Purinergic and Endothelin Receptors Control Cofilin Activation Pathways in Rat Aortic Smooth Muscle Cells Through $G\alpha_q$-Dependent Signalling

Smooth muscle cell (SMC) migration is vital for vascular development and tissue repair, however, pathological cell migration lead to atherosclerosis, hypertension and vessel occlusion. Understanding the mechanisms and signalling pathways that promote vascular SMC migration is important in order to control these processes. The vasoconstrictors, uridine 5'-triphosphate (UTP) and endothelin-1 (ET1), act primarily via the $G\alpha_q$ protein-coupled receptors $P2Y_2$ and ET_A to promote aortic SMC (ASMC) migration (1), but the signal transduction pathways regulated by these receptors to induce ASMC migration have yet to be fully resolved. Dynamic regulation and reorganisation of the actin cytoskeleton at the leading edge of the cell is a key component of cell migration. Cofilin is an actin binding protein responsible for depolymerisation (severing) of existing F-actin, and thus continuously supplying actin monomers for polymerisation and rapid turnover of actin microfilaments. Cofilin is regulated by LIM kinase (to phosphorylate and inactivate) and slingshot phosphatase (to dephosphorylate and activate) (2).

We have investigated whether UTP and ET1 activate cofilin in male Wistar rat ASMC and the mechanisms that lead to its activation. Utilizing standard western blotting techniques and a specific anti-phospho-cofilin antibody we show that cofilin is dephosphorylated and activated by UTP (100 μM) or ET1 (50 nM) with maximal cofilin dephosphorylation occurring 5 min after agonist addition. UTP had a greater (66±8%, p=0.0037 one-way ANOVA, n=5) and more prolonged (up to 30 min) effect on cofilin dephosphorylation compared to ET1 (47±8%, p=0.002 one-way ANOVA, n=5). Arrestin proteins have been implicated in cytoskeletal reorganisation and chemotaxis through the scaffolding of cofilin. Therefore, we examined whether UTP- and/or ET1-driven-cofilin activation is a $G\alpha_q$- or arrestin-dependent process. Single and double knockdown of arrestin-2 and/or arrestin-3 with small-interfering RNAs did not affect the extent or time-course of cofilin activation by UTP or ET1 in ASMC. In contrast, pharmacological inhibition with UBO-QIC (1 μM; 30 min), a specific $G\alpha_q$ inhibitor (3), completely blocked both UTP- and ET1-stimulated cofilin dephosphorylation (n=4). It is known that Rho kinase (ROCK) phosphorylates/activates LIM kinase. Addition of the ROCK inhibitor Y27632 (10 μM; 30 min) alone increased cofilin dephosphorylation (49±5%, $p<0.001$ compared to basal, n=4), however, subsequent addition of UTP or ET1 each induced further significant dephosphorylation of cofilin (78±2%, $p<0.001$ and 82±1%, $p<0.001$, respectively, one-way ANOVA).

These findings show that receptors for UTP and ET1 regulate signalling pathways in ASMC leading to cofilin activation and through such mechanisms can regulate SMC migration. We have identified that these pathways are primarily $G\alpha_q$-dependent and arrestin-independent. Stimulation with UTP or ET1 was still able significantly to increase cofilin dephosphorylation in the presence of a ROCK inhibitor suggesting that these agonists activate a cofilin phosphatase, such as slingshot, which likely dictates when cofilin is active.

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