Physiol. Res. 49: 251-259, 2000

Activation of Adenylate Cyclase System in the Preconditioned Rat Heart

Ľ. OKRUHLICOVÁ, T. RAVINGEROVÁ, D. PANCZA, N. TRIBULOVÁ, J. STYK, R. ŠTETKA

Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic

Received April 16. 1999
Accepted September 24, 1999

Summary

Ischemic preconditioning (IP) protects the heart against subsequent prolonged ischemia. Whether the β-adrenoceptor/adenylate cyclase pathway contributes to this cardioprotection is not yet fully known. Using enzyme catalytic cytochemistry we studied the adenylate cyclase activity and its distribution in the preconditioned rat heart. Adenylate cyclase activity was examined in Langendorff-perfused rat hearts subjected to the following conditions: control perfusion; 30 min regional ischemia; 5 min occlusion and 10 min reperfusion (IP); IP followed by ischemia. Ischemia-induced arrhythmias and the effect of ischemic preconditioning on the incidence of arrhythmias were analyzed. At the end of experiment the heart was shortly prefixed with glutaraldehyde. Tissue samples from the left ventricle were incubated in a medium containing the specific substrate AMP-PNP for adenylate cyclase and then routinely processed for electron microscopy. Adenylate cyclase activity was cytochemically demonstrated in the sarcolemma and the junctional sarcoplasmic reticulum (JSR) in control hearts, while it was absent after test ischemia. The highest activity of the precipitate was observed after ischemic preconditioning. In the preconditioned hearts followed by test ischemia, adenylate cyclase activity in the precipitate was preserved in sarcolemma and even more in JSR. Protective effect of ischemic preconditioning was manifested by the suppression of severe arrhythmias. These results indicate the involvement of the adenylate cyclase system in mechanisms underlying ischemic preconditioning.

Key words

Ischemic preconditioning • Adenylate cyclase • Cytochemistry • Rat • Heart

Introduction

It is well known that exposure of the heart to one or several episodes of transient myocardial ischemia reduces the extent of myocardial injury during subsequent prolonged ischemia. This phenomenon is denoted as ischemic preconditioning (IP) (Murry *et al.* 1986, Downey 1992) and is associated with a decrease of cellular damage as well as with suppression of life-threatening arrhythmias (Liu *et al.* 1991, Vegh *et al.* 1992, Štetka *et al.* 1999). Although many different endogenous substances have been proposed as mediators of the protective effects induced by ischemic preconditioning (Parratt 1993), the exact mechanisms underlying this phenomenon remain uncertain. It has been suggested that the protective effect of ischemic preconditioning can be mediated by endogenously released catecholamines *via* stimulation of α_1-

Fax +420 2 249 20 590
E-mail: physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

adrenoceptor/protein kinase C (Banerjee *et al.* 1993, Bankwala *et al.* 1994). On the other hand, catecholamines also affect the β-adrenoceptor/ adenylate cyclase system responsible for cyclic AMP (cAMP) synthesis. The role of cAMP in the modulation of intracellular pathways linked to Ca^{2+} homeostasis and the contraction-relaxation processes as well as to intercellular communication (De Mello 1988, 1992) is well established.

Whether the β-adrenoceptor/adenylate cyclase system is also implicated in mechanisms of the protection induced by ischemic preconditioning has not been fully elucidated. Some studies indicate the role of adenylate cyclase system in the cardioprotection induced by ischemic preconditioning against postischemic myocardial dysfunction (Iwase *et al.* 1993, Morita *et al.* 1997). The aim of the present study was, therefore, to examine the effect of ischemic preconditioning on the adenylate cyclase activity and its distribution in the rat myocardium. We used the method of enzyme catalytic cytochemistry which enables to detect activity at the cellular level *in situ* at the site of enzyme localization. Accordingly, it allows to reveal the topography of enzyme activity or subcellular distribution. We used a model of regional ischemia and one episode of ischemic preconditioning with respect to the effect on arrhythmias as the main end-point of protection.

Method

All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No 85-23, revised 1985).

Isolated heart preparation - Langendorff model

The hearts were excised from heparinized (500 IU i.p.) and anesthetized (sodium pentobarbitone 40 mg/kg i.p.) male Wistar rats (250 to 300 g) and perfused at a constant flow rate of 10 ml/min with Krebs-Henseleit solution containing (in mM): NaCl 118.0, KCl 3.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, NaH_2PO_4 1.18, $CaCl_2$ 2.5, glucose 11.1. The solution was saturated with a mixture of 95 % O_2 and 5 % CO_2, and maintained at 37 °C at pH 7.4.

Experimental protocol

All hearts were allowed to stabilize for 20 min. Control hearts were perfused for 75 min (n=20). The ischemic challenge was induced by occlusion of the left descendent coronary artery (LAD) lasting 30 min (test ischemia) (n=20). Ischemic preconditioning was induced by 5 min occlusion of the left descendent artery and 10 min reperfusion prior to the test ischemia (n=18). Arrhythmias were analyzed by determining the incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF), as well as by calculating the total number of ventricular premature beats (VPBs) in accordance with the Lambeth Convention (Walker *et al.* 1988). At the end of the experiments, the samples for cytochemistry were taken from the ischemic area of left ventricles as well as from the control hearts and processed for the cytochemistry of adenylate cyclase.

