J. Pharm. Pharmacol. 2000, 52: 539–546
Received October 19, 1999
Accepted December 20, 1999

The Upstream Regulation of p38 Mitogen-activated Protein Kinase Phosphorylation by Arachidonic Acid in Rat Neutrophils

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Abstract

The signal transduction pathways activated by arachidonic acid that lead to p38 mitogen-activated protein kinase (MAPK) activation in neutrophils remains unclear. In this study, selective inhibitors of several signalling pathways were utilized to investigate the mechanisms of activation of p38 MAPK by arachidonic acid in rat neutrophils.

Stimulation of p38 MAPK phosphorylation by arachidonic acid and its trifluoromethyl ketone analogue $AACOCF_3$ was transient, peaking at 1 min, and was concentration-dependent. Arachidonic acid-stimulated p38 MAPK phosphorylation was attenuated in cells pretreated with the $G_{i/o}$ inhibitor (pertussis toxin), but not with the dual cyclooxygenase/lipoxygenase inhibitor (BW755C) or the leukotriene biosynthesis inhibitor (MK886). Tyrosine kinase inhibitor (genistein), but not the extracellular signal-regulated kinase kinase inhibitors (PD98059 and U0126), attenuated the phosphorylation of p38 MAPK by arachidonic acid. Phosphoinositide 3-kinase inhibitors (wortmannin and LY294002) did not affect the arachidonic acid-induced response. After pretreatment of the cells with protein kinase C inhibitors (Gö6976, Gö6983 and GF109203X), only Gö6976 significantly attenuated the phosphorylation of p38 MAPK by arachidonic acid. In addition, phosphorylation of p38 MAPK by arachidonic acid was greatly attenuated by the phospholipase C inhibitor (U73122) and the Ca^{2+} chelator BAPTA ((1,2-bis-*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid), but not altered by the nitric oxide synthase inhibitor, *N*-nitro-L-arginine methyl ester. Arachidonic acid did not cause an increase in cellular cyclic GMP level.

This study revealed the involvement of pertussis toxin-sensitive G protein, non-receptor tyrosine kinase, phospholipase C/Ca^{2+}, and probably Ca^{2+}-dependent protein kinase C in arachidonic acid-stimulated p38 MAPK activation.

The mitogen-activated protein kinases (MAPKs), a family of serine/threonine kinases, are members of discrete signalling cascades that form focal points for diverse extracellular stimuli and function to regulate fundamental cellular processes. At least three distinct classes of MAPK exist in mammalian cells, the extracellular signal-regulated kinases, *c-Jun* amino-terminal kinases/stress-activated protein kinases and p38 MAPK, each having different physiological roles (Lopez-Ilasaca 1998). It is well established that p38 MAPKs play a role in gene expression through the phosphorylation and activation of transcription factors (Han et al 1997). Although p38 MAPKs have been reported to participate in the processes of cytokine and eicosanoid production, respiratory burst, and chemotaxis (Zu et al 1998; Syrbu et al 1999), the specific role of p38 MAPK in neutrophils remains unclear and the mechanisms of activation of p38 MAPK are poorly understood. Four distinct isoforms of p38 MAPK have been identified, p38α, β, γ, and δ (Li et al 1996; Jiang et al 1997). All of these isoforms share a common TGY motif in kinase subdomain VIII. Activation of p38 MAPK occurs after dual phosphorylation of threonine and tyrosine residues within the TGY motif by the upstream MAPK kinases (Moriguchi et al 1996). In mammalian cells, a novel kinase cascade consisting of TAK1, MAPK kinase 3/MAPK kinase 6, and p38 MAPK (Moriguchi et al 1996) and the Rac/Cdc42

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signalling through p21-activated kinase (Zhang et al 1995) have been suggested as upstream regulators of the kinase cascades leading to p38 MAPK activation. To date, intracellular events preceding p38 MAPK phosphorylation and activation in neutrophils remain largely undefined.

