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THE ROLE OF CA2+/CALMODULIN-DEPENDENT PROTEIN KINASE II IN NECROPTOTIC AND APOPTOTIC CELL DEATH IN MYOCARDIUM SUBJECTED TO ISCHEMIA-REPERFUSION

A Szobi,[1] T Rajtik,[1] S Carnicka,[2] T Ravingerova,[2] A Adameova[1] [1]*Faculty of Pharmacy, UK;* [2]*Institute for Heart Research, SAS*

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Background Necroptosis, alternatively termed programmed necrosis, is a relatively recently discovered form of cell death. Cellular pathways responsible for necroptosis execution are currently being slowly elucidated and based on current findings it seems that necroptosis should be an important determinant of the extent of cell death due to ischemia (I) and reperfusion (R). While it is established that calcium overload during myocardial ischemia-reperfusion injury is important in triggering cardiomyocyte death, it is not known whether Ca^{2+}-dependent signaling can modulate necroptosis. Therefore, our aim was to determine if

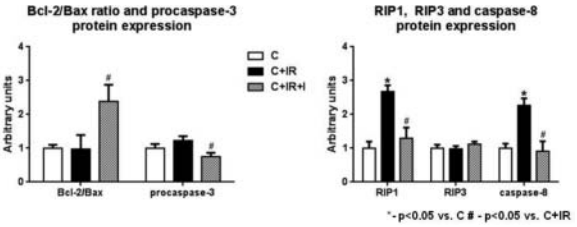

Figure 1

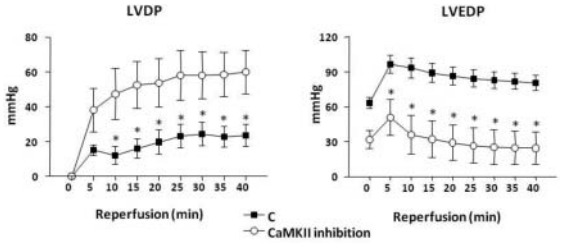

Figure 2

inhibition of Ca^{2+}/calmodulin dependent protein kinase II, a critical calcium homeostasis regulator in the heart whose increased activity is associated with deleterious effects on heart function, could affect necroptotic processes. In this study, we investigated the role of CaMKII in this process and detected changes in the content of necroptotic proteins RIP1, RIP3 and caspase-8 and apoptosis markers such as Bax, Bcl-2 and procaspase-3 in hearts injured by ischemia-reperfusion.

Methods Isolated Langendorff-perfused rat hearts were subjected to 30 min global ischemia followed by 40 min reperfusion. CaMKII inhibitor (KN-93, 0.5 μmol/l) was present in the perfusion solution during I and the first 10 minutes of R. During the whole IR protocol, hemodynamic parameters including left ventricular developed pressure (LVDP) and left ventricular end diastolic pressure (LVEDP) were being continuously monitored. Protein expression in left ventricles was determined by using Western blot analysis.

Results In the hearts subjected to I and R, characterized by worsened contractile function, the expression of necroptotic proteins RIP1 and caspase-8 ($p>0.05$) was significantly increased, while there were no changes in protein content of RIP3 nor of apoptotic Bcl-2, Bax and procaspase-3 ($p>0.05$). On the other hand, CaMKII inhibition normalized the expression of RIP1 and caspase-8 ($p>0.05$) and significantly reduced the expression of procaspase-3 and Bax ($p>0.05$). These antinecroptotic and antiapoptotic effects of CaMKII inhibition were accompanied by improvement of postischemic recovery of LVDP and abolishment of myocardial stunning.

CONCLUSION The results of this study imply that CaMKII activity may modulate both necroptotic and apoptotic pathways activated during myocardial I and R. Furthermore, CaMKII inhibition exerts cardioprotective effects which are likely to be, at least partially, due to necroptosis modulation.