Cytochemistry of adenylate cyclase (E.C.4.6.1.1.)

Adenylate cyclase localization and activity were evaluated in the ischemic tissue of the left ventricle from four experimental groups (n=4 per each group): 1) control hearts; 2) hearts subjected to test ischemia; 3) IP alone; 4) IP followed by test ischemia. At the end of the experiment, the hearts were prefixed by perfusion with 2 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, containing dimethylsulfoxide (Slezák and Geller 1979). Perfusion lasted for about 1-2 min. After that, tissue samples (about 1 mm^3) were incubated for 30 min at 37 °C in an incubation medium (Schulze *et al.* 1972) containing (in mM): Tris-maleate buffer 80.0, AMP-PNP 0.5, $Pb(NO_3)_2$ 2.0, $MgSO_4$ 10.0, tetramisole 4.0, sucrose 0.25. The tissue was then postfixed in 40.0 mM OsO_4 in 0.1 M cacodylate buffer, pH 7.4 for 1 h at 4 °C, dehydrated with graded alcohol and propylene oxide and embedded in Epon 812. Ultrathin unstained sections were analyzed in EM Tesla 500. Standard processing and the incubation of samples made it possible to evaluate semiquantitatively the changes in the intensity of the precipitate reaction under different pathophysiological conditions. The results were graded as follows: ± absence or scarce precipitate; + weak, finely granular precipitate; ++ coarse, finely granular homogeneous precipitate.

Results

Cytochemistry of adenylate cyclase

In control hearts, the reaction product of adenylate cyclase activity was found on the sarcolemma including T-tubules and intercalated discs, as well as on the junctional sarcoplasmic reticulum (Fig. 1). The same localization of the adenylate cyclase activity was observed after IP itself and after IP followed by

prolonged ischemia (Fig. 2). After 30 min ischemia, the precipitate was absent or scarce (Fig. 3) and the cardiomyocytes were edematous with swollen mitochondria and broken cristae.

