Ischaemic preconditioning preserves antioxidant enzyme activity and reduces cell damage of in vivo rat hearts undergoing acute ischaemia

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Acute myocardial ischaemia causes increased production of oxygen free radicals¹, which may react with cellular membrane lipids causing lipid peroxidation². Intrinsic antioxidant enzymes such as, superoxide dismutase (SOD) and glutathione peroxidase (GPx) may protect cellular integrity from oxygen free radicals³. In addition preconditioning (IPC) of the myocardium, has been proved useful in reducing ischaemic and reperfusion injury⁴.

The present study focuses on the role of myocardial preconditioning on the antioxidant enzyme activity, the cell energy status and on lipid peroxidation, in rat hearts undergoing 20 minutes of acute ischaemia. The experiments were carried out in the Department of Pathophysiology of the Medical University of Warsaw, according to the regulations for investigations on animals. Three groups of 21 male wistar rats were used for the experiments. Group A (7 rats, control group) in which the experimental animals were subjected to 30 minutes of stabilisation, without preconditioning or acute ischaemia. Group B (7 rats, ischaemia group) in which the rats were subjected to 10 minutes of stabilisation followed by 20 minutes of acute ischaemia and Group C (7 rats, IPC group) in which the rat hearts were subjected to 10 minutes of preconditioning (5 min of closure followed by 5 min of opening of the artery) followed by 20 minutes of acute ischaemia.

Anaesthesia was induced with intraperitoneal urethane (1g/kg). The animals were mechanically ventilated and their heart rhythm was monitored. Sternotomy was done, the heart was exposed and acute ischaemia was applied by ligating the proximal left coronary artery with a 6/0 silk thread. Preconditioning was achieved by tightening for 5 minutes the silk thread and then loosening it for another 5 minutes. Acute ischaemia was achieved by tightening the silk thread for 20 minutes. Acute ischaemia was confirmed by ECG changes and by the development of cyanosis on the affected myocardium.

At the end of the 30 minutes the animals were sacrificed and the left ventricle was homogenised, centrifuged and taken for estimation of the antioxidant activity, ATP and MDA.

Estimation of the activity of superoxide dismutase (SOD) was done according to Misra and Fridovich⁵, glutathione peroxidase (GPx) was assayed according to Paglia and Valentine⁶, malondialdehyde (MDA) was determined by the thiobarbituric acid method⁷ and adenosine triphosphate  (ATP) was determined according to Noronha-Dutra and Steen⁸.

Values are presented as mean plus standard error. Unpaired Student’s t-test was used to test for differences between groups. A two-tailed test with a p value less than 0.05 was considered significant.

CONCLUSION: The present study shows that preconditioning of 5 minutes duration provides cardioprotection to the myocardium subjected to 20 minutes of acute ischaemia, through a mechanism that involves preservation of the ATP levels and of the antioxidant enzymes SOD and GPx and by reduction in cell membrane lipid peroxidation.
Study limitations: The present study is limited by the fact that myocardial infarct size was not measured. Such measurement could be associated better to our findings.

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References