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**AT1 RECEPTORS AND CA<sup>2+</sup>/CALMODULIN-DEPENDENT PROTEIN KINASE II INHIBITION UNLIKE THEIR COMBINATION DURING ISCHEMIA-REPERFUSION IS ASSOCIATED WITH CHANGES IN CONTENT OF OXIDATIVE STRESS AND APOPTOTIC MARKERS AND IMPROVED CONTRACTILE FUNCTION IN HEART**

T Rajtik,<sup>1</sup> A Szobi,<sup>1</sup> S Carnicka,<sup>2</sup> K Siskova,<sup>1</sup> I Paulikova,<sup>1</sup> P Svec,<sup>1</sup> T Ravingerova,<sup>2</sup> A Adameova<sup>1</sup> <sup>1</sup>Faculty of Pharmacy, UK; <sup>2</sup>Institute for Heart Research, SAV  
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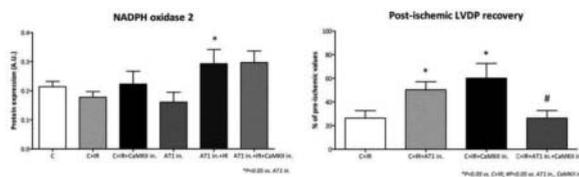


Figure 1

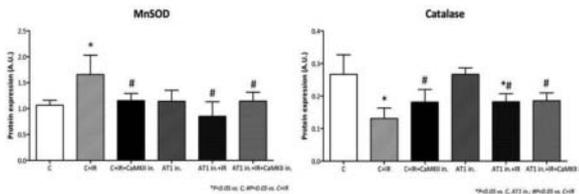


Figure 2

**Introduction**  $Ca^{2+}$ /calmodulin-dependent protein kinase II (CaMKII) is a crucial regulator of  $Ca^{2+}$ -handling and excitation-contraction coupling in cardiomyocytes. Lately, an oxidative activation of CaMKII has been described. Oxidative stress is an integral component of ischemia/reperfusion (IR) injury which results in a complex of subcellular and molecular changes leading to impaired cardiac function and cell death. As AT1 receptor activation promotes reactive oxygen species production through NADPH oxidase system it may be hypothesized that this signaling cascade might play an important role in oxidative activation of CaMKII under IR conditions.

**Methods** To test this, the isolated Langendorff-perfused hearts of Wistar rats treated with losartan (20 mg/kg, p.o., 14 days) as well as control ones were subjected to ischemia (30 min) followed by reperfusion (40 min). A role of CaMKII was studied by using a specific CaMKII inhibitor (KN-93, 0,5  $\mu$ mol/dm<sup>3</sup>) which was present in the perfusion solution 10 min before onset of ischemia, during ischemia and first 10 min of reperfusion. During the whole IR protocol, myocardial parameters were being continuously recorded. Protein content of total, oxidized CaMKII, oxidative stress markers, such as pro-oxidant NADPH oxidase (NOX2) and antioxidant superoxide dismutase (MnSOD) and catalase (CAT) as well as apoptotic markers (Bax, Bcl-2, cyt c) was evaluated in left ventricles by Western blotting.

**Results** In the IR hearts, inhibition of AT1 receptors or CaMKII alone normalized the protein content of antioxidant proteins MnSOD and CAT to the values of the non-ischemic hearts. Likewise, the protein content of oxidized CaMKII was modified. Interestingly, the protein content of NOX2 was significantly increased in IR-subjected group treated with losartan, suggesting a negative feedback due to a permanent AT1 inhibition. Moreover, the protein content of anti-apoptotic Bcl-2 was not changed upon losartan treatment nor CaMKII inhibition in the ischemic hearts. On the other hand, pro-apoptotic Bax and cytochrome C were upregulated in the non-ischemic losartan group. Simultaneous inhibition of AT1 receptors and CaMKII had no additive effect on content of any protein studied. On the other hand, although AT1 receptor inhibition or CaMKII inhibition improved postischemic recovery of left-ventricular developed pressure (LVDP), the simultaneous inhibition of AT1 receptors and CaMKII abolished this beneficial effect.

**Conclusion** In conclusion, it is likely that AT1 receptor signaling cascade plays a role in oxidative activation of CaMKII documented by normalized anti-oxidant protein content and by improved contractile

function of heart after AT1 inhibition. However, a simultaneous inhibition of CaMKII and AT1 receptors resulted in worsened LVDP recovery suggesting that excessive inhibition of CaMKII activation and therefore diminished activation of CaMKII target proteins of  $Ca^{2+}$ -handling may be deleterious.