Signalling pathways mediated by endothelin receptors located at the nuclear membrane

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Endothelins (ETs) are a family of neurohormonal factors comprising three distinct isoforms (ET-1, ET-2 and ET-3). ETs have potent vasoactive effects on vascular endothelial cells and mediate a variety of biological activities in non-vascular tissues. In the heart, Endothelins (ETs) are implicated in regulating contractility and in the initiation and progression of hypertrophy. ETs act by binding two subtypes of receptors (ETRs), ETA and ETB, which belong to the G-protein coupled receptor family (GPCR). Activation of cellular signalling by the interaction of ETs with its cell surface receptor has been well described. The best-described signalling pathway involves ETA and Gq/11 protein at the cell surface and results in the activation of the Erk1/Erk2 cascade. Several studies showed that functional ETRs are also located on the nuclear membranes of target cells. However the function of ETRs at this locus remains to be determined. ETB being the major subtype detected on nuclear membranes in cardiac myocytes, the present study was to determine the signalling pathway(s) modulated in the nuclear membrane of cardiac myocytes by ETB. Hence, the association of G proteins with ETB in nuclei isolated from rat heart was assessed by coimmunoprecipitate assays. Gαi, Gαs and Gαq co-immunoprecipitated with ETB. In each case, stimulation of isolated nuclei with ET-1 increased the amount of G protein immunoreactivity that co-immunoprecipitated with ETB. Moreover, incubation of nuclei with ET-1 resulted in an increase in the phosphorylation of Akt at serine-473. This phosphorylation was inhibited by the PI3K inhibitor wortmannin. In contrast, ET-1 did not increase the phosphorylation of p38 or Erk1/2 MAPKs. When nuclei are isolated from the intact ventricular myocardium, they originate from a heterogeneous population of cells. In order to selectively study the signaling associated with ETB in nuclear membranes in intact adult rat cardiac ventricular myocytes (ACVMs), a cell permeant and photoactivable caged ET-1 analog ([Trp-ODMNB^{21}]ET was used in conjunction with the fluorescent calcium dye, Fluo4/AM. In Fluo4/AM-loaded ACVMs, intracellular photolysis of caged ET-1 induced an increase in nuclear [Ca^{2+}] that was partially inhibited by the IP3 receptor inhibitor, 2-APB. Photolysis of ACVMs not preloaded with caged ET-1 failed to increase Fluo4 fluorescence. Concentrations of 2-APB as high as 100 μM failed to completely block the increase in Fluo4 fluorescence induced by photoloysis of caged ET-1. Thus, the signaling pathways associated with endothelin receptors in the nuclear membrane appear to differ from those same receptors when stimulated at the cell surface.