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THE ROLE OF NITRIC OXIDE AND CALCIUM REGULATION IN CARDIOPROTECTION FROM REMOTE ISCHAEMIC PRECONDITIONING

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We have previously shown that ischaemic preconditioning (IPC) of the whole heart protects the isolated ventricular myocytes against Ca^{2+}-overload injury during simulated ischaemia [1]. Nitric oxide (NO) signalling is known to modulate Ca^{2+}-regulation in cardiac myocytes [2] and plays a central role in IPC [3]. We have compared the involvement NO-signalling in cardioprotection in IPC versus remote ischaemic preconditioning (rIPC).

We used an isolated ventricular myocyte model of IPC of whole hearts [1] and compared this to rIPC myocytes, where naÃ¯ve cardiomyocytes are remotely conditioned with the superfusate from preconditioned hearts. Two models of ischaemia-reperfusion (I/R) injury were used to determine protection. 1) Ischaemia was simulated in myocytes centrifuged to a dense pellet and layered with mineral oil to prevent gaseous diffusion (37° C, 30 min), and reperfusion by dispersing the myocyte pellet into oxygenated 2mM Ca^{2+} Tyrode [1] and cell death was assessed by Calcein and Propidium Iodide staining. 2) $[Ca^{2+}]_i$ was recorded from field-stimulated myocytes loaded with Fura-2 and subjected to metabolic inhibition (2 mM NaCN and 1 mM Iodoacetic acid) for 8 min followed by re-energization with 2mM Ca^2 Tyrode for 12 min. Cell injury was determined as the inability to maintain low diastolic $[Ca^{2+}]_i$ and to contract in response to electrical stimulation. Data are mean±S.E.M (n=number of experiments, hearts; one-way ANOVA followed by Tukey's multiple comparison post-hoc test).

IPC and rIPC both significantly reduced the degree of necrotic injury compared to control myocytes [IPC 29.7±2.1% (n=32, 6; $P<0.001$); rIPC 30.9±3.7% (n=17, 12; $P<0.001$); control 55.1 ±2.9% (n=18, 13)]. The protective effect of rIPC was abolished by the non-specific NOS inhibitor L-NAME (100 μM) at 56.5±3.8% (n=13, 10; $P<0.001$), when present during the rIPC stimulus and the I/R protocol. Both IPC and rIPC increased the percentage of myocytes that recovered contractile function on re-energization following metabolic inhibition, from 39.8±3.7% of control myocytes (n=30, 12), to 63.8±2.3% (n=24, 7; $P<0.001$) and 55.7±3.2% (n=24, 9; $P<0.01$) respectively. This increased recovery of contractile function was not blocked by L-NAME present during the rIPC stimulus. However, only IPC increased the percentage of cells able to maintain a low diastolic $[Ca^{2+}]_i$ (fura-2 ratio<1) following re-energization, from 19.4±3.2% of control myocytes (n=30, 14) to 48.3±3.8% of IPC myocytes (n=24, 7; $P<0.001$) and 24.9±4.5% of rIPC myocytes (n=23, 9; ns).

Our data show that the protection against necrotic cell death of rIPC involves NOS-signalling, whereas, the improved recovery of contractile function was NOS-independent. Further, rIPC was not associated with maintained Ca^{2+}-regulation, as seen in true IPC.