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Title of Thesis : Adenosine A_3 Receptor Mediated Vasoconstriction In Diabetic Mouse

Abstract

Vascular dysfunction contributes significantly to mortality and morbidity in various cardiac and metabolic diseases. Among endogenous molecules regulating vascular tone is adenosine, with the adenosine A_3 receptor (A_3AR) exerting cardioprotective properties in ischemia and reperfusion. However, overexpression of A_3AR is suggested to result in vascular dysfunction and inflammation. The present study investigates the role of A_3AR in mediating vasoconstriction response in diabetic mice. The leukocyte enzyme Myeloperoxidase (MPO) is an important modulator of vascular function with nitric oxide-consuming and pro-inflammatory properties. Increased MPO plasma levels are observed in patients with cardiovascular disorders like heart failure, acute coronary syndromes and arrhythmias. Given that vascular dysfunction and inflammation are also hallmarks of diabetes, the role of MPO in adenosine-dependent vasomotor function was also investigated in a murine model of diabetes mellitus.

Streptozotocin treated mice aorta were exposed to A_3AR agonist 2-Chloro-N6-(3-iodobenzyl)-N-methyl-5-carbamoyladenosine (Cl-IBMECA) and antagonist 2,3-diethyl-4,5-dipropyl-6-phenylpyridine-3-thiocarboxylate-5-carboxylate (MRS1523) to measure the dose-response relationship.

Wild type (WT) and MPO-deficient ($Mpo^{-/-}$) mice were treated with Streptozotocin (STZ), which led to an increase in MPO plasma levels in WT mice and leading to enhanced aortic

superoxide generation, a marker for the presence of ROS, which was assessed by dihydroethidium staining in STZ-treated WT mice as compared to controls. Lipid peroxidation was also measured by colorimetry in control and diabetic groups.

The vasoconstriction of aortic segments in response to the A_3AR agonist Cl-IBMECA (2-Chloro-*N*6-(3-iodobenzyl)-*N*-methyl-5-carbamoyladenosine) as determined by isometric force measurements was augmented in diabetic WT as compared to $Mpo^{-/-}$ mice. In presence of antagonist it was observed that Cl-IBMECA mediated A_3AR response was almost completely inhibited in control, while diabetic showed response at low doses of Cl-IBMECA. This implies that MRS1523 is a specific antagonist against Cl-IBMECA. Moreover at higher concentrations of Cl-IBMECA, there a complete abolition of agonist mediated vasoconstriction response was observed this might be due to the receptor saturation or inhibition.Moreover, A_3AR protein expression demonstrates that A_3AR protein expression was enhanced in STZ treated mice but was attenuated by MPO-deficiency.

The data generated in this study demonstrates that ROS, MPO plasma levels and LPO are increased upon STZ-mediated induction of diabetes in mice this increase is associated with augmented endothelial expression of A_3AR and enhanced constriction of explanted aortic sections in response to A_3AR agonist Cl-IBMECA.

Finally, our studies also suggest that the A_3AR-mediated contraction through endothelium may play a role in cardiovascular inflammation, including hypertension and atherosclerosis, by affecting ROS signaling pathways. MPO is responsible for increase in vascular A_3AR expression under diabetic conditions which leads to enhanced vasoconstriction response to A_3AR agonists Cl-IBMECA. This increase in vascular response may be responsible for vascular dysfunction in diabetes. It also discloses an additional mechanism of MPO-mediated vascular dysfunction. This could play an important role in the pathophysiology of vascular diseases and could provide newer therapeutic approaches.