without TNFα (100 U/ml for 30 minutes) and examined for ROS production (lucigenin emiluminescence) and MAPK activation (Western blot). Compared with cells transfected with an empty vector control or cells transfected with wild-type p47phox (overexpression), S303/304A mutation had no significant effect on the basal level (without TNFα) of ROS production. When cells were stimulated with TNFα, there was a twofold increase in ROS production by control and p47phox overexpressed cells. TNFα-induced ROS production was significantly reduced (~60%) in cells transfected with the S303/304A mutation. However, there was no significant difference in TNFα-induced phosphorylation of ERK1/2, p38MAPK and JNK between controls and the cells transfected with the S303/304A mutation. In conclusion, p47phox phosphorylation at S303/S304 plays an important role in TNFα-induced ROS production, but is not involved in TNFα-induced MAPK activation.

**008 NEURONAL NITRIC OXIDE SYNTHASE-MEDIATED REGULATION OF MYOCARDIAL REDOX STATE AND β3 ADRENERGIC RECEPTOR RESPONSES**

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Recent evidence suggests that increased superoxide production may decrease the bioavailability of endothelial nitric oxide synthase (eNOS)-derived nitric oxide in the myocardium of neuronal nitric oxide synthase (nNOS)-knockout mice. To test the functional relevance of this observation, we compared the eNOS-dependent isotropic and [Ca²⁺]i transient effect of β3-adrenergic receptor (β3-AR) stimulation in left ventricular myocytes from eNOS−/− and nNOS−/− mice and their wild-type littermates. β3-AR stimulation (with BRL 37344, 10 μmol/l in the presence of the β1 and β2-AR blocker nadolol, 10 μmol/l) resulted in a significant reduction in cell shortening and [Ca²⁺]i transient amplitude in wild-type myocytes. In contrast, BRL plus nadolol had no effect in myocytes from eNOS−/− and nNOS−/− myocytes (in the absence of changes in β3-AR messenger RNA levels or in eNOS protein expression in the latter) or in nNOS−/− myocytes pretreated with the eNOS-specific inhibitor, SMTC (0.1 μmol/l). Inhibition of xanthine oxidoreductase (XOR) or NADPH oxidases caused a similar reduction in basal superoxide production in nNOS−/− myocytes; however, whereas apocynin had no effect, XOR inhibition restored the negative isotropic response to β3-AR stimulation in nNOS−/− myocytes but not in eNOS−/− myocytes. eNOS activity was uncoupled in the myocardium of nNOS−/− mice in the absence of changes in BH4 and biopterin levels or arginase activity. However, l-arginine transport was impaired in nNOS−/− myocytes and excess l-arginine (10 mmol/l) restored the response to β3-AR stimulation. In summary, increased superoxide production by XOR (but not by NADPH oxidase) selectively abolished the negative isotropic effect of β3-AR stimulation in nNOS−/− myocytes by decreasing l-arginine transport and the bioavailability of eNOS-derived nitric oxide. These findings demonstrate that the source and subcellular localisation of superoxide production can account for the diverse and specialised actions of reactive oxygen species in the heart.

**010 CARDIOMYOCTE-SPECIFIC OVEREXPRESSION OF NOX4 ATTENUATES ADVERSE CARDIAC REMODELLING AFTER MYOCARDIAL INFARCTION**

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**Background** Reactive oxygen species (ROS) production is implicated in the development of cardiac remodelling after myocardial infarction (MI). NADPH oxidases are major sources of cardiovascular ROS, with cardiomyocytes expressing both Nox2 and Nox4 isoforms. We previously showed that Nox2 contributes significantly to the processes underlying adverse cardiac remodelling and contractile dysfunction post-MI, but the effect of Nox4 remains unclear. The aim of this study was to investigate the role of Nox4 in cardiac remodelling after MI.

**Methods and Results** We generated transgenic mice with cardiomyocyte-specific overexpression of Nox4, expressing the mouse full-length Nox4 complimentary DNA under control of the α-MHC promoter. Transgenic mice were backcrossed onto a C57BL6/J background and wild-type littermates (wt) used as controls. Nox4 transgenic mice were grossly similar to wt in terms of body weight, growth, activity, fertility and basal cardiac function. Transgenic mice displayed heart-specific expression of the Nox4 transgene, had significantly increased Nox4 protein expression, over 90% higher heart NADPH oxidase activity and 42% increased hydrogen peroxide production (all p<0.05). Compared with wt, p22phox protein expression was increased by 2.4-fold, which may be related to an increased protein stability. There was no change in the protein expression level of Nox2 or eNOS. MI was achieved by permanent left coronary ligation for 4 weeks. Compared with wt/MI, transgenic/MI groups displayed significantly high survival rate (72% vs 95%) and less hypotrophy at 4 weeks post-MI in terms of heart/body weight ratio (6.95±0.16 vs 6.44±0.14, p<0.01), although the