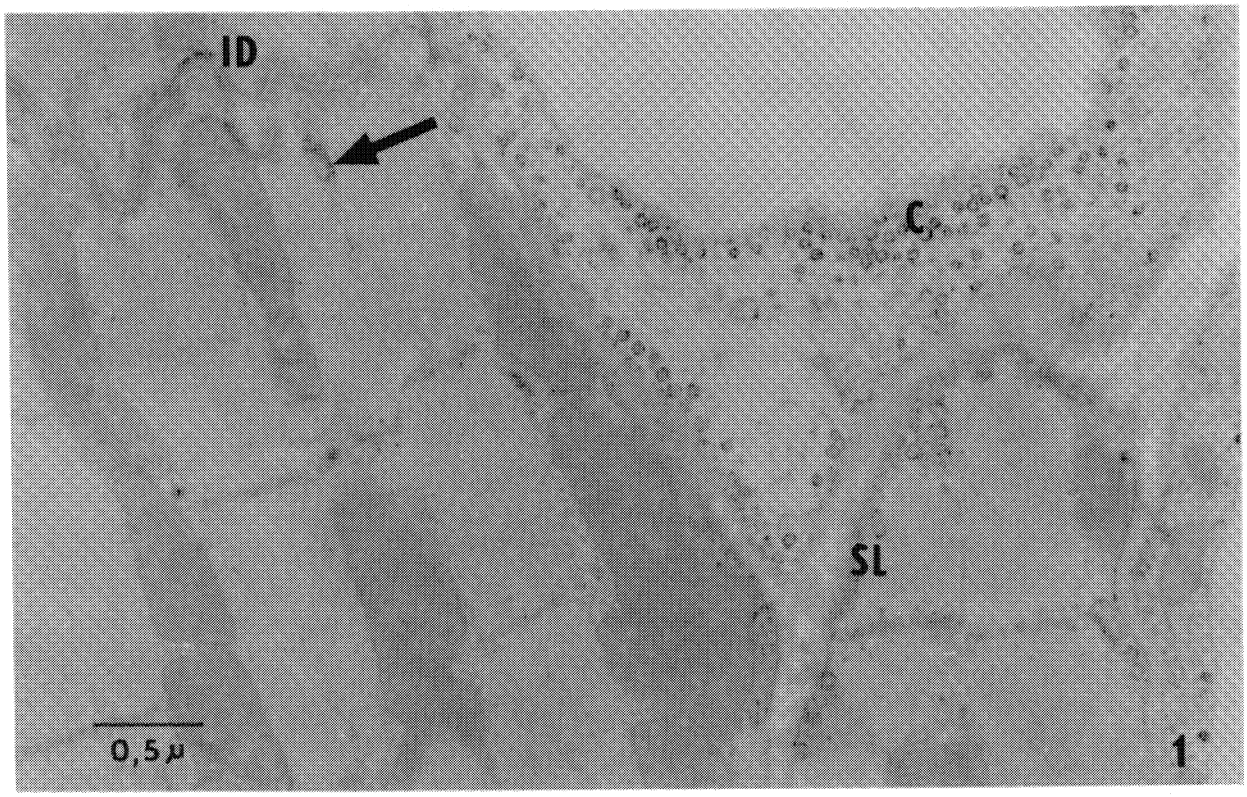

Fig. 1. *Ultrastructural localization of adenylate cyclase activity in the sarcolemma (SL) and the junctional sarcoplasmic reticulum (arrow) in control rat heart. c - capillary, ID- intercalated disc.*

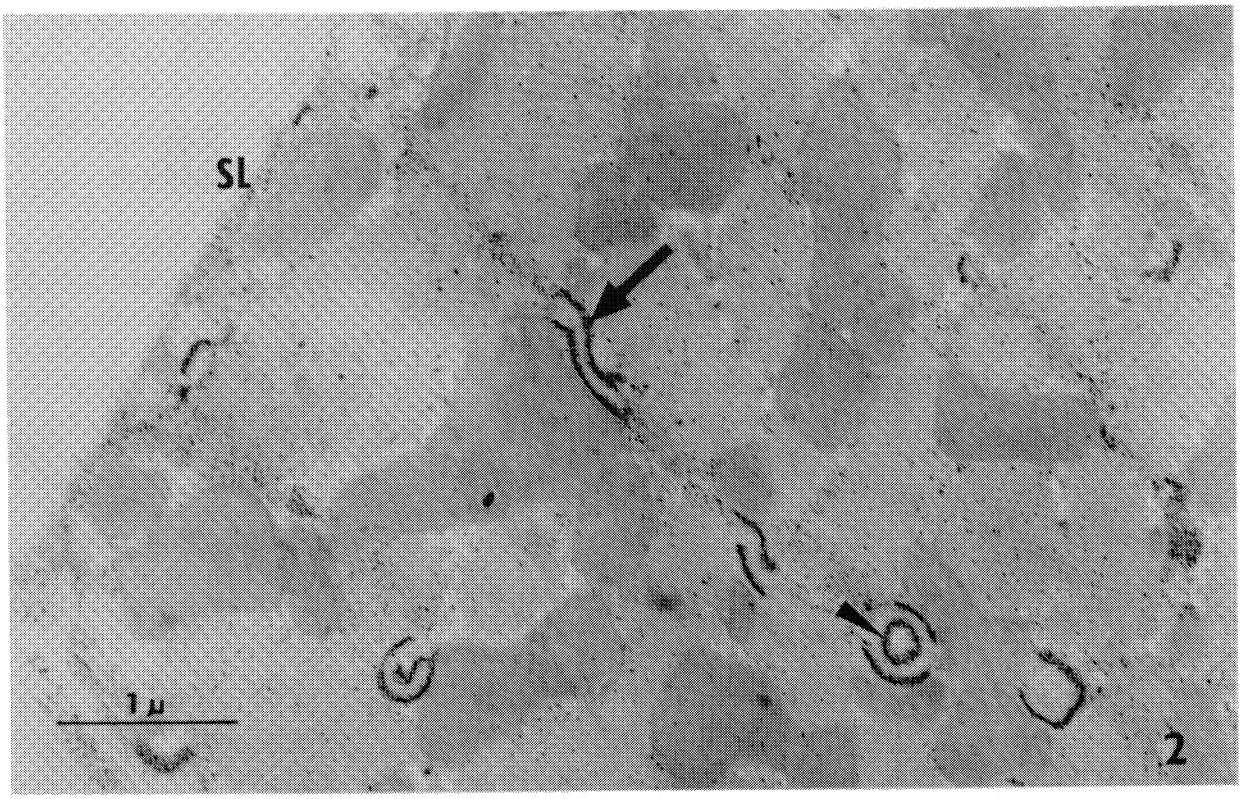

Fig. 2. *Maintenance of adenylate cyclase activity was found in the sarcolemma (SL) including T-tubules (arrow head) and the junctional sarcoplasmic reticulum (arrow) in the preconditioned rat heart subjected to test ischemia.*

