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Regulation of brown adipocyte gene expression by protein kinase A and PPAR gamma signalling pathways

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The brown thermogenic genes, uncoupling protein 1 (UCP1) and peroxisome proliferator activated receptor gamma coactivator 1 α (PGC1α) are regulated by protein kinase A (PKA)-dependent transactivation of the cAMP response elements (CRE) in the enhancer and proximal promoters[1,2]. Agonists PPARγ also increases UCP1 and PGC1α gene expression[3]. The aim of the study is to establish the interaction between PKA and PPARγ signalling pathways on stimulation of UCP1 and PGC1α in response to forskolin, an activator of cAMP, and rosiglitazone, a PPARγ agonist.

Brown preadipocytes (HIB-1B) were transfected with either the UCP1 (3.1 kb) or PGC1α (2.6 kb) promoter luciferase reporter construct in the presence and absence of forskolin and rosiglitazone. UCP1 and PGC1α transcriptional activity were measured by luciferase assay and gene expression by real-time PCR (RT-PCR). All data were analysed by ANOVA.

Forskolin and rosiglitazone significantly increased UCP1 ($P<0.001$) and PGC1α ($P<0.001$) transcriptional activity. (Fig. 1). When forskolin and rosiglitazone were combined together, there was a synergistic increase in UCP1 and PGC1α ($P<0.001$) transcription. These results were confirmed in experiments measuring gene expression by RT-PCR. Inclusion of a PKA inhibitor (H89) down regulated both forskolin and rosiglitazone stimulated PGC1α and blocked forskolin stimulated UCP1 expression whereas the PPARγ antagonist (rosiglitazone) only inhibited UCP1 expression when forskolin and rosiglitazone were combined.

It is concluded that the PKA- and PPARγ-dependent pathways interact to induce synergistic regulation of UCP1 and PGC1α expressions.

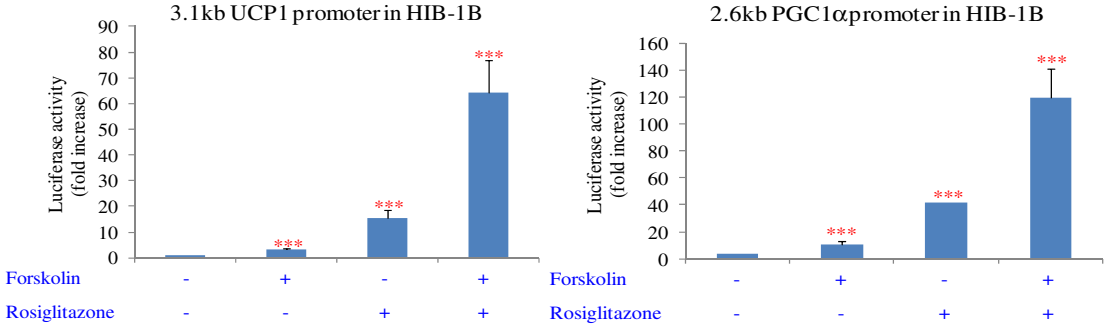

Fig. 1. Luciferase activities of 3.1 kb UCP1 and 2.6 kb PGC1α promoters. Values are from three independent experiments. ***$P<0.001$.

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