

Cellular mechanisms involved in intermittent hypoxia-induced heart damage in rat

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Background: Obstructive sleep apnoea (OSA), characterised by intermittent hypoxia (IH) during sleep, is increasingly recognised as an independent risk factor of cardiovascular diseases (CVD). OSA has been reported to be associated with changes in the levels of circulating oxidative stress and inflammatory markers as well as dyslipidemia, supporting their mediating roles in cardiovascular pathogenesis. This study aimed to investigate the effect of IH on heart tissue using an IH-exposed rat model and to explore the potential mechanisms involved in the occurrence of cellular injury in the heart.

Methods and Results: Male Sprague–Dawley rats were divided into intermittent air (IA)- or IH-exposed groups, and sacrificed after 4 weeks. IH caused elevations in serum malondialdehyde (MDA) and cytokine-induced neutrophil chemoattractant-1 (CINC-1) and reduction in serum adiponectin levels. In contrast, cardiac oxidative stress and pro-inflammatory markers were suppressed while cardiac adiponectin and cholesterol levels were elevated after IH exposure. In parallel, there was an increase in apoptosis in the heart tissue of IH-exposed rats, demonstrated by TUNEL staining and elevations of Bax and cleaved caspase-3 protein. Myocardial damage was further evident with decreased arterial vessel and capillary densities, increased cardiac fibrosis and the loss of troponin I.

Conclusions: Our data demonstrated for the first time that IH exposure caused systemic oxidative and inflammatory responses but “protective” responses in heart tissue. Despite such a local compensatory protective mechanism, heart damage was still observed that may have likely resulted from IH-induced caspase-dependent apoptosis cell death via cholesterol accumulation in the heart.

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UCP4 is a target effector of NF-κB c-Rel pro-survival pathway against oxidative stress

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Objectives: To examine the role of the cRel, subunit of NF-κB, involved in the protective mechanism of UCP4 in alleviating mitochondrial dysfunction and oxidative stress.

Methods: c-Rel construct was co-transfected with UCP4-promoter-luciferase-fusion expression vector into SH-SY5Y cells. Luciferase activity was measured to determine the promoter activity of UCP4. Changes in protein expression of UCP4 were measured by western blot. Binding of c-Rel on UCP4 promoter region was shown by gel-shift assay (EMSA). Superoxide levels were measured by DHE staining. Reduced, oxidised glutathione and mitochondrial membrane potential (MMP) were measured after H₂O₂ treatment.

Results: We showed that c-Rel overexpression induced NF-κB activity without affecting p65 levels. Overexpression of c-Rel increased UCP4 promoter activity and protein expression. Electrophoretic mobility shift assay showed increased specific binding of c-Rel protein complexes to a NF-κB site on the UCP4 gene promoter. Under H₂O₂-induced oxidative stress, UCP4 knockdown significantly increased superoxide levels, decreased GSH (reduced glutathione) and increased GSSG (oxidised glutathione) levels, compared to controls. UCP4 expression induced by c-Rel overexpression significantly decreased superoxide levels, and preserved GSH levels and MMP under similar stress.

Conclusions: In conclusion, our findings demonstrate the link between UCP4 and NF-κB c-Rel against oxidative stress in an in-vitro model of oxidative stress. We have shown that UCP4 can exert protective effects against H₂O₂ by being up-regulated by c-Rel overexpression, thereby reducing ROS levels, preserving cellular GSH levels and maintaining MMP. The protective effects of c-Rel overexpression were significantly decreased after UCP4 knockdown. These findings demonstrated that UCP4 is a target effector of the NF-κB c-Rel pro-survival pathway, and that UCP4 may act as a mitochondrial surveillance factor that mitigates the effects of oxidative stress through activation of this pathway.