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Short communication

11,12-Epoxyeicosatrienoic acid stimulates tyrosine kinase activity in porcine aortic endothelial cells

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Abstract

Although epoxyeicosatrienoic acids, cytochrome *P*-450 mono-oxygenase metabolites of arachidonic acid, have been demonstrated to play a crucial role in endothelial cell Ca^{2+} homeostasis and endothelium-dependent vasorelaxation, the understanding of the actions of epoxyeicosatrienoic acids is limited. In this study, the effect of epoxyeicosatrienoic acids on tyrosine kinase in endothelial cell homogenate was investigated. 11,12-Epoxyeicosatrienoic acid increased tyrosine kinase activity in a concentration dependent manner (EC_{50} = 11.7 nM). Arachidonic acid in much higher concentrations (20 μM) mimicked the effect of the epoxyeicosatrienoic acid on tyrosine kinase. This effect of arachidonic acid was abolished in the presence of the cytochrome *P*-450 mono-oxygenase inhibitor thiopentone sodium, indicating that arachidonic acids needs to be converted to epoxyeicosatrienoic acids by the endothelial cytochrome *P*-450 mono-oxygenase to stimulate tyrosine kinase. These data describe a novel aspect of the actions of epoxyeicosatrienoic acids, and show that in addition to K^+ channel activation, epoxyeicosatrienoic acids also regulate tyrosine kinase activated signaling pathways in endothelial cell activation. © 1998 Elsevier Science B.V.

Keywords: Tyrosine kinase; Epoxyeicosatrienoic acid; Endothelial cell

1. Introduction

The arachidonic acid metabolites, epoxyeicosatrienoic acids, have been demonstrated to relax smooth muscle cells and may represent the endothelium-derived hyperpolarizing factor (EDHF; Hecker et al., 1995). Thus, epoxyeicosatrienoic acids activate K^+ channels in vascular smooth muscle (Hu and Kim, 1993), possibly via a G protein-dependent mechanism (Graier et al., 1996; Li and Campbell, 1997). In endothelial cells, epoxyeicosatrienoic acids elicit hyperpolarization, also by activation of K_{Ca} channels (Hoebel et al., 1997). They mediate endothelial Ca^{2+} signaling through yet unknown mechanisms (Graier et al., 1995). Since tyrosine kinase activation is involved in endothelial Ca^{2+} signaling (Fleming et al., 1995) and K^+ channel activity (Hoebel et al., 1997), the effect of epoxyeicosatrienoic acids on tyrosine kinase activity was investigated in endothelial cell homogenates to rule out effects on tyrosine kinase linked to epoxyeicosatrienoic acid-mediated changes on endothelial Ca^{2+} homeostasis and K^+ channel activity.

2. Materials and methods

2.1. Cell isolation and culture

Endothelial cells were isolated from porcine aortae by enzymatic digestion with 200 U/ml collagenase (type II) in dulbecco's minimal essential medium (DMEM) containing dilutions of commercially available stock solutions of (v/v) 0.02 amino acids and 0.01 vitamins (Gibco, Vienna, Austria) and trypsin inhibitor (soybean type I; 1 mg/ml) as described previously (Graier et al., 1995). Cells were cultured in Opti-MEM containing 2% fetal calf serum.

2.2. Tyrosine kinase activity

Tyrosine kinase activity was measured using a photometric assay from Calbiochem–Novabiochem (Vienna, Austria) as previously described (Hoebel et al., 1997). Cell

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lysates were obtained by sonication in chilled buffer containing in mM: 20 Tris, 50 NaCl, 1 EDTA, 1 EGTA, 0.2 phenylmethylsulfonyl fluoride, 0.2 Na_3VO_4, 5 mercaptoethanol, 1 μg/ml pepstatin and 0.5 μg/ml leupeptin, pH adjusted at 7.4. Phosphorylation was determined by a horse radish peroxidase labeled phosphotyrosine specific antibody and monitored at 450 nm.

