NOX4 NADPH OXIDASE IS A KEY REGULATOR OF ENDOTHELIAL CELL FUNCTION IN EXPERIMENTAL DIABETES

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Introduction The characteristic hyperglycaemia of diabetes drives reactive oxygen species (ROS) production in endothelial cells (ECs), leading to microvascular dysfunction and cardiovascular complications. NADPH oxidases are enzymes whose primary function is to generate ROS, and which contribute to development and progression of cardiovascular disease. This study aimed to investigate the role of the NOX4 isoform, which is highly expressed in ECs, under hyperglycaemic conditions, in EC signalling and paracrine communication with fibroblasts, as a major determinant of diabetic cardiovascular remodelling.

Methods Human aortic endothelial cells (HAoECs) were treated with normal (NG, 5.5 mM) or high (HG, 25 mM) glucose for up to 5 days with or without NOX4 siRNA knockdown (KD) prior to assessment of mRNA expression (real-time RT-PCR; relative to β-actin) and superoxide generation (DHE fluorescence). NIH 3 T3 fibroblasts were treated with conditioned media from NOX4-modified HAoECs for 24 hour to interrogate effects on paracrine signalling.

Results HG treatment of HAoECs for 5 days increased mRNA expression of NOX4 (NG 1.01±0.06, HG 1.27±0.06; n=9, p<0.05) and associated antioxidant and proinflammatory genes (e.g. NRF2: 0.91±0.04 vs 1.22±0.04; IL-6: 1.03±0.13 vs 2.59±0.58; n=6, p<0.05). This was associated with increased superoxide production (262±12 vs 338±11 arbitrary units; p<0.05) and associated antioxidant and proinflammatory genes (e.g. NRF2: 0.91±0.04 vs 1.22±0.04; IL-6: 1.03±0.13 vs 2.59±0.58; n=6, p<0.05). This was associated with increased superoxide production (262±12 vs 338±11 arbitrary units; p<0.05) and associated antioxidant and proinflammatory genes (e.g. NRF2: 0.91±0.04 vs 1.22±0.04; IL-6: 1.03±0.13 vs 2.59±0.58; n=6, p<0.05). This was associated with increased superoxide production (262±12 vs 338±11 arbitrary units; p<0.05) and associated antioxidant and proinflammatory genes (e.g. NRF2: 0.91±0.04 vs 1.22±0.04; IL-6: 1.03±0.13 vs 2.59±0.58; n=6, p<0.05).

Conclusions HG-induced NOX4 signalling regulates ROS production and endogenous antioxidant expression in ECs, driving paracrine stimulation of fibroblast differentiation. It therefore seems likely that EC NOX4 NADPH oxidase signalling contributes significantly to adverse diabetic cardiovascular remodelling.