ADRENOMEDULLIN-INDUCED CARDIOPROTECTION: THE ROLE OF PEROXYNITRITE

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The nitric oxide (NO) pathway has been widely implicated in the cardiovascular effects of adrenomedullin (AM) (Hinson et al., 2000). We have previously demonstrated that AM exhibits NO-dependent cardioprotective effects against ischaemia-induced arrhythmias (Looi et al., 2002). NO and superoxide, when generated by the same, or closely interacting, cells react with each other to form peroxynitrite. Thus the aim of this study was to investigate the role of peroxynitrite in these observed effects exerted by AM.

Pentobarbitone (60 mg/kg)-anaesthetised male Sprague-Dawley rats (290-400g) were subjected to ligation of the left main coronary artery for 30 min. The number and incidence of cardiac arrhythmias, mean arterial blood pressure (MABP) and heart rate were monitored throughout the experiments. Ex vivo ROS generation in response to zymosan was determined in whole blood by luminol chemiluminescence (CL). Intravenous AM (1 nmol/kg; n=19) or saline (n=29) was administered 5 min prior to occlusion. The peroxynitrite scavenger N-mercaptopropionyl-glycine (MPG) was infused (20 mg/kg/h, i.v.) from 30 min prior to i.v. administration of AM (1 nmol/kg; n=10) or saline (n=10). Time-matched controls were also performed (n=10). In another set of experiments, immunocytochemical staining for nitrotyrosine, a marker for peroxynitrite deposition, was performed in heart sections from sham operated animals that were subjected to bolus AM (1 nmol/kg) or saline as well as in AM or saline treated animals that were subjected to 30 min myocardial ischaemia and 2 h reperfusion. Data is expressed as mean±s.e.m. Statistical comparison was performed using one-way ANOVA and Dunnett's post-hoc test. The percentage incidence of ventricular fibrillation (VF) was compared using Fisher's exact test. $P<0.05$ indicates statistical significance.

AM administration resulted in a significant reduction in the total number of ventricular ectopic beats that occurred during ischaemia (520±74 vs. 1274±175). However, the total number of ventricular ectopic beats that occurred during ischaemia in AM and saline treated animals receiving MPG were not significantly different from control (1064±155 and 1136±329 respectively). The percentage incidence of VF was significantly lower in the AM treatment group compared to control (26% vs. 66%) but was not significantly different from the time-matched control in the MPG-treated groups. AM treatment also caused a significant increase in CL generation 1 min (135±15%) preocclusion and subsequently at 15 min (134±16%) postocclusion compared to controls. In MPG treated animals, CL generation was not significantly different from the time-matched controls at any time points. Semi-quantification of staining showed that nitrotyrosine positivity in the endothelium of coronary arteries was significantly greater in the heart sections from animals treated with AM compared to saline treatment in both sham operated animals (17% vs. 58%) and in animals subjected to myocardial ischaemia and reperfusion (23% vs. 56%).

We conclude that the effects of AM in reducing ischaemia-induced arrhythmias and in increasing ROS generation from whole blood are both peroxynitrite-dependent. AM also increased nitrotyrosine staining in coronary endothelium, suggesting that peroxynitrite may play a role in the cardioprotective effect of AM.

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