

ABSTRACT

Adenosine plays a critical role in the heart signalling while affecting heart rate, contractility or coronary flow. Nowadays, four adenosine receptor subtypes are distinguished which are present in most of tissues and cells: A₁, A_{2A}, A_{2B} and A₃. All these receptors belong to the family of G protein-coupled receptors. Upon activation, their main target is an enzyme adenylyl cyclase which produces an important second messenger cAMP. The main goal of this thesis was characterization of adenosine receptors in the rat myocardium, assessment of their distribution, binding properties and signalling. We examined a possible disparity in receptors distribution between the left and right ventricles using SDS-PAGE electrophoresis and Western blotting. The same methods have been used in studies of adenosine receptor distribution in lipid rafts. Samples of lipid rafts and soluble fraction were prepared using a nonionic detergent Triton X-100. We did not find any evidence of different distribution between the left and right ventricles and our results did not confirm compartmentation of the receptors either. For determination of binding properties of the receptors we used radioligand binding assays with the A₁ selective radioligand [³H]DPCPX. We did not observe any significant difference between the receptor number in the ventricles. We observed that the amount of adenosine receptors in the heart is very low, which was a consistent result in all of our experiments. Our last experiment aimed to determine the activity of adenylyl cyclase by using radioactively labeled [α -³²P]. Adenylyl cyclase was stimulated by forskolin, isoprenaline and CGS-21680 (A_{2A} receptor agonist) and inhibited by CPA (A₁ receptor agonist). A significant stimulation was observed in forskolin stimulated enzyme, but there was only negligible stimulation induced by isoprenalin and CGS. The activity of adenylyl cyclase was not decreased by CPA as well. Because of the low number of adenosine receptors in the heart, these studies are rather challenging. However, due to widespread distribution and function of adenosine in physiological as well as pathological states of the cells, the understanding of expression of adenosine receptors and their signalling is important for the development of new drugs.