β-Adrenoceptor-stimulated cAMP response-element binding protein (CREB) signalling in myometrial cells is mediated by Src, PI3K, p38 MAPK and arrestin proteins

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During gestation, elevated cAMP levels in the myometrium play a significant role in the maintenance of smooth muscle quiescence, which is essential to prevent preterm labour (Price et al., 2001). This process is mediated through short-term mechanisms including PKA-mediated inactivation of myosin light chain kinase, and longer-term regulation of gene expression, which has been linked to activation of cAMP response-element binding protein (CREB) (Bailey et al., 2000). Although CREB expression and activity have been shown to decline during labour (Bailey et al., 2000), the actual molecular mechanisms which control agonist-driven CREB signalling have not been fully determined in myometrial cells. Therefore, the effects of increasing cAMP levels on CREB signalling were examined in the immortalized human ULTR myometrial cell line stimulated with the β-adrenoceptor (Gs-coupled) receptor agonist isoprenaline (ISO).

Agonist-driven CREB phosphorylation was detected using standard western blotting techniques and a specific anti-phospho-(p)CREB antibody. To ensure that all samples contained the same levels of protein, all membranes were stripped and re-probed for total CREB immunoreactivity, using an anti-CREB antibody. Protein expression was quantified using an image analysis system.

Stimulation of ULTR cells with ISO caused time- and concentration-dependent increases in pCREB immunoreactivity, with a peak at 30 min in the presence of 1μM ISO. ISO-stimulated CREB phosphorylation was inhibited 80 ± 9, and 52 ± 8% (means ± SEM, n=5; $p<0.05$, one-way ANOVA, Bonferonni's *post-hoc* test, n=5), following pre-incubation with the phosphoinositide-3-kinase (PI3K) inhibitor LY294002 (100nM, 30 min) and the Src-kinase inhibitor PP1 (5μM, 30 min), respectively. Pre-incubation with the PKA inhibitor KT5720 (1μM, 30 min) had no effect on ISO-stimulated CREB phosphorylation. Both ERK and p38 MAPK have been reported to induce CREB phosphorylation. In ULTR cells, ISO (1μM) caused a time-dependent inhibition of ERK phosphorylation, but stimulated p38 MAPK phosphorylation (peaking at 5 min and declining to basal at 60 min). Inclusion of the p38 inhibitor SB203580 (20μM, 30 min pre-incubation) significantly attenuated the time-dependent ISO-induced CREB phosphorylation ($p<0.05$ two-way ANOVA, Bonferonni's *post-hoc* test; n=5). Arrestin proteins have recently been reported to play a role in the regulation of CREB signalling in different cell backgrounds. To investigate whether arrestins play a similar role in ULTR cells, CREB signalling was examined following depletion of the endogenous arrestin2 and arrestin3 populations using small-interfering RNAs. Following arrestin2 depletion ISO-stimulated CREB phosphorylation was significantly enhanced ($p<0.05$ compared to negative-control transfected cells, two-way ANOVA, Bonferonni's *post-hoc* test; n=5). In contrast, depletion of arrestin3 had no effect.

In summary, we have shown that β-adrenoceptor-stimulated CREB phosphorylation in ULTR myometrial cells is dependent on PI3K, Src and p38 MAPK, and is independent of PKA activity. Furthermore, we have identified a role for arrestin2 in the regulation of β-adrenoceptor-stimulated CREB signalling however, it is unclear whether this is an arrestin scaffolding effect or due to a decreased β-adrenoceptor desensitization.

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