Arachidonic acid is a second messenger that is released by the action of phospholipase A_2 in activated cells. Arachidonic acid and its metabolites mediate a number of biological processes of activated neutrophils including adhesion, chemotaxis, aggregation, degranulation, and respiratory burst (Abramson et al 1991; Capodici et al 1998). However, the molecular mechanisms through which the actions of arachidonic acid are mediated are unclear. Arachidonic acid has been demonstrated to stimulate the activity of extracellular signal-regulated kinase and p38 MAPK in a variety of cells including human neutrophils (Hii et al 1998). The activation of extracellular signal-regulated kinase by arachidonic acid has been proposed via its metabolites, and regulated by G protein and protein kinase C in human neutrophils. However, the mechanism of activation of p38 MAPK by arachidonic acid is incompletely understood.

In this study, we have evaluated the phosphorylation of p38 MAPK by immunoblot analysis with phospho-specific antibody as an index of p38 MAPK activation, and utilized selective inhibitors of several signalling pathways to investigate the mechanisms of activation of p38 MAPK by arachidonic acid.

Materials and Methods

Materials

Dextran T-500, enhanced chemiluminescence reagent and cyclic GMP enzyme immunoassay kit were from Amersham Pharmacia Biotech (Uppsala, Sweden). Hanks' balanced salt solution was from Gibco (Gaithersburg, MD). Rabbit polyclonal antibodies to phospho-p38 MAPK and to p38 MAPK were from New England Biolabs (Beverly, MA). Pertussis toxin was from Research Biochemicals International (Natick, MA). $AACOCF_3$, U73122, MK886, and LY294002 were from Biomol Res. (Plymouth Meeting, PA). Arachidonic acid, GF109203X (2-[1-(3-dimethylaminopropyl)-1*H*-indol-3-yl]-3-(1*H*-indol-3-yl)-maleimide), Gö6976 (13-cyanoethyl-12-methyl-6,7,12,13-tetrahydroindolo[2,3-*a*]pyrolo[3,4-*c*]carbazole·7-one), Gö6983 (2-[1-(3-dimethylaminopropyl)5-methoxy-1*H*-indol-3-yl]-3-(1*H*-indol-3-yl)-maleinide), PD98059, and wortmannin were from Calbiochem-Novabiochem (San Diego, CA). U0126 was from Promega (Madison, WI). BAPTA-AM was from Molecular Probes (Eugene, OR) and polyvinylidene difluoride membrane was from Millipore (Bedford, MA). BW755C was kindly provided by Wellcome Research (Kent, UK). All other chemicals were purchased from Sigma (St Louis, MO).

Neutrophil isolation

Neutrophils were isolated from Sprague-Dawley rats as described by Wang et al (1995). Briefly, fresh blood was withdrawn from the abdominal aorta and mixed with EDTA. The neutrophils were purified by dextran sedimentation, centrifugation through Ficoll-Hypaque, followed by the hypotonic lysis of contaminating erythrocytes. The purified neutrophils with viability $>95\%$ were suspended in Hanks' balanced salt solution containing 10 mM HEPES, pH 7·4, 4 mM $NaHCO_3$, and kept in an ice-bath before use.

Detection of p38 MAPK phosphorylation

Neutrophils (2×10^7 cells mL^{-1}) were incubated with dimethylsulphoxide (DMSO) or test drugs at 37°C for the indicated time before stimulation with arachidonic acid. Reactions were terminated by adding a stopping solution (20% trichloroacetic acid, 1 mM PMSF (phenylmethyl sulphanyl fluoride), 2 mM *N*-ethylmaleimide, 10 mM NaF, 2 mM Na_3VO_4, 2 mM *p*-nitrophenyl phosphate, 7 μg mL^{-1} aprotinin and pepstatin).

Protein pellets were washed twice and lysed in Laemmli sample buffer. Proteins (60 μg per lane) were resolved by 10% SDS-polyacrylamide gel electrophoresis, then transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% non-fat dried milk in TBST buffer (10 mM Tris-HCl, pH 7·5, 150 mM NaCl, and 0·1% Tween 20), probed with rabbit polyclonal anti-phospho-p38 MAPK antibodies and sheep anti-rabbit IgG conjugated with horseradish peroxidase at room temperature. The phospho-p38 MAPKs were visualized by chemiluminescence, and quantified by densitometry. After detection of p38 MAPK phosphorylation, the same blots were stripped with strip buffer (62·5 mM Tris-HCl, pH 6·8, 100 mM 2-mercaptoethanol, 2% SDS) at 50°C for 30 min for standardization of protein loading. The blots were washed extensively and then reprobed with antibody against p38 MAPK in TBST buffer with 0·1% non-fat dried milk. The membranes were incubated in secondary antibody and treated for chemiluminescence as in the above procedure.

