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Activation of the Nuclear Factor Kappa B Pathway Following Ischemia-Reperfusion of the Murine Testis

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Ischemia-reperfusion (IR) of the testis results in testicular oxidative stress and germ cell-specific apoptosis. Nuclear factor kappa B (NF-κB) is a nuclear transcription factor involved in the control of a number of cellular processes, and its activation is part of the cellular stress response to a variety of factors including

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cytokine stimulation, irradiation, and IR. The present study investigates NF- κ B activation after IR of the murine testis and potential downstream target genes of that activation. Mice were subjected to a period of testicular ischemia followed by 0-4 hours of reperfusion. Activation of NF- κ B was assessed by 1) Western blot analysis of the NF- κ B inhibitory protein, $l\kappa$ B α ; 2) immunohistochemistry for $l\kappa$ B α ; and 3) TranSignal NF- κ B target gene array (107 genes) analysis. Results demonstrate that $l\kappa$ B α is phosphorylated on serine 32 reaching a peak by 2 hours after IR of the testis. A decrease in total $l\kappa$ B α was also noted at 2 hours after IR, consistent with the rapid degradation of the phosphorylated protein. Phosphorylation and degradation of $l\kappa$ B α is indicative of NF- κ B activation. Imunnolocalization revealed $l\kappa$ B α specifically in Sertoli cells of the murine testis. Results of the TranSignal target gene array revealed that the expression of 9 genes was consistently changed 2 hours after IR of the testis, 3 of which increased in expression and 6 of which were down-regulated. Most notably, high-mobility group nucleosomal binding domain 1 increased in expression while platelet-derived growth factor B and Wilms tumor homolog decreased. These results suggest that testicular IR releases the suppression of NF- κ B by $l\kappa$ B α in Sertoli cells. Activation of the NF- κ B pathway in the testis resulted in an alteration of expression of potential NF- κ B target genes, some increased while others decreased. The specific roles of these genes in the testicular response to IR remains to be determined.

Key words: Testicular oxidative stress, apoptosis, NF-*x*B activation

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