2.3. Materials

Cell culture materials are obtained from Life Technologies (Vienna, Austria), fetal calf serum was from PAA (Linz, Austria) and petri dishes were from Corning (Vienna, Austria). All other chemicals used were obtained from Sigma (Vienna, Austria). Epoxyeicosatrienoic acids were from Cascade Biochem (Reading, UK).

2.4. Statistics

Analysis of variance was performed and statistical significance of differences were estimated by Scheffe's *F*-test. Level of significance was defined as $P < 0.05$.

3. Results

Addition of 11,12-epoxyeicosatrienoic acid to endothelial cell lysates increased tyrosine kinase activity in a concentration dependent manner (Fig. 1; $EC_{50} = 11.7 \pm 2.3$ nM). Similar results were obtained with 8,9-epoxyeicosatrienoic acid (data not shown). A time course of 11,12-epoxyeicosatrienoic acid on tyrosine kinase activity indicated increases in tyrosine kinase activity by 7.6 ± 5.3 (n.s. vs. control) 29.0 ± 7.5 ($P < 0.05$ vs. control), 52.7 ± 14.0 ($P < 0.05$ vs. control) and $77.4 \pm 11.8\%$ ($P < 0.05$ vs. control) after 3, 10, 20 and 30 min incubation of the cell lysates with 30 nM 11,12-epoxyeicosatrienoic acid ($n = 4$ for each time point). Increasing the free Ca^{2+} concentration to 100 μM free Ca^{2+} did not affect 11,12-epoxyeicosatrienoic acid-induced increase in tyrosine kinase activity (data not shown).

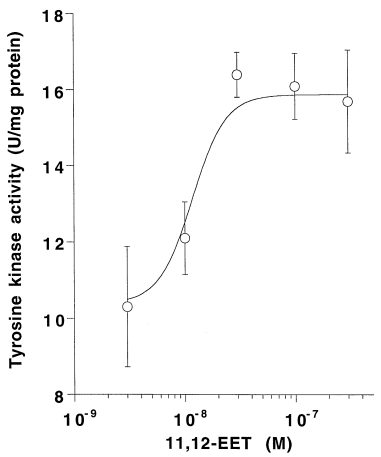

Fig. 1. Tyrosine kinase activity in endothelial cells lysates is increased by 11,12-epoxyeicosatrienoic acid. Tyrosine kinase activity in endothelial lysates was measured during 30 min at 37°C in the presence of the 11,12-epoxyeicosatrienoic acid (11,12-EET) in concentrations indicated. Each point represents the mean ± S.E.M. ($n = 4$–8).

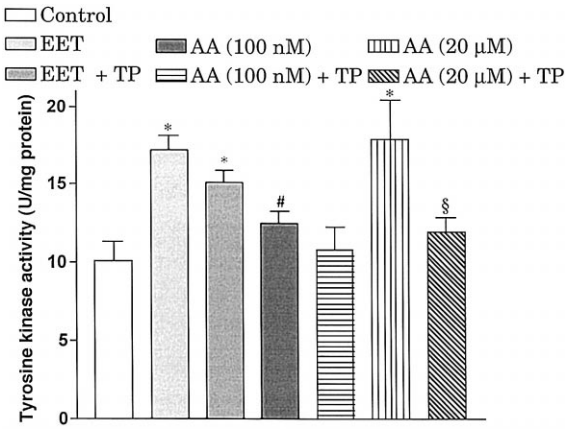

Fig. 2. Arachidonic acid stimulates tyrosine kinase activity in a cytochrome P450 mono-oxygenase-dependent manner. Tyrosine kinase activity in endothelial lysates was measured for 30 min at 37°C in the presence of the solvent (1% ethanol; Control), 100 nM 11,12-epoxyeicosatrienoic acid (EET) or arachidonic acid (AA) in concentrations of 100 nM and 20 μM, respectively. As indicated 300 μM thiopentone sodium (TP) which had no effect on tyrosine kinase activity itself was present. Each column represents the mean ± S.E.M. ($n = 6$–8). $*$ $P < 0.05$ vs. the solvent control, $\#P < 0.05$ vs. 100 nM 11,12-epoxyeicosatrienoic acid and $\S P < 0.05$ vs. 20 μM arachidonic acid.