Determination of cellular cyclic GMP

Neutrophils (2×10^7 cells mL^{-1}) were incubated with DMSO, arachidonic acid or sodium nitroprusside plus zaprinast for 10 min at 37°C. Reactions were terminated by adding stopping solution (50 mM acetate buffer, pH 6·2, and 50 μM zaprinast). After being boiled for 5 min, the suspension was kept in ice, sonicated and sedimented. The supernatant was acetylated by the addition of 0·025 vols triethylamine : acetic anhydride (2 : 1, v/v). The cyclic GMP content in aliquots of the acetylated samples was assayed using an enzyme immunoassay kit.

Statistical analysis

The densitometric data of the immunoblots were expressed as means ± s.e.m. Statistical analysis was performed by the Bonferroni's *t*-test method after analysis of variance. $P < 0{\cdot}05$ was considered significant for all tests. Analysis of the regression line test was used to calculate the IC50 values.

Results and Discussion

Activation of p38 MAPK by arachidonic acid and $AACOCF_3$

Hii et al (1998) have demonstrated the parallel changes in phosphorylation and kinase activity of p38 MAPK in neutrophils. In this study, Western blot analysis with anti-phospho-p38 MAPK antibodies showed that arachidonic acid and its trifluoromethyl ketone analogue $AACOCF_3$ stimulated p38 MAPK activation in rat neutrophils in a concentration- and time-dependent manner (Figure 1). After exposure of cells to arachidonic acid for 1 min, p38 MAPK activation could be detected at 10 μM arachidonic acid, and phosphorylation increased with increasing concentrations of arachidonic acid up to 100 μM, the maximum concentration tested. Stimulation of p38 MAPK phosphorylation was transient, detectable at 30 s and peaking at 1 min, with only a very weak signal left at 5 min after exposure to 30 μM arachidonic acid. Compared with arachidonic acid, $AACOCF_3$ was a less potent stimulus of p38 MAPK phosphorylation, with a significant effect occurring at 30 μM for 1 min activation. The effect of $AACOCF_3$ was more transient, detectable at 30 s and decreasing to baseline by 2 min at 100 μM. Reprobing of blots with antibody against p38 MAPK confirmed that the individual lanes were loaded with equal amounts of proteins. These findings suggested that

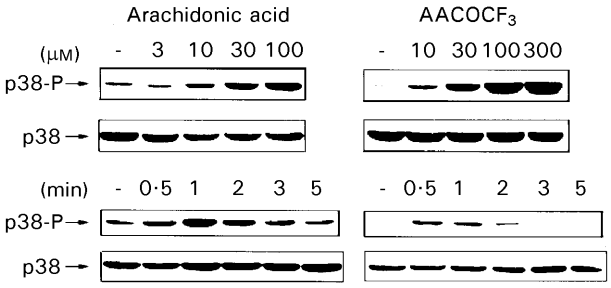

Figure 1. Concentration- and time-dependent activation of p38 MAPK by arachidonic acid and $AACOCF_3$ in rat neutrophils. Cells were treated with various concentrations of arachidonic acid or $AACOCF_3$ for 1 min, or stimulated with 30 μM arachidonic acid or 100 μM $AACOCF_3$ for various time-intervals at 37°C. The levels of phosphorylated p38 MAPK were determined by immunoblot analysis using anti-phospho-p38 MAPK antibodies. Data are representative of three independent experiments.

the free carboxyl group of arachidonic acid was not essential to p38 MAPK activation, but essential to extracellular signal-regulated kinase phosphorylation (data not shown). Rat neutrophils were stimulated with 30 μM arachidonic acid for 1 min in subsequent experiments. It has been reported that p38 MAPK is involved in the increase in cytosolic phospholipase A_2 activity in activated cells (Syrbu et al 1999), and suggested that arachidonic acid liberated by ligand-stimulated cytosolic phospholipase A_2 may participate in sustaining/amplifying MAPK activity and the activity of cytosolic phospholipase A_2.