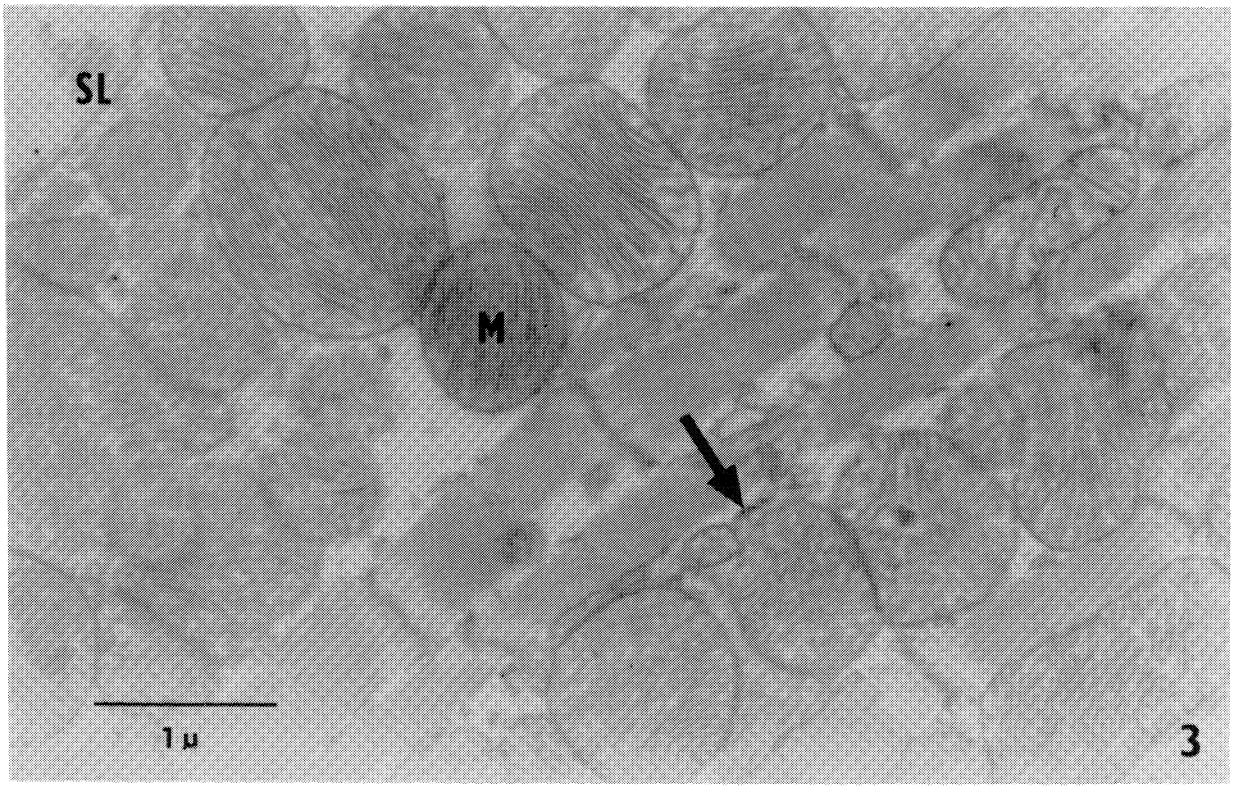

Fig. 3. *After 30 min regional ischemia, scarce formation of the specific precipitate was observed in the junctional sarcoplasmic reticulum (arrow) and/or the sarcolemma (SL), respectively. Cardiomyocytes are edematous, mitochondria (M) are swollen with broken cristae.*

Table 1. The intensity of adenylate cyclase reaction product in rat hearts under various experimental conditions

	Intensity of adenylate cyclase reaction product			
Controls		**30 min ischemia**	**IP**	**IP followed by ischemia**
SL	+	±	++	+
JSR	+	±	++	++

Classification: + normal reaction, ± scarce, ++ strong. IP - ischemic preconditioning, SL - sarcolemma, JSR - junctional sarcoplasmic reticulum.

The variability in the intensity of the electrondense precipitate was observed in each experimental group and the density is shown in Table 1. The strongest intensity of the reaction product was observed immediately after ischemic preconditioning. In the preconditioned hearts followed by test ischemia, the precipitate was preserved in the sarcolemma and was even more pronounced in the junctional sarcoplasmic reticulum.

Antiarrhythmic protection by ischemic preconditioning

Test ischemia induced high incidence of ventricular arrhythmias: 100 % ventricular tachycardia and 70 % ventricular fibrillation. The protective effect of ischemic preconditioning were manifested by a reduced incidence and severity of arrhythmias. Only 30 % of the hearts exhibited ventricular tachycardia, whereas ventricular fibrillation was completely abolished. The total number of VPBs was decreased from 800±150 in the controls to 30±10 ($p<0.05$) (Fig. 4).

Discussion

In the present study, we demonstrated the protective effect of ischemic preconditioning on adenylate cyclase activity and its localization in rat cardiomyocytes using the method of enzyme catalytic cytochemistry *in situ*.

