Proteins Released from Liver after Ischaemia Induced an Elevation of Heart Resistance Against Ischaemia-Reperfusion Injury: 2. Beneficial Effect of Liver Ischaemia *in situ* *

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Abstract. We have shown earlier that proteins released from the heart during preconditioning may protect non-preconditioned heart during sustained ischaemia, similarly as preconditioning itself. In other our experiments we have documented that also proteins released from isolated rat liver during reperfusion after global ischaemia performed a protective effect on isolated rat heart against ischaemia-reperfusion injury. In the current study we examined the effect of liver ischaemia *in situ* on resistance of rat heart to ischaemia and reperfusion injury. Wistar rats (male) were subjected to liver ischaemia maintained by occlusion of portal vein and hepatic artery for 20 min, followed with 30-min reperfusion after reopening of both vessels. Then the hearts were isolated and perfused according to Langendorf. Hearts, after initial stabilisation (15 min), were subjected to 20-min ischaemia and 30-min reperfusion. During reperfusion, the haemodynamic parameters of hearts were measured. The protein pattern of high soluble fraction (HS fraction) isolated from rat blood by precipitation with ammonium sulphate was detected by SDS-PAGE.

Our results showed improved parameters of pressure and contractility in the group after liver ischaemia (ischaemic group), presented by decreased diastolic pressure and increased $\mathrm{LVDP_{(S\text{-}D)}}$ in comparison with levels of these parameters in the control group. We also observed improved heart contraction-relaxation cycles parameters $(\mathrm{d}P/\mathrm{d}t)_{\max}$ and $(\mathrm{d}P/\mathrm{d}t)_{\min}$ in ischaemic group as compared with the control group. On the other hand, there were no significant differences in heart rate

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and coronary flow between both experimental groups. SDS-PAGE showed changed protein pattern in HS fraction, particularly the levels of several low molecular weight proteins increased. We conclude that liver ischaemia induced a higher resitance of heart against ischaemia-reperfusion injury. We propose that release of some cardioprotective proteins present in HS fraction can also contribute to this cardioprotection.

Introduction

Ischaemic preconditioning is the phenomenon whereby brief periods of ischaemia have been shown to protect the myocardium against a sustained ischaemic insult (Murry et al. 1986; Reimer et al. 1990). Many substances and pathways have been proposed to play a role in the signal transduction mediating the cardioprotective effect of ischaemic preconditioning (Schulz et al. 2001). There is also evidence that release of some protein-like substances such as heat shock proteins may be involved in molecular mechanisms of preconditioning phenomenon (Marber et al. 1993; Sakamoto et al. 2000; Zhou et al. 2001). These findings indicate that during ischaemia, some cardioprotective proteins can be released from the heart tissue. Ziegelhöffer et al. (1995) showed that a high soluble protein fraction (HS fraction) released from the dog myocardium during ischaemic preconditioning was found to be cardioprotective against ischaemia similarly as ischaemic preconditioning itself.

It was documented that preconditioning can exist also in other organs than the heart, such as the central nervous system (Matsuyama et al. 1997), skeletal muscle (Pang et al. 1997), lung (Du et al. 1996) and liver (Kume et al. 1996). In addition, it has become apparent that ischaemia in other organs, such as kidneys and small intestine in rats (Gho et al. 1996), and skeletal muscle in rabbits (Birnbaum et al. 1997) can afford protection of the myocardium called remote preconditioning.

Our previous experiments have indicated that during postischaemic reperfusion of isolated rat liver some cardioprotective proteins were released into the perfusion solution (Barteková et al. 2003). It was shown that HS fraction isolated from the perfusate after ischaemia and reperfusion of perfused rat liver can protect isolated rat heart during ischaemia-reperfusion injury.

The aim of the current study was to examine whether some peptides are released into the blood also after *in situ* liver ischaemia, and whether *in situ* liver ischaemia may perform a beneficial effect on isolated heart during ischaemia and consequent reperfusion.

Materials and Methods

Treatment of animals

The male Wistar rats with body weight of 200–250 g kept on standard pellet diet and tap water *ad libitum* were used. The animals were anaesthetized with sodium pentobarbitone (40 mg/kg) i.p. and heparinized (500 IU) before each experiment.

In situ liver ischaemia

Intact male Wistar rats of both groups – control and liver ischaemic, were anaesthetized and heparinized. *In situ* ischaemia of liver was performed by occlusion of hepatic artery and portal vein (total liver ischaemia) for 20 min in ischaemic group of rats. After the 20-min liver ischaemia, consequent 30-min reperfusion was performed by reopening of both occluded vessels. The rats from control group were shame-operated without liver ischaemia. The hearts from both animal groups were than isolated and used for Langendorf perfusion. The whole body blood sera from all rats were then used for ammonium sulphate precipitation to isolate the HS fraction.