Activation of p38 MAPK by arachidonic acid through G protein

Arachidonic acid has been reported to stimulate GTPγS loading of the heterotrimeric G protein in membrane fractions and to induce pertussis toxin-sensitive superoxide anion generation in human neutrophils (Abramson et al 1991). In addition, activation of extracellular signal-regulated kinase by arachidonic acid is pertussis toxin sensitive (Capodici et al 1998). However, the role of G protein in regulating p38 MAPK activation is incompletely understood. In this study, cells pretreated with 1 μg mL^{-1} of pertussis toxin for 2 h inhibited the phosphorylation of p38 MAPK by arachidonic acid (about 53% inhibition) (Figure 2) as well as by 100 μM $AACOCF_3$ (about 61% inhibition) (data not shown). These results indicated that the pertussis toxin-sensitive G protein is involved in the regulation of p38 MAPK activation by arachidonic acid.

Activation of p38 MAPK by arachidonic acid is independent of its cyclooxygenase/lipoxygenase metabolites

Cell-associated arachidonic acid can directly serve as an endogenous second messenger and is also metabolized by cyclooxygenase, lipoxygenase or cytochrome P450 into a variety of derivatives. In rat neutrophils, arachidonic acid is metabolized to PGD_2, PGE_2, 6-keto $PGF_{1\alpha}$, TXA_2, LTB_4 and 5-HETE (Yamaki & Oh-ishi 1992). Arachidonic acid activation of extracellular signal-regulated kinase through its metabolites has been identified in human neutrophils (Capodici et al 1998). Therefore, we investigated the role of arachidonic acid metabolites in p38 MAPK activation in rat neutrophils. The results indicated that arachidonic acid-stimulated p38 MAPK phosphorylation was independent of its metabolism by cyclooxygenase/lipoxygenase because the effect was not affected by 30 μM BW755C, a dual cyclooxygenase/lipoxygenase inhibitor (Higgs et al 1979), or 30 nM MK886 (Figure 2), a leukotriene biosynthesis inhibitor (Gillard et al 1989). This is consistent with the report by Hii et al (1998) that the ability of arachidonic acid to stimulate p38 MAPK phosphorylation was not diminished by either the lipoxygenase inhibitor nordihydroguaiaretic acid or by indomethacin in human neutrophils. Arachidonic acid is metabolized by a cytochrome P450-dependent mechanism in human neutrophils (Lutton et al 1989). It is unlikely that arachidonic acid directly activates a G protein-coupled cell surface receptor. Whether arachidonic acid stimulates p38 MAPK phosphorylation in rat neutrophils through cytochrome P450- or other enzyme-dependent metabolites needs further investigation.

Tyrosine kinase but not extracellular signal-regulated kinase kinase is required for p38 MAPK activation by arachidonic acid

Stimulation of extracellular signal-regulated kinase by arachidonic acid is accompanied by extracellular signal-regulated kinase kinase activation (Capodici et al 1998). The observation that PD98059 and U0126, two selective and non-competitive inhibitors of extracellular signal-regulated kinase kinase (Favata et al 1998), had no effect on p38 MAPK phosphorylation by arachidonic acid (Figure 3) excludes the involvement of extracellular signal-regulated kinase kinase. Recent studies have shown also the G protein-coupled stimulation of p38 MAPK through the Src family kinase-dependent signalling pathway (Nagao et al 1998). In addition, genistein, the general tyrosine kinase inhibitor (Akiyama et al 1987), inhibited p38 MAPK activation by bacterial phagocytosis, tumour necrosis factor α, and granulocyte-monocyte colony stimulating factor in human neutrophils (McLeish et al 1998). We have demonstrated in rat neutrophils that the increase in p38 MAPK phosphorylation by arachidonic acid was inhibited (about 43%) by 30 μM genistein (Figure 3), and a comparable inhibition of $AACOCF_3$-induced response was observed (data not shown). Hence, arachidonic acid may also activate p38 MAPK through non-receptor tyrosine kinase.