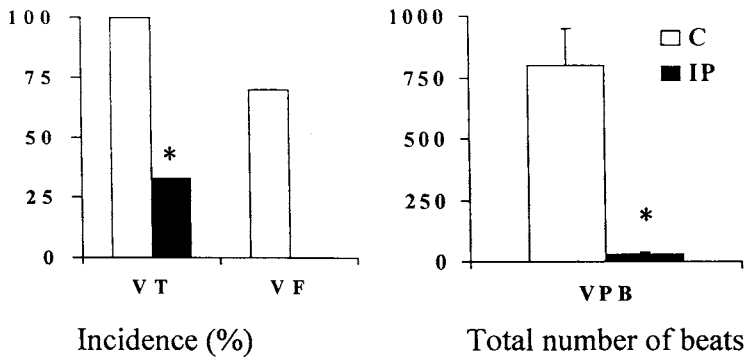

Fig. 4. *Effect of ischemic preconditioning on the incidence of ischemia-induced ventricular tachycardia (VT), ventricular fibrillation (VF) (left) and the total number of ventricular premature beats (VPB) (right). C – control ischemia (n=20), IP – ischemic preconditioning (n=18). Data are means ± S.E.M. * $p<0.05$*

The cytochemical localization of adenylate cyclase activity in the sarcolemma and the junctional sarcoplasmic reticulum has already been shown in previous studies (Schulze *et al.* 1972, Katz *et al.* 1974, Slezák and Geller 1979, Okruhlicová *et al.* 1988). The principle of the enzyme catalytic cytochemical procedure is the visualization of the primary product of enzyme reaction which forms an electrondense precipitate with heavy metal Pb^{2+} ions at the site of enzyme localization. This method includes prefixation which preserves the structure and integrity of cellular membranes and allows the precise localization of adenylate cyclase. Because ATP can be hydrolyzed by endogenous ATPases, we used the specific substrate AMP-PNP (Yount 1974). Despite the high specificity of AMP-PNP, it is not possible to eliminate the presence of endogenous ATPases. However, ions such as Ca^{2+}, Na^{+} or K^{+} necessary for the activation of main ATPases in the cardiac sarcolemma are absent in the medium. Thus, ATPases cannot interfere appreciably in the formation of the specific precipitate. To eliminate AMP-PNP hydrolysis by membrane alkaline phosphatase (Johnsson and Welden 1977), tetramisole was added into the incubation medium. AMP-PNP as a nonhydrolyzable ATP analogue has also been applied in some biochemical studies to demonstrate cAMP-modulated processes (Aiello *et al.* 1995). The appropriate incubation medium for the adenylate cyclase reaction and the standard processing of samples make it possible to compare changes in the activity and/or localization of the enzyme under various pathophysiological conditions.

In our present experiments, the adenylate cyclase activity was localized in the sarcolemma, including transverse T-tubules and intercalated discs as well as in the junctional sarcoplasmic reticulum adjacent to the T-tubules. Adenylate cyclase *via* its intracellular second messenger cAMP modulates multiple processes including intercellular communication (De Mello 1992, Kwak and Jongsma 1996) and Ca^{2+}-transport regulating processes on the sarcolemmal L-type channel (Sperelakis 1994) and the sarcoplasmic reticulum (Vittone *et al.* 1990, Sasaki *et al.* 1992). The adenylate cyclase reaction was also detected along the intercalated discs, which are responsible for the intercellular electromechanical coupling. Gap junctions as a special part of the intercalated discs mediate the propagation of the action potentials and intercellular signal transduction (such as second messengers, cAMP and cGMP) between adjacent cells *via* gap junctional channels (Imanaga 1989, De Mello 1996). This allows cardiomyocytes to synchronize their electrical and contractile activities. Since the sarcolemma is known to control Ca^{2+} fluxes and the junctional sarcoplasmic reticulum plays a specific role in the electromechanical coupling, the cytochemical detection of adenylate cyclase activity on these membranes supports its role in the regulation of heart contractions.

It is known that adenylate cyclase activity and cAMP levels differ in the normal and ischemic myocardium (Krause *et al.* 1978, Tribulová *et al.* 1998). This can reflect increased local catecholamine release in the myocardium (Schömig *et al.* 1984). During early ischemia, higher catecholamine levels result in a rapid increase of cAMP. In confirmation of these data, we observed the enhanced adenylate cyclase activity resulting in the formation of more intensive reaction products in the hearts subjected to 5 min ischemia and also in the hearts subjected to 5 min ischemia followed by 10 min reperfusion as compared to the controls. These results correspond to our previous work (Okruhlicová *et al.* 1988) in which we already demonstrated the

stimulating effect of 5 min ischemia on the adenylate cyclase activity in the rat myocardium using both methods, enzyme catalytic cytochemistry and biochemistry. The predominance of sympathetic regulation in early ischemia can increase the gap junction conductance and intercellular communication as well as accelerate sarcoplasmic Ca^{2+} exchange and prevent the Ca^{2+} overload *via* cAMP-dependent protein phosphorylation.

In long-lasting ischemia, elevated cytoplasmic free Ca^{2+} persists and may result in subcellular damage (Okruhlicová *et al.* 1988), electrical uncoupling (Manoach *et al.* 1996), contractile dysfunction (Opie *et al.* 1980, Rona 1985) and, moreover, may inhibit adenylate cyclase activity (Iwami *et al.* 1995, Ebina *et al.* 1997). Furthermore, we observed scarce formation of the electrondense precipitate of the adenylate cyclase reaction, together with swollen mitochondria and broken cristae in edematous cardiomyocytes and with a high incidence of severe ventricular arrhythmias.

