[gw22-e0337]

ISCHEMIC PRECONDITIONING ACTIVATES CAVEOLAE-MEDIATED ENOS SIGNALLING AND ELICITS CARDIOPROTECTION THROUGH S-NITROSYLATION OF THE SUBSARCOLEMMAL MITOCHONDRIA

Junhui Sun, Tiffany Nguyen, Elizabeth Murphy *Systems Biology Center, National Heart Lung and Blood Institute, National Institutes of Health, USA*

10.1136/heartjnl-2011-300867.172

Background Recent studies suggest that caveolae transduce endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) signalling in ischemic preconditioning (IPC) hearts. And the NO-mediated protein S-nitrosylation (SNO) has been shown to play cardioprotective roles against ischemic reperfusion injury. Interestingly, most of these identified IPC-induced SNO proteins are mitochondrial proteins, suggesting mitochondria are the major end effector for NO-mediated cardioprotection (*Circ Res*, 2007, 101:1155–1163; 2010, 106:285–296; 2011, 108:418–426).

Aims Given the diffusion-limited NO signalling in the cells and the close ultrastructural distance in cardiomyocytes between the subsarcolemmal mitochondria (SSM) and the sarcolemma, the SSM rather than the interfibrillar mitochondria (IFM) are hypothesised to be the preferential targets for caveolae-associated eNOS signalling in IPC hearts.

Methods C57BL/6J male mice (12–16 weeks) were Langendorff perfused, and the control hearts were perfused for 40 min while IPC hearts were subjected to four cycles of 5 min of ischemia and 5 min of reperfusion followed with 20 min of equilibrium perfusion. After perfusion control and IPC protocol, the SSM and IFM were isolated quickly in the presence of 1 mM of EDTA and 0.1 mM of neocuproine in the dark to prevent the decomposition of SNO. The protein compositions in these two populations of mitochondria were analysed by immunoblot using various marker antibodies, and the SNO was measured by a modified biotin switch method using DyLight Fluor-maleimide.

Results In the samples isolated from the perfusion control hearts, eNOS and caveolin-3 (the specific caveolae marker in muscles) were found to be present only in the SSM but not in the IFM, suggesting a close subcellular co-localisation and interaction between the sarcolemma and the SSM. IPC not only caused the phosphorylation and activation of AKT/eNOS signalling pathway, but also led to a translocation of eNOS to the SSM via caveolae-mediated trafficking. Consistent with the presence of eNOS and caveolin-3 in the SSM but not in the IFM, the total SNO content in the SSM was significantly higher than that present in the IFM in perfusion control hearts. More interestingly, IPC significantly increased SNO only in the SSM but not in the IFM, parallel with the findings that IPC led to a translocation of eNOS only to the SSM.

Conclusion In conclusion, these results demonstrated that the SSM are the major targets for caveolae/eNOS/NO-mediated protein S-nitrosylation in IPC hearts, suggesting that the SSM might be preferentially targeted by post-translational protein modifications derived from the sarcolemmal signalling (caveolae-associated eNOS and NO-mediated protein S-nitrosylation in this study) and then to elicit cardioprotection.