Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol19Issue1abst042P.pdf

Differential regulation of beta$_2$-adrenoceptor stimulated CREB signalling by G protein-coupled receptor kinases in vascular smooth muscle

J. Willets, L. Jones. Dept of Molecular & Cell Biology, University of Leicester, Leicester, United Kingdom

In vascular smooth cells (VSMC) cAMP generation through the activation of G_s-coupled receptors like the β_2-adrenergic receptor (β_2AR), promotes an anti-proliferative phenotype. cAMP mediates many of its action through the transcriptional regulator cAMP response element binding protein (CREB), decreasing expression of cell-cycle and mitogenic genes. GPCR signalling is regulated by G protein-coupled receptor kinases (GRKs), and in VSMC GRK2 and GRK5 negatively regulate β_2AR/cAMP accumulation. Thus, we utilised RNAi techniques to deplete GRK isozymes and identify their roles in the regulation of β_2AR activated CREB signalling in adult male Wistar rat aortic smooth muscle cells (ASMC). ASMC were transfected with siRNAs targeting GRKs 2, 5 or 6 or a negative-control (NC) siRNA. After 24h ASMC were serum-starved (24h) before stimulation with the βAR agonist isoprenaline (1μM). Agonist-driven CREB phosphorylation was detected using standard immunoblotting techniques using a specific anti-phospho-(p)CREB antibody. pCREB absorbance levels for each treatment were corrected for differences in total CREB immunoreactivity. siRNAs transfection depleted targeted GRK expression by ≥80% when compared to NC-transfected cells with no effects on non-targeted GRKs. In NC-transfected cells isoprenaline-stimulated a time-dependent increase in pCREB accumulation which peaked at 5 min, and remained above basal levels for 60min. Isoprenaline-stimulated pCREB immunoreactivity was attenuated over the first 10 min of the time-course after GRK2 knockdown (pCREB immunoreactivity at 5 min; NC-siRNA, 51995±5563 *vs* anti-GRK2, 14313±6898; data are means±SEM for n=5; $p<0.01$ two-way ANOVA; Tukey's *post hoc* test). Conversely, GRK5 knockdown, significantly enhanced isoprenaline-induced pCREB immunoreactivity over the first 10 min of the time course (pCREB immunoreactivity at 5 min; NC-siRNA, 51995±5563 *vs* anti-GRK5 siRNA, 67779±2318; data are means ± SEM for n=5; $p<0.05$ two-way ANOVA; Tukey's *post hoc* test). Furthermore, GRK6 depeltion significantly enhanced the peak (pCREB immunoreactivity at 5min; NC-siRNA, 51995±5563 *vs* anti-GRK6 siRNA, 74518±6017; data are means±SEM for n=5; $p<0.01$ two-way ANOVA; Tukey's *post hoc* test) and prolonged (pCREB immunoreactivity at 45 min; NC-siRNA, 45720±6939 *vs* anti-GRK6 siRNA, 78325±6982; data are means±SEM for n=5; $p<0.01$ two-way ANOVA; Tukey's *post hoc* test) isoprenaline-mediated pCREB immunoreactivity over the 60 min time-course. These data indicate that GRKs differentially regulate β_2AR-driven CREB activity in ASMC, with GRK2 activating, and GRK5 inhibiting short-term CREB phosphorylation. Moreover, GRK6 negatively regulates short-term and sustained CREB-phosphorylation. Thus, targeting GRK activity in vascular disease may provide an alternative mechanism to regulate CREB signals and prevent ASMC proliferation. We gratefully acknowledge the British Heart Foundation (Grant No. PG11/60/29007) for funding this work.