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Molecular insight in apoM-S1P-induced cardioprotection against ischemia/reperfusion injury

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Purpose: Apolipoprotein M (apoM) is a plasma lipoprotein that mainly associates with high-density lipoproteins (HDL) and that serves as a carrier of the bioactive lipid Sphingosine-1-Phosphate (S1P). Recent studies indicate that S1P binding to G-protein-coupled receptors, known as S1P-receptors, in the heart activates signalling pathways promoting cardiomyocyte survival, but downstream targets are largely unknown. Here, we investigate the putative role of the apoM-S1P axis in relation to cardioprotection against ischemia/reperfusion (IR) injury.

Methods and Results: ApoM transgenic (Apom-Tg) mice, in which plasma S1P is increased by >250%, and wild-type (WT) mice were subjected to 30 min of left coronary artery ligation and 24 hrs reperfusion in vivo. We found a reduction of infarct size in Apom-Tg mice ($15 \pm 1\%$) in comparison with WT mice ($29 \pm 4\%$, N=8-9, $p<0.01$). In agreement, neutrophil infiltration into the infarcted area was lower in Apom-Tg mice ($14.8 \pm 0.2\%$ vs. 25.9 ± 5.1 in WT, N=3, $p<0.05$). Interestingly, 5 min of S1P treatment at the onset of reperfusion reduced infarct size in response to 30 min of no-flow global ischemia (control: $23 \pm 3\%$, S1P-treated: $11 \pm 2\%$, N=5, $p<0.05$) in ex vivo Langendorff perfused hearts, suggesting that S1P exerts a direct protective effect on cardiomyocytes. Moreover, the sensitivity to ex vivo IR of Apom-Tg mice was not different from WT mice, further supporting that the cardioprotective effect observed in vivo is due to increased plasmatic S1P in these mice. To obtain further insight into the mechanism underlying S1P-induced cardioprotection, neonatal rat ventricular cardiomyocytes were treated for 5 min with S1P after pre-incubation with PKC kinase inhibitors or with specific antagonists of S1P receptors. We found by Western blot that S1P induced phosphorylation of the gap junction protein Connexin43 (Cx43) on Serine 368 by a PKC-dependent mechanism and that this phosphorylation was mediated by S1P2 and S1P3 but not by S1P1 receptors. Finally, 5 min of S1P treatment reduced gap junctional communication between cardiomyocytes (9 ± 1 cells, N=29) in comparison to control conditions (15 ± 2 cells, N=34, $p<0.01$), as assessed by dye coupling assay.

Conclusion: Increased plasma apoM-S1P in mice protects the heart against IR injury. The molecular mechanism might involve reduced cardiomyocyte death by activation of S1P2 and S1P3 receptors, which leads to PKC-dependent phosphorylation of Cx43 and reduction of cell-to-cell coupling.