in MAPK-blocked cells, demonstrating MAPK and CREB independent inhibition of Egr1 expression by cAMP. Inhibition of EGR1 transcription was associated with a significant (to $21.4 \pm 6.4\%$, $p < 0.05$) inhibition of SRF activity, suggesting a role for SRF inhibition in cAMP-mediated EGR1 repression and inhibition of proliferation.

In summary, our results demonstrate that PKA and Epac synergise to inhibit VSMC proliferation by rapidly inhibiting EGR1 expression via a mechanism that probably involves inhibition of SRF activity. This also provides a mechanistic explanation for the CREB and MAPK-independent anti-mitogenic effects of cAMP in VSMC.

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TRANSCRIPTOME ANALYSIS IDENTIFIES A NOVEL MECHANISM OF CAMP-MEDIATED GROWTH ARREST IN VSMC: PKA AND EPAC SYNERGISE TO SUPPRESS EGR1 EXPRESSION

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Cyclic AMP signalling promotes VSMC quiescence in healthy vessels and during vascular healing following injury. We recently demonstrated that cAMP-sensitive PKA and Epac signalling pathways synergise to inhibit VSMC proliferation but the underlying mechanisms are not understood.

Using PKA and Epac-selective agonists and microarray analysis we tested the hypothesis that PKA and Epac block VSMC proliferation via coordinate regulation of immediate early genes required for cell-cycle progression. Epac agonist significantly enhanced PKA-dependent inhibition of EGR1 mRNA (4.6 ± 0.38 fold for PKA vs 13.8 ± 1.09 fold for PKA+EPAC, $p < 0.05$) and protein expression (1.9 ± 0.18 fold for PKA vs 7.1 ± 0.8 fold for PKA+EPAC, $p < 0.05$). This inhibition was replicated by elevating endogenous cAMP with forskolin (4.8 ± 1.9 fold, $p < 0.05$), adenosine (3.4 ± 0.7 , $p < 0.05$), A2B receptor agonist BAY60-6583 (4 ± 1.2 fold, $p < 0.05$) or Cicaprost (3.7 ± 0.5 fold, $p < 0.05$). Importantly, dominant-negative EGR1 inhibited VSMC proliferation.

cAMP-dependent inhibition (3.9 ± 0.8 fold, $p < 0.05$) of EGR1 transcription (reporter gene activity) was associated with a potent stimulation of CREB activity (225 ± 72 fold, $p < 0.05$). However, expression of dominant-negative CREB did not prevent cAMP-dependent inhibition of EGR1 expression. cAMP-mediated EGR1 repression was also associated with MAPK inhibition. However, inhibition of EGR1 transcription preceded MAPK inhibition. cAMP retains the ability to inhibit EGR1 expression