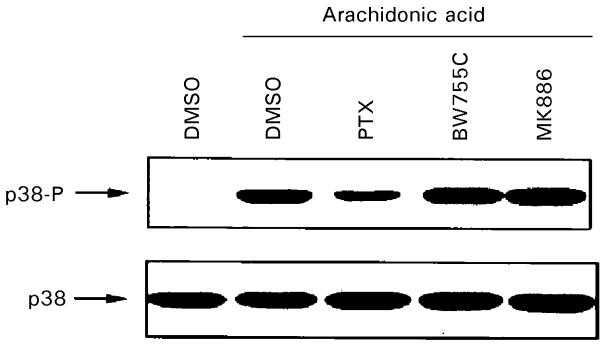

Figure 2. Effects of pertussis toxin (PTX), BW755C, and MK886 on arachidonic acid-stimulated p38 MAPK activation. Cells were preincubated at 37°C for 2 h with DMSO, 1 μg mL^{-1} of pertussis toxin, or for 10 min with 30 μM BW755C or 30 nM MK886 before stimulation with 30 μM arachidonic acid or without stimulation. One minute later, the levels of phosphorylated p38 MAPK were determined by immunoblot analysis using anti-phospho-p38 MAPK antibodies. Data are representative of three independent experiments.

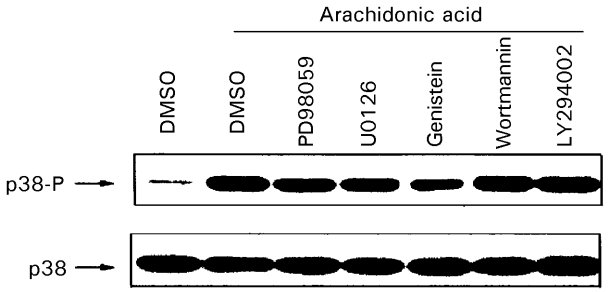

Figure 3. Effects of PD98059, U0126, genistein, wortmannin, and LY294002 on arachidonic acid-stimulated p38 MAPK activation. Cells were preincubated at 37°C for 30 min with DMSO, 10 μM PD98059, 30 μM genistein, or for 10 min with 0·3 μM U0126, 1 μM wortmannin or 10 μM LY294002 before stimulation with 30 μM arachidonic acid or without stimulation. One minute later, the levels of phosphorylated p38 MAPK were determined by immunoblot analysis using anti-phospho-p38 MAPK antibodies. Data are representative of three to four independent experiments.

Activation of p38 MAPK by arachidonic acid is independent of phosphoinositide 3-kinase

Activation of phosphoinositide 3-kinase, which catalyses the phosphorylation of phosphatidylinositols at the D3 position, is one of the earliest responses of neutrophils to G protein-coupled stimulation. Neutrophils contain two classes of phosphoinositide 3-kinase, a classical tyrosine kinase-regulated phosphoinositide 3-kinase α (p85/p110α) and a novel Gβγ-regulated phosphoinositide 3-kinase γ (p101/p110γ) (Stephens et al 1997). It has been reported that formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated p38 MAPK activation is dependent on phosphoinositide 3-kinase in human neutrophils (Krump et al 1997). Wortmannin, a potent phosphoinositide 3-kinase inhibitor (Powis et al 1994), attenuated the activation of p38 MAPK by granulocyte-monocyte colony stimulating factor, but not tumour necrosis factor a in human neutrophils (McLeish et al 1998). However, the role of phosphoinositide 3-kinase in regulating p38 MAPK activation by arachidonic acid in neutrophils is unclear. This study demonstrated that arachidonic acid-stimulated phosphorylation of p38 MAPK in rat neutrophils was independent of phosphoinositide 3-kinase because the effect was affected neither by 1 μM wortmannin nor by 10 μM LY294002 (Figure 3), a more selective phosphoinositide 3-kinase inhibitor (Vlahos et al 1994).

