Proceedings of the Nutrition Society (2008), **67 (OCE8)**, E393 doi:10.1017/S0029665108000670

Summer Meeting 30 June–3 July 2008

The cJun kinase inhibitor SP600125 stimulates expression of PPARγ co-activator 1α (PGC-1α) and uncoupling protein 1 (UCP1) in the HIB-1B brown fat preadipocyte cell line

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Mitogen-activated protein kinase signalling pathways such as the c-Jun NH_2-terminal kinase (JNK) pathway play an important role in transducing environmental and growth factor signals into changes in cell proliferation and differentiation. Blockade of JNK stimulates conversion of mesenchymal stem cells to adipocytes[1], but deletion of JNK1 leads to protection from high-fat-diet-induced obesity and insulin resistance[2], suggesting that energy expenditure by brown adipose tissue may be increased. The transcriptional regulation of a gene (PGC-1α) involved in brown adipocyte differentiation has previously been investigated[3]. Here the effect of an inhibitor of JNK, SP600125, on the expression of PGC-1α and the brown-fat-specific marker gene UCP1 is reported.

A mouse brown preadipocyte cell line (HIB-1B) was seeded into culture dishes and transfected at 70% confluence with a PGC-1α luciferase reporter construct (PGC1α-PGL3) containing 264 bp of the PGC-1α proximal promoter, and a CCAAT-enhancer-binding protein-β (C/EBPβ) overexpression vector as described previously[3]. At confluence (48 h later) cells were incubated with SP600125 and forskolin for 12 h followed by measurement of luciferase activity. Another group of cells received similar treatments except that transfection with PGC1α-PGL3 was omitted and the cells were harvested for RNA after incubation with SP600125 and forskolin.

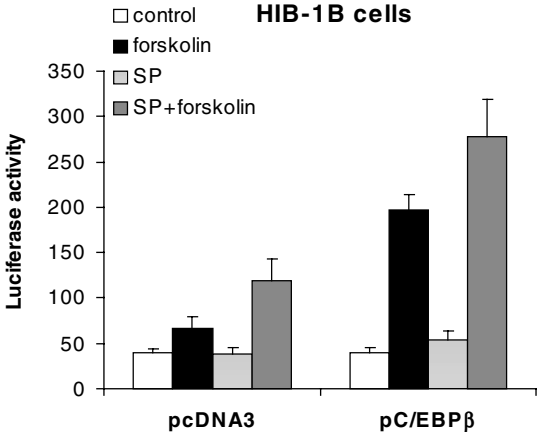

Figure. Effect of forskolin, C/EBPβ overexpression and SP600125 on the transcriptional activity of a PGC1α-PGL3 proximal promoter promoter construct transfected into HIB-1B cells.

It has been reported)previously that overexpression of C/EBPβ significantly increases forskolin-stimulated expression of both the PGC-1α reporter construct and accumulation of PGC-1α mRNA[3]. Addition of the JNK inhibitor SP600125 significantly increased both PGC-1α reporter activity (Figure) and mRNA expression in HIB-1B cells treated with both forskolin and forskolin + C/EBPβ overexpression. These effects were matched by an increase in mRNA for C/EBPβ and UCP1 with both forskolin and forskolin + SP600125.

These results are interpreted as demonstrating that JNK blockade increases the expression of key genes involved in the brown adipose tissue differentiation programme, which may be of relevance to the protection from obesity observed in JNK1 (-/-) mice. Previous results[3] combined with those from the present study suggest that JNK blockade promotes cAMP-dependent transcriptional activation of a number of genes involved in brown adipogenesis.

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3. Karamanlidis G, Karamitri A, Docherty K, Hazlerigg DG & Lomax MA (2007) *J Biol Chem* **282**, 24660–23669.