Despite the fact that increased sympathetic activity and cAMP might be arrhythmogenic in ischemia and reperfusion, it has been suggested that short-lasting ischemia and/or the effect of exogenous catecholamines can be antiarrhythmic (Vegh *et al.* 1992, Lukas and Botsford 1997, Ravingerová *et al.* 1997) through adrenergic activation of the α_1-adrenoceptor/protein-kinase C pathway. Several studies, however, have suggested that the adrenergic activation of the β-adrenoceptor/adenylate cyclase complex might also be involved in the cardioprotective mechanisms of ischemic preconditioning. Iwase *et al.* (1993) have observed that the preconditioned heart in rabbits exhibited a delayed ischemia-induced reduction in β-adrenergic signal transduction, G_s proteins and the activity of catalytic unit. Asimakis *et al.* (1994), Morita *et al.* (1997) and Nasa *et al.* (1997) have shown that a brief period of β–adrenoceptor stimulation exerts a preconditioning-mimetic protective effect against postischemic contractile dysfunction in perfused rat hearts and also results in the preservation of adenylate cyclase (Morita *et al.* 1997). In agreement with these data, we observed enhanced adenylate cyclase activity in the sarcolemma and the junctional sarcoplasmic reticulum in preconditioned rat hearts subjected to 30 min ischemia. The ischemic preconditioning-induced enhancement of adenylate cyclase activity in this model was accompanied by a significant reduction of ischemia-induced fibrillations and, furthermore, by the prevention of the changes in cardiomyocyte ultrastructure. The maintenance of adenylate cyclase function in the sarcolemma and the junctional sarcoplasmic reticulum in preconditioned cardiomyocytes might be due to the contribution of this enzyme system to the protective effect of ischemic preconditioning *via* cAMP-dependent Ca^{2+}-transport regulating systems as well as the cAMP-dependent upregulation of gap junction channels as has been demonstrated in cultured cardiac myocytes (Miyachi *et al.* 1995, Darrow *et al.* 1996).

In the mammalian heart, two major type V and type VI adenylate cyclase isoforms have been found (Strasser and Marquetant 1991, Krupinski *et al.* 1992). In the adult rat heart, type V has been reported as highly predominant (Mercadier *et al.* 1996) and with different sensitivity to various stress conditions (Ebina *et al.* 1997). This variability and sensitivity of adenylate cyclase can be responsible for a wide range of potential regulations especially under pathophysiological conditions.

Due to species-related differences (Schott *et al.* 1990, Cohen *et al.* 1991) and/or a modification of the experimental protocols of ischemic preconditioning, it is difficult to identify exactly which signaling pathway serves as the prevailing mechanism of protection. It is possible that both the β-adrenoceptor and the α-adrenoceptor pathway might regulate cAMP signaling, since two isozymes of protein kinase C, α and ξ, have been observed to activate type V cardiac adenylate cyclase *in vitro* (Strasser and Marquetant 1991, Kawabe *et al.* 1994). Therefore, different pathways can be activated by ischemic preconditioning and operate independently.

Our results indicate that the function of the adenylate cyclase system is preserved in the ischemic preconditioned heart and that it is involved in the protective mechanisms of ischemic preconditioning in the rat myocardium. By using cytochemical methods, the effect of ischemic preconditioning on adenylate cyclase enzyme complex can be specified. However, the variability in the intensity of the enzyme reaction product in our experiments can reflect the effects of ischemic preconditioning on single components of the adenylate cyclase system. Therefore, further studies and methods such as immunocytochemistry are needed to define the precise mechanisms and relations between the β-adrenoceptor/adenylate cyclase pathway and the effect of ischemic preconditioning.

Acknowledgements

This work was supported by the Slovak Grant Agency, grant No. VEGA 4045. We express our thanks to Mrs. A. Brichtová for skilful laboratory and technical assistance.

References

AIELLO EA, WALSH MP, COLE WC: Phosphorylation by protein kinase A enhances delayed rectifier K^+ current in rabbit vascular smooth muscle cells. *Am J Physiol* **268**: H926-H934, 1995.

ASIMAKIS GK, INNERS-McBRIDE K, CONTI VR, YANG CJ: Transient β-adrenergic stimulation can precondition the rat heart against postischaemic contractile dysfunction. *Cardiovasc Res* **28**: 1726-1734, 1994.

BANERJEE A, LOCKE-WINTER C, ROGERS KB, MITCHELL MB, BREW EC, CAIRNS CB, BENSARD DD, HARKEN AH: Preconditioning against myocardial dysfunction after ischemia and reperfusion by an $alpha_1$-adrenergic mechanism. *Circ Res* **73**: 656-670, 1993.

BANKWALA Z, HALE SL, KLONER RA: α-adrenoceptor stimulation with exogenous norepinephrine or release of endogenous catecholamines mimics ischemic preconditioning. *Circulation* **90**: 1023-1028, 1994.

