Hyperosmotic stress activates p65/RelB NFκB in cultured cardiomyocytes with dichotomic actions on caspase activation and cell death

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Abstract

NFκB is a participant in the process whereby cells adapt to stress. We have evaluated the activation of NFκB pathway by hyperosmotic stress in cultured cardiomyocytes and its role in the activation of caspase and cell death. Exposure of cultured rat cardiomyocytes to hyperosmotic conditions induced phosphorylation of IKKα/β as well as degradation of IκBα. All five members of the NFκB family were identified in cardiomyocytes. Analysis of the subcellular distribution of NFκB isoforms in response to hyperosmotic stress showed parallel migration of p65 and RelB from the cytosol to the nucleus. Measurement of the binding of NFκB to the consensus DNA κB-site binding by EMSA revealed an oscillatory profile with maximum binding 1, 2 and 6 h after initiation of the hyperosmotic stress. Supershift analysis revealed that p65 and RelB (but not p50, p52 or cRel) were involved in the binding of NFκB to DNA. Hyperosmotic stress also resulted in activation of the NFκB-lux reporter gene, transient activation of caspases 9 and 3 and phosphatidylserine externalization. The effect on cell viability was not prevented by ZVAD (a general caspase inhibitor). Blockade of NFκB with AdIκBα, an IκBα dominant negative overexpressing adenovirus, prevented activation of caspase 9 (more than that caspase 3) but did not affect cell death in hyperosmotically stressed cardiomyocytes. We conclude that hyperosmotic stress activates p65 and RelB NFκB isoforms and NFκB mediates caspase 9 activation in cardiomyocytes. However cell death triggered by hyperosmotic stress was caspase- and NFκB-independent.