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Effect of hypoxia on the vascular sphingolipid system

Background: Sphingosine kinase 1 (SK1) catalyses the conversion of sphingosine to the bioactive lipid sphingosine-1-phosphate (S1P).Considerable evidence has emerged that the SK1/S1P pathway plays an important role in vascular tone regulation and cardioprotection against ischaemia/reperfusion injury. Our previous work has demonstrated that S1P is a vasodilator in the rat coronary artery and in this study we wanted to investigate the effect of hypoxia on the expression of SK1 and how this may regulate vascular function through the generation of S1P.

Methods and results: Hearts were removed from male Sprague-Dawley rats (200-250g) and the coronary arteries dissected out, treated with specific inhibitory agents outlined below (all at a concentration of 10□M), exposed to hypoxia/normoxia (30 min), and then fixed using paraformaldehyde. The artery was then incubated with fluorescently-labelled anti-SK1 antibodies and SK1 expression and localisation visualized by confocal microscopy. Immunostaining (The percentage of the tissue which exhibited fluorescence binding above the background value) was measured from 3 different images taken from 3 different areas in each artery by image J software. Results (expressed as mean ± SEM)were analysed using one-way ANOVA (Dunnett'spost-test). Rat thoracic aortic rings were set-up in a wire myographunder normoxic and hypoxic conditions to test the vasodilator responses to S1P and synthetic analogues of S1P. Results (expressed as mean ± SEM) were analysed using two-way ANOVA (Bonferroni's post hoc). In rat coronary artery endothelium, hypoxia caused a marked increase in SK1 expression (9.7±1.2%, vs 2.6 ± 0.4% under normoxic conditions; $p<0.05$,n=3) and this increase was abolished by pre-treatment with cycloheximide(1.9±0.6% n=3). Moreover, pre-treatment with a sphingosine kinase inhibitor (2-(p-hydroxyanilino)-4-(p-chorophenyl) thiazole) (SKi) blocked the effect of hypoxia on SK1 expression (1.2±0.6%, n=3) while addition of a combination of the proteasomal inhibitor, MG132 and the lysosomal inhibitor, CA-074ME reversed the decrease in SK1 expression induced by SKi (14.6±1.9%, n=4). In the rat aorta, S1P caused an endothelium-dependent relaxation (E_{max}; 19.8±5.9%, n=10). Hypoxia significantly enhanced the relaxant response to S1P (E_{max}; 31.8±6.3%, n=10) and also the contractile response to U46619 in endothelium intact vessels and this effect was abolished by pre-incubation with SKi.

Conclusion: Hypoxia increases the SK1 expression in rat coronary artery endothelial cells. This effect can be blocked by cycloheximide, suggesting that hypoxia may enhance SK1 protein synthesis. Furthermore, the data obtained using SKi, CA-074ME and MG132 suggest that SKi might promote the proteasomal and lysosomal degradation of SK1.In rat aortic rings, hypoxia-induced upregulation of SK1 enzyme may enhance S1P receptor-dependent relaxation, which is likely mediated by S1P receptors.