ROLE OF ADENOSINE A_{2A} RECEPTOR IN THE REGULATION OF ANGIOTENSIN II INDUCED ENDOTHELIAL CELL OXIDATIVE STRESS AND DYSFUNCTION

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Adenosine, a potent vasodilator, is produced abundantly during cellular metabolism and acts via its 4 G protein-coupled receptors, A_1, A_{2A}, A_{2B}, and A_3 expressed in the cardiovascular system (Varani *et al.,* 2003). Among those adenosine receptors, the adenosine A_{2A} receptor ($A_{2A}R$) has been found to be largely expressed in vascular endothelial cells and involved in the regulation of endothelial function. Endothelial cells express constitutively an NADPH oxidase, which generates reactive oxygen species (ROS) and participates in cellular redox-signalling. The activity of endothelial NADPH oxidase can be activated by angiotensin II (Ang II), a potent vasoconstrictor, resulting in endothelial oxidative stress and dysfunction (Li *et al*., 2004). Endothelial dysfunction contributes to the pathogenesis of many cardiovascular diseases such as hypertension and atherosclerosis. Although pharmacologically adenosine has been found to play an important role in the cardiovascular system, little is known about the function of the $A_{2A}R$ in the regulation of endothelial ROS production. In this study, we investigated the role and the mechanisms of $A_{2A}R$ in the regulation of angiotensin II-induced endothelial ROS production by NADPH oxidase, using a mouse microvascular endothelial cell line (SVEC 4-10) (*in vitro)* and aortic sections from mice (*ex vivo)* (n=3 for all experiments).

Firstly we examined the $A_{2A}R$ mRNA expression by quantitative real-time PCR in SVEC 4-10 cells treated for 30 min with or without Ang II (100 nM). We found that $A_{2A}R$ mRNA was detected in control endothelial cells and the expression level was significantly ($p<0.05$) increased 2 fold after Ang II treatment. Next we examined the NADPH-dependent ROS production in SVEC 4-10 cells treated for 30 min with or without a specific $A_{2A}R$ antagonist (SCH 58261, 100 nM). We found that the levels of ROS production found in endothelial cells treated with SCH 58261, as detected by lucigenin (5 μM)-chemiluminescence, was significantly ($p<0.01$) less (26±5%) then that in control. Treatment of endothelial cells with Ang II (100 nM, 30 min.) significantly increased (119±5%) NADPH-dependent ROS production ($p\leq0.001$), which could be significantly reduced to the basal level in the presence of SCH 58261 (100 nM) ($p\leq0.01$). Angiotensin II-induced ROS production could be inhibited by tiron (a specific O_2^- scavenger) and apocynin (an NADPH oxidase inhibitor) suggesting that the enzymatic source of ROS generation was NADPH-oxidase. Finally we examined ERK1/2 activation in aortic vessels obtained from mice (CD1, male, 10 weeks old) killed by schedule 1 procedure 90 min after intraperitoneal injection of SCH 58261 (10 mg/kg body weight) or vehicle (10% DMSO in PBS) as control. Aortic vessels were then treated for 30 min with or without Ang II (100 nM) and examined for the ERK1/2 phosphorylation by confocal microscopy. High ERK1/2 phosphorylation was found in aortic sections treated with Ang II. Compared with the sections treated with PBS only there was reduced ERK1/2 phosphorylation in sections from SCH 58261 treated mice.

These results indicate that the $A_{2A}R$ plays a role in promoting endothelium NADPH oxidase activation, as an antagonist of $A_{2A}R$ (SCH 58261) inhibits endothelial ROS production. Understanding the underlying mechanism of $A_{2A}R$ and Ang II interactions will further broaden our knowledge of their involvement in the production of ROS that results in oxidative stress contributing to vascular damage in cardiovascular disease.

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Varani K *et al.,* (2003) The FASEB Journal, 17:280-282

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