Protein kinase C is probably required in the activation of p38 MAPK by arachidonic acid

Protein kinase C is a family of phospholipid-dependent serine/threonine protein kinases that play a central role in signal transduction. Arachidonic acid has been demonstrated to activate protein kinase C in human neutrophils (Hii et al 1998). We, therefore, investigated the involvement of protein kinase C in p38 MAPK activation by arachidonic acid. We used three different protein kinase C inhibitors, Gö6976, Gö6983, and GF109203X (Martiny-Baron et al 1993; Gschwendt et al 1996). Gö6976 preferentially inhibits protein kinase C α, β, and μ isoforms, Gö6983 inhibits protein kinase C α, β, γ, δ, and ξ isoforms, and GF109203X inhibits protein kinase C α, β, δ, ε, ξ, and μ isoforms in in-vitro kinase assays. Gö6983 and GF109203X inhibited protein kinase C isoforms with a specificity broader than Gö6976, however only Gö6976 significantly attenuated p38 MAPK phosphorylation by arachidonic acid in a concentration-dependent manner (about 73% inhibition at 10 μM) with IC50 values of 5·4 ± 1·2 μM (Figure 4). This is in contrast with a previous study where GF109203X partially inhibited the appearance of phosphorylated p38 MAPK by arachidonic acid in human neutrophils (Hii et al 1998). The reasons for this discrepancy are not clear but might be attributed to differences in species, because GF109203X inhibited greatly the extracellular signal-regulated kinase activity in arachidonic acid-stimulated human neutrophils (Hii et al 1998) but failed to inhibit the extracellular signal-regulated kinase phosphorylation in rat neutrophils (data not shown). Moreover, the inhibitory effect of Gö6976 was significantly more pronounced than those of Gö6983 and GF109203X on fMLP-stimulated extracellular signal-regulated kinase phosphorylation in rat neutrophils (Chang & Wang 1999). Alternatively, the Ca^{2+}-dependent protein kinase C probably acts as the major protein kinase C isoform in the regulation of arachidonic acid-stimulated p38 MAPK phosphorylation in rat neutrophils. Blockade of the novel and atypical protein kinase C isoforms failed to improve, but instead eliminated, the effect of protein kinase C inhibitor on p38 MAPK phosphorylation. Arachidonic acid has been reported to stimulate Ca^{2+} mobilization (Hardy et al 1995). It remains to be determined which protein kinase C isoform is involved in arachidonic acid-stimulated p38 MAPK activation.

$[Ca^{2+}]_i$ elevation is essential to the p38 MAPK activation

It is well established that the activation of inositol-specific phospholipase C results in $[Ca^{2+}]_i$ elevation. Since arachidonic acid has been shown to

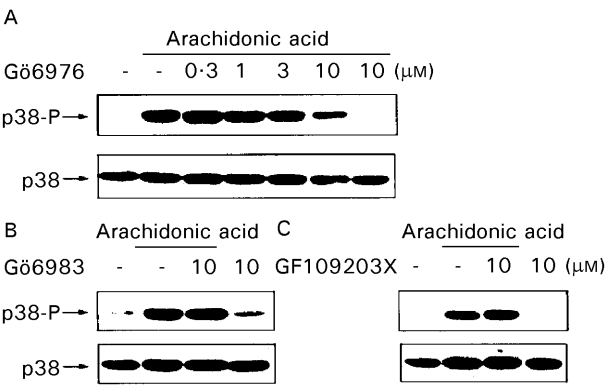

Figure 4. Effects of Gö6976, Gö6983, and GF109203X on arachidonic acid-stimulated p38 MAPK activation. Cells were preincubated at 37°C for 10 min with DMSO, (A) various concentrations of Gö6976, (B) 10 μM Gö6983, or (C) 10 μM GF109203X before stimulation with 30 μM arachidonic acid or without stimulation. One minute later, the levels of phosphorylated p38 MAPK were determined by immunoblot analysis using anti-phospho-p38 MAPK antibodies. Data are representative of three to four independent experiments.

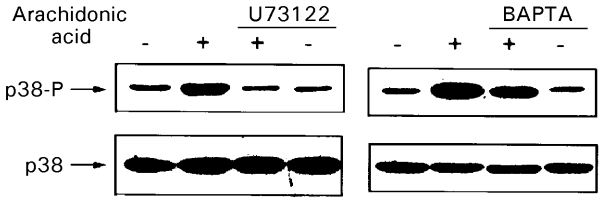

Figure 5. Effects of U73122 and BAPTA on arachidonic acid-stimulated p38 MAPK activation. Cells were preincubated at 37°C for 10 min with DMSO, 10 μM U73122, or for 1 h with DMSO or 10 μM BAPTA-AM before stimulation with 30 μM arachidonic acid or without stimulation. One minute later, the levels of phosphorylated p38 MAPK were determined by immunoblot analysis using anti-phospho-p38 MAPK antibodies. Data are representative of three to four independent experiments.

