

## ISCHEMIC AND PHARMACOLOGICAL INDUCTION OF DELAYED CELLULAR PROTECTION IN iNOS GENE-DISRUPTED MICE MYOCYTES

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Inducible nitric oxide synthase (iNOS) has been implicated as a mediator in myocardial protection, but this property of iNOS is still conflicting. Therefore, the present study was designed to assess whether iNOS really contributes to the ischemically and pharmacologically induced delayed cellular protection (DCP) in mice myocytes. The following groups of cultured iNOS gene-knockout (iNOS<sup>-/-</sup>), and its respective wild-type (wt) mice myocytes subjected to simulated ischemia (SI) at 20 h were studied: (a) wt + SI: with ischemia alone; (b) iNOS<sup>-/-</sup> + SI: with ischemia alone; (c) iNOS<sup>-/-</sup> + heat shock (HS): iNOS<sup>-/-</sup> and HS; (d) iNOS<sup>-/-</sup> + sub-lethal simulated ischemia (SSI): iNOS<sup>-/-</sup> and SSI; (e) iNOS<sup>-/-</sup> + A<sub>1</sub>AR agonist 2-chloro-N<sup>6</sup>-cyclopentyladenosine (CCPA): iNOS<sup>-/-</sup> and 1 μM CCPA; (f) iNOS<sup>-/-</sup> + A<sub>1</sub>AR agonist (2S)-N<sup>6</sup>-[2-endo-norbornyl]adenosine (S-ENBA): iNOS<sup>-/-</sup> and 1 nM S-ENBA; (g) iNOS<sup>-/-</sup> + K<sub>ATP</sub> channel opener pinacidil (Pin): iNOS<sup>-/-</sup> and 0.05 μM Pin, and (h) iNOS<sup>-/-</sup> + mitochondrial K<sub>ATP</sub> channel opener diazoxide (Diazo): iNOS<sup>-/-</sup> and 100 μM Diazo. The release of LDH into the medium as well as the amount of LDH remaining in the cells was used as a marker of cellular injury and cell viability. The cellular resistance was acquired by iNOS<sup>-/-</sup> mice myocytes due to HS, SSI, CCPA, S-ENBA, pinacidil and diazoxide treatment, which was evidenced by reduction of LDH (U/L) release from 51.14 ± 1.35 (iNOS<sup>-/-</sup>) to 42.20 ± 1.01 (iNOS<sup>-/-</sup> + HS); 45.57 ± 0.75 (iNOS<sup>-/-</sup> + SSI); 42.87 ± 0.87 (iNOS<sup>-/-</sup> + CCPA); 43.21 ± 0.70 (iNOS<sup>-/-</sup> + S-ENBA); 37.81 ± 0.99 (iNOS<sup>-/-</sup> + Pin) and 36.79 ± 0.68 (iNOS<sup>-/-</sup> + Diazo), p < 0.01. Our data suggest that heat shock (HS), sub-lethal simulated ischemia (SSI), A<sub>1</sub> adenosine agonists CCPA, S-ENBA and K<sub>ATP</sub> channel openers pinacidil (membrane K<sub>ATP</sub> channel), diazoxide (mitochondrial K<sub>ATP</sub> channel) induce delayed cellular protection in mice myocytes against subsequent sustained simulated ischemia without the involvement of iNOS. Further, our data also suggest that pinacidil and diazoxide are more potent inducers of delayed cellular protection among others in iNOS<sup>-/-</sup> mice myocytes against sustained simulated ischemia.

**Key words:** *simulated ischemia, K<sub>ATP</sub> channel, iNOS, late protection, heat shock*

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