PGE_2 Regulates the Actin Cytoskeleton to Promote EPAC1-Dependant Cell Spreading

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Background: EPAC1 is a key guanine nucleotide exchange factor for Rap1 GTPase and is activated by increases in intracellular cAMP induced by Gs-coupled GPCR ligands, including Prostaglandin E2 (PGE_2). EPAC1-activated Rap1 induces a range of cell responses, including cell spreading, adhesion and cohesion, all of which are regulated through cytoskeletal restructuring. In the present study we attempt to dissect the signalling pathways through which EPAC1 controls these effects. Results indicate that EPAC1 can regulate the activities of modulators of actin cytoskeleton dynamics, including RhoA GTPase and the FERM-domain protein, Ezrin.

Methods: Immunofluorescent confocal microscopy was carried out on HEK-293 cells stably transfected with either EPAC1 or a vector construct. Cells were seeded at $1x10^5$ per sterilised coverslip and allowed to adhere overnight. Cells were then stimulated with the cAMP elevating agents (Forskolin/Rolipram 10□M), the EPAC specific cAMP analogue (8-CPT-2'-O-Me-cAMP; 007; 10□M) or the physiological ligand PGE_2 (10□M) for one hour prior to fixation with 4% (w/v) paraformaldehyde + 1% (w/v) sucrose and permeabilised with 0.1%(v/v) Triton X-100. Primary antibodies against EPAC1, ezrin and ERM and secondary fluorescent antibodies (anti-mouse/anti-rabbit FITC/Rhodamine conjugates) (or Rhodamine-Phalloidin in actin stained cells) were incubated 1hr RT sequentially before treatment with DAPI for 20minutes RT. Cytoskeletal effects were assayed following treatment with the RhoA inhibitor, C3-ribosyltransferase (5ug/ml, 6hr pretreatment), or the actin destabilisers latrunculin A (LatA, 10ng/ml, 1.5hrs), or cytochalaisin D (cytD, 10ng/ml, 1.5hrs). Western blotting against total and phospho-specific proteins (T-ERM, P-ERM) allowed levels of phosphorylation to be observed.

Results: Treatment of vector or EPAC1-overexpressing and cells with Forskolin/Rolipram demonstrated that EPAC1 overexpression was required for cAMP to promote spreading, as observed by immunofluorescent confocal microscopy of actin-stained cells. This involvement of EPAC1 was further verified using an EPAC1-specific cyclic AMP analogue, 007, which also produced a significant spreading effect in EPAC1-transfected cells. Interestingly, PGE_2 was able to replicate this effect, demonstrating that EPAC1 induces cell spreading in response to physiological stimuli. To dissect the pathways involved in these actions of EPAC1, C3 endotoxin, cytD and latA were employed to investigate the role of actin de-polymerisation in these processes. Although all treatments resulted in a decrease in stress fibres, inactivation of RhoA or chemical induced actin depolymerisation appeared to be insufficient to promote cell spreading alone. Cell spreading also correlated with translocation of ezrin, which was recruited to the cell cortex, however this seems to be regulated by EPAC1 independently of it's classical phospho-site; threonine 567.

Conclusions: Results show that EPAC1 is vital for cAMP-mediated cell spreading in HEK-293 cells. EPAC1-dependent cell spreading correlates with a reduction in stress fibre abundance and an increase in cortical actin. Ezrin relocates to cortical actin following EPAC1 activation, which probably involves the membrane-actin linking action of the ezrin. Although EPAC1 activation appears to induce ezrin translocation, the mechanisms producing this effect appear to be atypical.