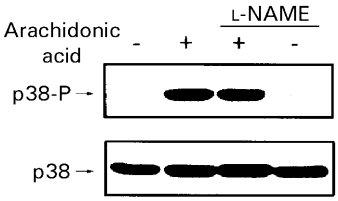

Figure 6. Effects of *N*-nitro-L-arginine methyl ester (L-NAME) on arachidonic acid-stimulated p38 MAPK activation. Cells were preincubated at 37°C for 10 min with DMSO or 3 mM L-NAME before stimulation with 30 μM arachidonic acid or without stimulation. One minute later, the levels of phosphorylated p38 MAPK were determined by immunoblot analysis using anti-phospho-p38 MAPK antibodies. Data are representative of three independent experiments.

stimulate Ca^{2+} mobilization in neutrophils, we determined the role of phospholipase C and $[Ca^{2+}]_i$ in p38 MAPK activation. Cells pretreated with 10 μM U73122, a phospholipase C inhibitor (Smith et al 1990), or preloaded with a Ca^{2+} chelator BAPTA-AM (Lincoln & Cornwell 1993) greatly inhibited (approximately 86% and 79%, respectively) p38 MAPK phosphorylation by arachidonic acid (Figure 5). A comparable inhibition of $AACOCF_3$-induced response was also observed (data not shown). Thus, the phospholipase C/Ca^{2+} signalling pathway may be involved in mediating the effect of arachidonic acid on p38 MAPK activation.

Activation of p38 MAPK by arachidonic acid is independent of nitric oxide/cyclic GMP

Nitric oxide is an intercellular messenger that performs a number of important functions. Nitric oxide exerts many of its effects by activating soluble guanylyl cyclase, leading to cyclic GMP accumulation (Kessels et al 1991). Blockade of the nitric oxide/cyclic GMP pathway attenuated phosphorylation of p38 MAPK by lipopolysaccharide in human neutrophils has been reported by Browning et al (1999). To examine the relevance of nitric oxide/cyclic GMP in p38 MAPK phosphorylation by arachidonic acid in rat neutrophils, cells were treated with the nitric oxide-synthase inhibitor *N*-nitro-L-arginine methyl ester (L-NAME) before stimulation with arachidonic acid. The increase in phospho-p38 MAPK levels was not altered by L-NAME even at a concentration 1000-fold the published IC50 (Figure 6). Incubation of neutrophils in medium with 300 μM sodium nitroprusside, a nitric oxide-releasing agent (Kowaluk et al 1992), resulted in a significant increase in cellular cyclic GMP levels ($2{\cdot}67 \pm 0{\cdot}28$ pmol per 2×10^7 cells, $P < 0{\cdot}01$). Arachidonic acid (30 μM) had no effect ($0{\cdot}63 \pm 0{\cdot}15$ vs $0{\cdot}58 \pm 0{\cdot}14$ pmol per 2×10^7 cells as control value, $P > 0{\cdot}05$). Hence, it is unlikely that arachidonic acid activated p38 MAPK through the nitric oxide/cyclic GMP signalling pathway.

In summary, the phosphorylation of p38 MAPK by arachidonic acid in rat neutrophils is upstream regulated by pertussis toxin sensitive-G protein, non-receptor tyrosine kinase, phospholipase C/Ca^{2+}, and protein kinase C. The phosphoinositide 3-kinase and nitric oxide/cyclic GMP signalling pathways are probably not involved. Although four members of the p38 MAPK family exist, only α and δ isoforms are detected in neutrophils (Nick et al 1999). Until specific antibodies to p38 subtypes become available commercially, it is not possible to determine which p38 isoform is activated by arachidonic acid.

Acknowledgements

This work was supported by grants from the National Science Council (NSC88-2314-B-075A-002) and Taichung Veterans General Hospital (TCVGH-887323C), Republic of China.

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