EP1_10

TNF alpha Protects Against Hypoxia in Heart Cultures Via Activation of PKA, Mitogen Activated Protein Kinases (MAPKs), SERCA2a and Phospholamban

Dalia El-Ani, Irena Philipchik, Hagit Stav, Moran Levi, Asher Shainberg
The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Israel

Tumor necrosis factor alpha (TNF) levels rise in ischemic injury. We have shown that heart cultures were protected against hypoxic injury if they were first pretreated by TNF. The aim of this study was to elucidate the mechanism by which TNF exerts its protection against hypoxia. TNF (30 ng/ml) protected rat and mice heart cultures against hypoxic damage, but not heart cultures of TNFR/KO mice, as revealed by lactate dehydrogenase (LDH) or creatine kinase (CK) released to the medium. $^{45}Ca^{2+}$ accumulation into the sarcoplasmic reticulum (SR) in normoxic heart cultures of TNFR/KO mice was 2 fold higher than in wild type mice. In wild type heart cultures, but not in TNFR/KO mice, TNF increased, in dose dependent manner, $^{45}Ca^{2+}$ accumulation into the SR. Intracellular Ca^{2+} [Ca^{2+}] levels was determined fluorescently by indo-1. TNF induced immediately decrease in [Ca^{2+}] that was elevated following hypoxia or extracellular Ca^{2+} ($[Ca^{2+}]_o$) application. However, TNF did not decrease [Ca^{2+}] after its increase by thapsigargin – a SERCA2a inhibitor. H-89 or KT5720- PKA inhibitors, but not KN93 a calmodulin kinase II inhibitor, attenuated the protective effect of TNF against hypoxic damage as determined by attenuating of Ca^{2+} elevation or LDH or CK released to the medium. Hypoxia decreased phosphorylated phospholamban. However, TNF increased phosphorylated phospholamban following hypoxia. Additionally, Protein Arrays of MAPKs reveal that TNF increased MAPKs during normoxia. However, in hypoxia the increase of MAPKs by TNF was prevented. The cardioprotective effect of TNF against hypoxic damage was inhibited by SB203580 – a p38 MAPK inhibitor or by PD98059- an ERK-1 inhibitor.

Conclusions:

TNF activates SERCA2a via activation of PKA, Mitogen Activated Protein Kinases (MAPKs) and phosphorylated phospholamban to pump Ca^{2+} into the SR in hypoxia and thus preventing Ca^{2+} overload.