The effect of arachidonic acid on tyrosine kinase activity was also assessed (Fig. 2). In concentrations where the epoxyeicosatrienoic acids had maximal effect on tyrosine phosphorylation (i.e., 100 nM; Fig. 2), arachidonic acid failed to stimulate tyrosine kinase activity. In contrast 20 μM arachidonic acid increased tyrosine kinase activity by 77% within 30 min (Fig. 2; $P < 0.05$ vs. control). The time course of the effect of 20 μM arachidonic acid on tyrosine kinase activity showed a slower onset of increased enzyme activity compared with that obtained with 11,12-epoxyeicosatrienoic acid. Tyrosine kinase activity increased by 2.1 ± 3.1 (n.s. vs. control) 6.7 ± 4.9 (n.s. vs. control), 23.6 ± 8.7 ($P < 0.05$ vs. control) and $69.1 \pm 12.5\%$ ($P < 0.05$ vs. control) after 3, 10, 20 and 30 min incubation of the cell lysates with 20 μM arachidonic acid ($n = 4$ for each time point).

A co-incubation of 20 μM arachidonic acid with the cytochrome P450 mono-oxygenase inhibitor thiopentone sodium (300 μM) abolished the stimulatory effect of arachidonic acid on tyrosine kinase activity (Fig. 2; $P < 0.05$ vs. the effect of arachidonic acid alone; n.s. vs. control). In contrast to the effect of arachidonic acid, tyrosine kinase stimulation by 11,12-epoxyeicosatrienoic acid was not significantly affected by 300 μM thiopentone sodium (Fig. 2; n.s. vs. the effect of 11,12-epoxyei-

cosatrienoic acid alone; $P < 0.05$ vs. control). Unlike many other cytochrome *P*-450 inhibitors (Sargeant et al., 1994) thiopentone sodium alone does not diminish tyrosine kinase activity, while it inhibits microsomal cytochrome *P*-450 mono-oxygenase in endothelial cells (Hoebel et al., 1997).

4. Discussion

These data for the first time describe that epoxyeicosatrienoic acids increase tyrosine kinase activity in endothelial cell homogenates in a concentration and time-dependent manner. This represents a new type of physiological action of epoxyeicosatrienoic acids and might explain their role in endothelial Ca^{2+} signaling. Moreover, these findings open a new perspective for the potential role of epoxyeicosatrienoic acids in cell homeostasis and cell activation.

Our findings that the stimulatory effect of arachidonic acid was abolished by the cytochrome *P*-450 mono-oxygenase inhibitor thiopentone sodium (Hoebel et al., 1997) suggest that arachidonic acid needs to be converted to epoxyeicosatrienoic acids by the endothelial cytochrome *P*-450 mono-oxygenase in order to stimulate tyrosine phosphorylation. In agreement with our findings, Cui and Douglas (1997) mentioned that in kidney epithelial cells arachidonic acid-initiated activation of extracellular signal-regulated protein kinase (ERK) depends on lipoxygenase and/or cytochrome *P*-450 metabolism.

These data for the first time indicate that cytochrome P450-derived metabolites of arachidonic acid have additional effects on cellular signal transduction pathways besides their effect on K^+ channels. Since tyrosine kinase is involved in autacoid- (Fleming et al., 1995, 1996) and shear stress- (Corson et al., 1996) induced endothelial cell activation, these additional properties of epoxyeicosatrienoic acids are of particular importance for the understanding of cellular signaling and cell activation. Additional work is necessary to verify the substrate proteins for epoxyeicosanotrienoic acid-activated tyrosine kinase to understand the importance for cell function of the findings presented herein.

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