et al*., 1993) and glomerular mesangial cells (Schulze-Lohoff *et al*., 1992; Huwiler & Pfeilschifter, 1994). A group of serine/threonine kinases, the MAPKs, have been identified as key enzymes in the signalling process leading from growth factor receptors to growth control of mammalian cells (Leevers & Marshall, 1992; Davis, 1993; Crews & Erikson, 1993). Extracellular nucleotides have been demonstrated to activate the MAPK module in mesangial cells (Huwiler & Pfeilschifter, 1994; Ishikawa *et al*., 1994), im astrocytes (Neary *et al*., 1994), in rat inner medullary collecting tubule cells (Heasley *et al*., 1994) and in EAhy 926 endothelial cells (Graham *et al*., 1996a,b).

Activation of the MAPK module is critical for the initiation of mesangial cell proliferation, a characteristic event in the early phases of most forms of glomerulonephritis (Kashgarian & Sterzel, 1992).

Pathological proliferation of mesangial cells is opposed by the process of programmed cell death, or apoptosis. Recently, Baker *et al*. (1994) showed that mesangial cell apoptosis is induced during the course of Thy-1 nephritis in rats. The inhibition of mesangial cell proliferation and the removal of surplus mesangial cells by apoptosis guarantees a sophisticated orderly process of repair in this experimental model of glomerulonephritis. In this context it is worth mentioning that activation of SAPKs is critical for induction of apoptosis in different cell types (Xia *et al*., 1995; Verheij *et al*., 1996). A

dynamic balance between growth factor-stimulated ERK signalling pathway and stress-activated SAPK and p38 modules is important in determining whether cells survive or undergo programmed cell death (Xia *et al*., 1995).

The differential activation of the ERK and SAPK pathways by a wide variety of agents strongly suggest that these signalling cascades share few, if any, common members (Woodgett *et al*., 1996). In support of this, we have demonstrated that PKC activates the ERK pathway in mesangial cells exposed to extracellular nucleotides (Huwiler & Pfeilschifter, 1994) whereas activation of the SAPKs is independent of phorbol ester – sensitive PKC isoenzymes (Table 1). The exquisite sensitivity of ATP- and UTP-induced SAPK activity to pertussis toxin suggests that a G_i-type protein couples the P_{2U} receptor to the signalling events leading to c-Jun phosphorylation. Pertussis toxin causes ADP-ribosylation of the α-subunit of G_i in mesangial cells (Pfeilschifter & Bauer, 1986) and thus prevents the dissociation of α_i-GTP from $\beta\gamma$-dimers, blocking the downstream signalling events initiated by both, α_i-GTP and $\beta\gamma$-subunits (Ui, 1984). Pertussis toxin only partially blocks extracellular nucleotide-stimulated phosphoinositide hydrolysis (Pfeilschifter, 1990a,b), thus suggesting that in addition to G_i other G-proteins, most likely G_q couple nucleotide receptors to phospholipase C. In contrast, pertussis toxin completely inhibits ATP- and UTP-induced activation of SAPK (Table 1) thus emphasizing the essential role of G_i in the activation of this signalling pathway. Recently Coso *et al*. (1996) showed that signalling from muscarinic cholinoceptors to SAPK involves $\beta\gamma$ subunits of heterotrimeric G-proteins, acting on small G-proteins like Ras or Rac-1-dependent pathways. From our data with genistein and NAC we suggest that activation of this pathway may include the activation of a yet to be defined protein tyrosine kinase and a step sensitive to redox regulation.

Interestingly, ATP and UTP are effective activators of SAPKs (this paper) as well as powerful activators of ERKs (Huwiler & Pfeilschifter, 1994). It is important to understand the extent of cross-communication between the SAPK and ERK pathways, given that integration of both signals determines whether cells proliferate in response to extracellular nucleotides (Schulze-Lohoff *et al*., 1992; Huwiler & Pfeilschifter, 1994) or eventually undergo apoptosis (Di Virgilio, 1995). We are currently investigating the environmental conditions that critically influence the decision of mesangial cells exposed to extracellular nucleotides either to proliferate or to die.

This work was supported by Swiss National Science Foundation Grant 31-43090.95, by a grant from the Commission of the European Union (Biomed 2, PL 950 979), by a grant from the Wilhelm Sander-Stifung, and by grants from the Roche Research Foundation and the CIBA-Geigy Jubiläumsstiftung. G.v.R. was an exchange student from the University of Utrecht supported by the Erasmus exchange programme.

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(Received August 16, 1996
Revised October 31, 1996
Accepted November 21, 1996)