COHEN MV, LIU GS, DOWNEY JM: Preconditioning causes improved wall motion as well as smaller infarcts after transient coronary occlusion in rabbits. *Circulation* **84**: 341-349, 1991.

DARROW BJ, FAST VG, KLEBER AG, BEYER EC, SAFFITZ JE: Functional and structural assessment of intercellular communication. *Circ Res* **79**: 174-183, 1996.

DE MELLO WC: Increase in junctional conductance caused by isoproterenol in heart cell pairs is suppressed by cAMP-dependent protein-kinase inhibitor. *Biochem Biophys Res Commun* **154:** 509-514 , 1988.

DE MELLO WC: Bioenergetics and cell communication; cell uncoupling seen as a protective mechanism in cardiac muscle. *Cell Biol Int Rep* **16:** 625-637, 1992.

DE MELLO WC: Impaired regulation of cell communication by β-adrenergic receptor activation in the failing heart. *Hypertension* **27**: 265-268, 1996.

DOWNEY JM: Ischemic preconditioning: nature's own cardioprotective intervention. *Trends Cardiovasc Med* **2**: 170-176, 1992.

EBINA T, TOKYA Y, OKA N, SCHWENCKE C, KAWABE J, ISHIKAWA Y: Isoform specific regulation of adenylyl cyclase by oxidized catecholamines. *J Mol Cell Cardiol* **29:** 1247-1254, 1997.

IMANAGA I: Cell-to-cell diffusion of large molecules in cardiac cells. In: *Cell Interactions and Gap Junctions.* Vol II, N SPERELAKIS, WC COLE (eds), CRC Press, Boca Raton, Florida, 1989, pp 50-63.

IWAMI G, KAWABE J, EBINA T, CANNON PJ, HOMCY CJ, ISHIKAWA Y: Regulation of adenylyl cyclase by protein kinase A. *J Biol Chem* **270**: 12481-1284, 1995.

IWASE T, MURAKAMI T, TOMITA T, MIKI S, NAGAI K, SASAYAMA S: Ischemic preconditioning is associated with a delay in ischemia-induced reduction of β-adrenergic signal transduction in rabbit hearts. *Circulation* **88**: 2827-2837, 1993.

JOHNSON RA, WELDEN J: Characteristic of the enzymatic hydrolysis of 5-adenylylimido-diphosphate implications for study of adenylate cyclase. *Arch Biochem Biophys* **183**: 216-220, 1977.

KATZ AM, TADA M, REPKE DI, IORIO JM, KIRCHBERGER MA: Adenylate cyclase: its probable localization in sarcoplasmic reticulum as well as sarcolemma of the canine heart. *J Mol Cell Cardiol* **6**: 73-79, 1974.

KAWABE J, IWAMI G, EBINA T, OHNO S, KATADA T, UEDA Y, HOMCY CJ, ISHIKAWA Y: Differential activation of adenylylcyclase by protein kinase C isoenzymes. *J Biol Chem* **269**: 16554-16558, 1994.

KRAUSE EG, ZIEGELHÖFFER A, FEDELEŠOVÁ M, STYK J, KOSTOLANSKY Š, GABAUER I, BLASIG I, WOLLENBERGER A: Myocardial cyclic nucleotide levels following coronary artery ligation. *Adv Cardiol* **25**: 119-129, 1978.

KRUPINSKI J, LEHMAN TC, FRANKENFIELD CD, ZWAAGSTRA JC, WATSON PA: Molecular diversity in the adenylyl cyclase family. Evidence for 8 forms of the enzyme and cloning of type VI. *J Biol Chem* **267**: 24858-24862, 1992.

KWAK BR, JONGSMA HJ: Regulation of cardiac gap junction channel permeability and conductance by several phosphorylating conditions. *Mol Cell Biochem* **157**: 93-99, 1996.

LIU GS, THORNTON J, VAN WINKLE DM, STANLEY AWH, OLSSON RA, DOWNEY JM: Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. *Circulation* **84**: 350-356, 1991.

LUKAS A, BOTSFORD MW: Cardioprotection induced by ischemic preconditioning in the mammalian heart: effects on arrhythmogenesis. *Can J Physiol Pharmacol* **75**: 316-325, 1997.

MANOACH M, TRIBULOVÁ N, IMANAGA I: The protective effect of d-sotalol against hypoxia-induced myocardial uncoupling. *Heart Vessels* **11**: 181-188, 1996.

MERCADIER JJ, ESPINASSE I, IOURGENKO V: Beta-adrenergic signal transduction and heart adenyl cyclase. *C R Seances Soc Biol Fil* **190**: 207-218, 1996.

MIYACHI EI, MANOACH M, UCHIYAMA H, WATANABE Y: Is cyclic AMP involved in the defibrillating effect of sotalol? *Life Sci* **57**: PL393-PL399, 1995.

MORITA Y, MURAKAMI T, IWASE T, NAGAI K, NAWADA R, KOUCHI I, AKAO M, SASAYAMA S: K_{ATP} channel contributes to the cardioprotection of preconditioning independent of anaesthetics in rabbit hearts. *J Mol Cardiol* **29**: 1267-1276, 1997.

