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The Role of Adenylate Cyclase in Ischemic Preconditioning in the Rat Heart: A Cytochemical Study

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Abstract. Using catalytic cytochemistry the AC activity was studied during ischemic preconditioning (IP) (5 min occlusion of LAD and 10 min reperfusion) followed by 30 min regional ischemia in isolated Langendorff-perfused rat heart. In controls the specific precipitate of AC reaction was found on the sarcolemma (SL) and the junctional sarcoplasmic reticulum (JSR) of cardiomyocytes. After prolonged ischemia the reaction product was absent, whereas IP followed by prolonged ischemia protected the AC activity on SL and JSR. IP-induced enhancement of AC activity in this model was accompanied by significant reduction of ischemia/reperfusion fibrillation. The results suggest involvement of AC system in mechanisms of IP.

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It is known that the exposure of the heart to one or few episodes of transient myocardial ischemia reduces the extent of the myocardial injury in a subsequent prolonged ischemia This phenomenon is known as ischemic preconditioning (IP) (Murry *et al* 1986, Liu and Downey 1992) and is associated with a decrease in cellular damage as well as in life-threatening arrhythmias Several studies suggest a role of catecholamines and adrenergic activation of α 1-adrenergic receptors in the cardioprotective effect of IP (Banerjee *et al* 1993, Ravingerová *et al* 1997) Whether β -adrenoceptor/adenylate cyclase system (AC) (E C 4 6 1 1) stimulated by catecholamines is also implicated in the mechanism of the protection induced by IP has not been fully elucidated Therefore, the aim of the present study was to examine the effect of IP on AC activity and its distribution in rat myocardium using cytochemical approach. The latter enables to detect the enzyme activity *in situ* in the myocardial tissue

For this purpose, the rat hearts were perfused (Langendorff preparation) at a constant flow of 10 ml/min with Krebs-Henseleit solution and subjected to 1) IP (a single 5 min occlusion of LAD and 10 min reperfusion), 2) regional ischemia (30 min occlusion of LAD) and 3) IP followed by 30 min ischemia During experiment the heart rate was continuously monitored and incidence of arrhythmias was classified according Lambeth Conventions (Walker *et al* 1988)

At the end of experiment the hearts were perfusion fixed with 1% glutaraldehyde and the tissue pieces from the ischemic left ventricle were processed for the cytochemistry of AC (Schulze *et al* 1978) Ultrathin unstained sections were analyzed in EM Tesla 500 *Cytochemistry of AC* In control hearts the reaction product of AC activity was found on the sarcolemma and on the junctional sarcoplasmic reticulum. The same localization of the specific precipitate was observed after IP itself and IP followed by prolonged ischemia However, the variability in the intensity of the electrondense precipitate was observed in each experimental group (Table 1). The strongest precipitate was absent, whereas in preconditioned heart followed by prolonged ischemia the precipitate was found on the sarcolemma and even more on the junctional sarcoplasmic reticulum.

	AC activity				
	control	30' Isch	IP	IP + 30' Isch	
Sarcolemma JSR	++++		++ ++	+ ++	

 Table 1. Localization and intensity of the specific product of the AC reaction in rat cardiomyocytes

Incidence of arrhythmias It has been demonstrated that both, incidence and duration of sustained ischemia-induced ventricular tachycardia (VT) and ventricular fibrillation (VF) were significantly attenuated after IP at the rat heart In the ischemic group, 30 min occlusion of LAD induced 100% and 67% incidence of VT and VF, respectively In the IP group, incidence of VT and VF were significantly reduced to 17% and 0%, respectively (p < 0.01)

In the present study we demonstrated the protective effect of IP on AC applying *in situ* method The enzyme activity was demonstrated on the subcellular membrane

structures directly involved in the regulation of intracellular Ca homeostasis and thus the contraction-relaxation processes and the intercellular communication, too (de Mello 1988) It is well-known that mentioned processes are modulated by cAMP. It is also known that the AC activity and cAMP levels differ in normal and ischemic myocardium (Krause et al 1978, Okruhlicova et al 1988, Tribulova et al 1998) and reflect the increased local catecholamine release (Schomig 1984) Despite of the fact that increased sympathetic activity and catecholamine release might be arrhythmogenic in ischemia and reperfusion, it has been suggested that short lasting effect of catecholamines can be an tiarrhythmic (Ravingerova et al 1997) A few studies refer to the contribution of the β -adrenoceptor/AC system in the cardioprotection of IP against postischemic myocardial dysfunction (Nasa et al 1997) The protection of cAMP levels during IP may be due to several factors, for example ischemia-induced dual sensitization of the β -adrenergic system (Strasser et al 1989), inhibition of phosphodiesterases (Lochner et al 1997) or IP-induced reduction of norepinephrine release in the heart. The results of the present study indicate that AC can be involved in the cardioprotective effect of IP in the rat heart

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Detection of Cytoskeletal Proteins in Small Cell Lung Carcinoma

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Abstract. Small cell lung carcinoma (SCLC) is the most aggressive of lung tumors, metastasize widely and are virtually incurable by surgical means. Therefore, the classification of lung cancer into SCLC and non-small cell lung carcinoma is essential for disease prognosis and treatment.

For this purpose we have compared the immunohistochemical distribution of different cytoskeletal proteins as tumor markers. Analysis was performed by using of monoclonal antibodies directed against cytokeratins, neurofilaments, β III-tubulin, epithelial membrane antigen and neuron-specific enolase. Our results indicate that keratin and epithelial membrane antigen are reliable epithelial markers for SCLC. In addition, the positive staining with monoclonal antibodies TU-20 against β III-tubulin and neuron-specific enolase was found in some cases of SCLC. We suggest, that these antibodies could be a useful tool for complex immunohistochemical diagnosis of SCLC.

Introduction

Small cell lung carcinoma (SCLC) is a clinicopathologically distinct form of pulmonary carcinoma characterized by specific morphological, ultrastructural, biochemical and cytogenic markers Ultrastructurally, a few dense-core neurosecretory-type granules were found in about 80% of the SCLC cells The granules are similar to those found in the APUD (Kultchitsky's) cells of the lung, which are originally supposed to be of ectodermal neural crest origin (de Leij *et al* 1985) According to the other hypothesis, SCLC originates in primitive cells of the basal bronchial epithelium, which in the process of neoplastic change undergoes partial differentiation towards neuroendocrine cells Immunohistochemically, the positivity of SCLC for keratin was described, often simultaneously with the neural markers such as neurofilaments, Leu-7, chromogranin, synaptophysin and neuron-specific enolase (Guinee *et al* 1994) In this study, we have used a monoclonal

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