MURRY CE, JENNINGS RB, REINER KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74**: 1124-1136, 1986.

NASA Y, YABE K, TAKEO S: β-adrenoceptor stimulation-mediated preconditioning-like cardioprotection in perfused rat hearts. *J Cardiovasc Pharmacol* **29**: 436-443, 1997.

OKRUHLICOVÁ L, SLEZÁK J, TRIBULOVÁ N, FICKOVÁ M: Changes of myocardial adenylate cyclase activity in early phases of ischemia. *Bratisl Lek Listy* **89**: 240-244, 1988.

OPIE LH, MULLER C, NATHAN D, DARIES P, LUBBE WF: Evidence for role of cyclic AMP as second messenger of arrhythmogenic effects of beta-stimulation. *Adv Cyclic Nucleotide Res* **12**: 63-69, 1980.

PARRATT JR: Endogenous myocardial protective (antiarrhythmic) substances. *Cardiovasc Res* **27**: 693-702, 1993.

RAVINGEROVÁ T, SONG W, PANCZA D, DŽURBA A, ZIEGELHÖFFER A, PARRATT JR: Pretreatment with catecholamines can suppress severe ventricular arrhythmias in rats: relevance to ischemic preconditioning. *Exp Clin Cardiol* **2**: 19-24, 1997.

RONA G: Catecholamine cardiotoxicity. *J Mol Cell Cardiol* **17**: 291-306, 1985.

SASAKI T, INUI M, KIMURA Y, KUZUYA T, TADA M: Molecular mechanism of regulation of Ca^{2+} pump ATPase by phospholamban in cardiac sarcoplasmic reticulum. Effects of synthetic phospholamban peptides on Ca^{2+} pump ATPase. *J Biol Chem* **267:** 1674-1679, 1992.

SCHÖMIG A, DART AM, DIETZ R, MAYER E, KUBLER W: Release of endogenous catecholamines in the ischemic myocardium of the rat. Part A. Locally mediated release. *Circ Res* **55**: 689-701, 1984.

SCHOTT RJ, ROHMANN S, BRAUN ER, SCHAPER W: Ischemic preconditioning reduces infarct size in swine myocardium. *Circ Res* **66**: 1133-1142, 1990.

SCHULZE W, HINTERBERGER U, WOLLENBERGER A, KRAUSE EG, JANISZEWSKI E: Problems of the cytochemical demonstration of adenylate cyclase. *Adv Cyclic Nucleotide Res* **1:** 249-260, 1972.

SLEZÁK J, GELLER AG: Cytochemical demonstration of adenylate cyclase in cardiac muscle. Effect of dimethyl sulfoxide. *J Histochem Cytochem* **27**, 774-781, 1979.

SPERELAKIS N: Regulation of calcium slow channels of the heart by cyclic nucleotides and effect of ischemia. *Adv Pharmacol* **31**: 1-24, 1994.

STRASSER RH, MARQUETANT R: Sensitization of of the β-adrenergic system in acute myocardial ischemia by a protein kinase C-dependent mechanism. *Eur Heart J* **12**: 48-53, 1991.

ŠTETKA R, RAVINGEROVÁ T, VOLKOVOVÁ K, PANCZA D, ZIEGELHÖFFER A, STYK J: Effects of ischemic preconditioning on the diabetic rat heart. *Physiol Res* **48:** 23P, 1999.

TRIBULOVÁ N, RAVINGEROVÁ T, OKRUHLICOVÁ L, GABAUER I, FICKOVÁ M, MANOACH M: Tedisamil modulates cAMP level in normal and ischemic guinea pig hearts. *J Mol Cell Cardiol* **30**: A68, 1998.

VEGH A, KOMORI S, SZEKERES L, PARRATT JR: Antiarrhythmic action of ischaemic preconditioning in anaesthetized dogs and rats. *Cardiovasc Res* **26**: 487-495, 1992.

VITTONE L, MUNDINA C, CINGOLANI GCD, MATTIAZZI A: cAMP and calcium-dependent mechanisms of phospholamban phosphorylation in intact hearts. *Am J Physiol* **258**: H318-H325, 1990.

WALKER MJA, CURTIS MJ, HEARSE DJ, CAMPBELL RW, JANSE MJ, YELLON DM, COBBE SM, COKER SJ, HARNESS JB, HARRON DW et al.: The Lambeth Conventions: guidelines for the study of arrhythmias in ischemia, infarction, and reperfusion. *Cardiovasc Res* **22**: 447-455, 1988.

YOUNT RG: Adenylylimidodiphosphate and guanylylomidodiphosphate. *Methods Enzymol* **38**: 420-427, 1974.

Reprint requests

Ľ.Okruhlicová, Institute for Heart Research, Slovak Academy of Sciences, Dúbravská cesta 9, 842 33 Bratislava, Slovak Republic. e-mail: usrdokru@savba.sk