Ischaemia of isolated rat hearts

Hearts from both groups of rats were cannulated through the aorta and perfused in a non-recirculating mode according to Langendorff at a constant perfusion pressure equivalent to 75 mm Hg at 37℃. As a perfusion medium, the modified Krebs–Henseleit buffer (pH 7.4) was used, gassed with 95 % O_2 and 5 % CO_2 and containing (in mmol/l): 118.0 NaCl, 27.2 $NaHCO_3$, 1.2 $MgSO_4$, 1.0 KH_2PO_4, 1.59 $CaCl_2$, 3.0 KCl and 11.1 glucose. The solution was filtered through a 5 μm porosity filter to remove contaminants. After 20-min stabilisation, perfusion hearts were submitted to 20-min global ischaemia followed by 30-min reperfusion. During reperfusion, the haemodynamic parameters of each heart were measured. An epicardial electrogram (EG) was registered by means of two stainless steel electrodes attached to the apex of the heart and the aortic cannula, and continuously recorded by Mingograph ELEMA (Siemens, Solna, Sweden). Heart rates were calculated from the EG. Coronary flow was measured by a time collection of coronary effluents. Left ventricular pressure was measured by means of a latex water-filled balloon inserted into the left ventricle *via* the left atrium (adjusted to obtain end-diastolic pressure of 7–10 mm Hg) and connected to a P23 Db pressure transducer (Gould Statham Instruments Inc., USA). Heart contractility was evaluated using left ventricular developed pressure ($LVDP_{(S\text{-}D)}$), i.e., value that represents the difference between systolic and diastolic pressures in left ventricle. Maximum rates of left ventricular pressure falls and developments $[dP/dt]_{min}$ and $[dP/dt]_{max}$ were used as measures for heart relaxation and contraction properties. Experimental protocol is schaematically documented in Fig. 1.

Isolation, characterisation and partial purification of proteins released from the liver into the blood during ischaemia and reperfusion

Blood sera obtained from the whole blood by centrifugation at 3000 × *g* were precipitated overnight with 2.66 mol/l ammonium sulphate (4 ml on each 1 ml of serum) at 4℃. Precipitates were separated at 3000 × *g* and supernatants were repeatedly precipitated with ammonium sulphate (added 200 mg of solid ammonium sulphate into each 1 ml of supernatant) for 3 h at 4℃ and then pH was adjusted to 4.3 by H_3PO_4. Mixtures were precipitated overnight and centrifuged at 3000 × *g*.

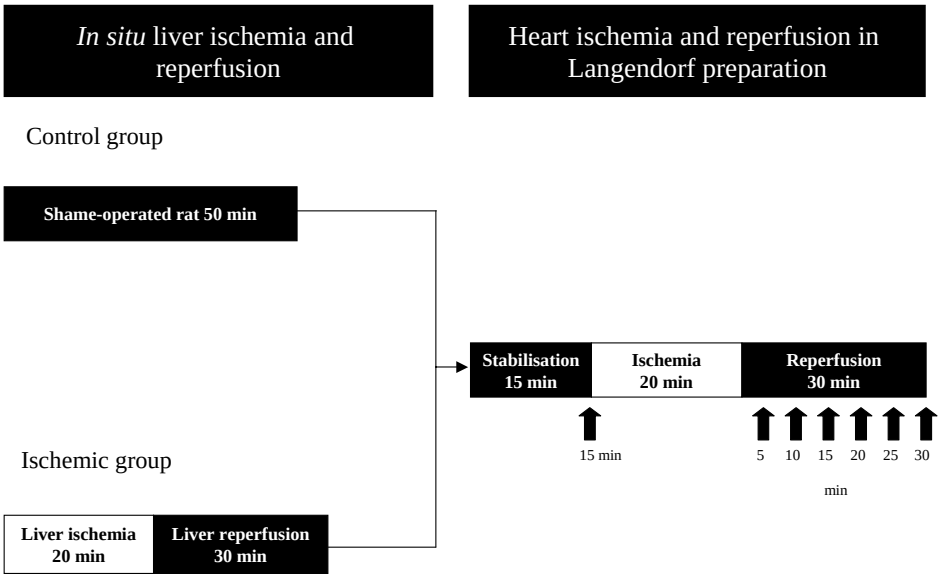

Figure 1. Scheme of experimental protocol of combined *in situ* liver ischaemia and consequent global ischaemia of Langendorf perfused rat hearts. ⬆ – haemodynamic parameters measured.

Pellets were dissolved in bi-distilled water and dialysed overnight against water. All dialysed samples were freeze-dried and stored for SDS-PAGE analysis. The protein patterns of all fractions were determined by means of SDS-PAGE in 5–15 % gradient gel using Laemmli procedure. Protein bands were visualised by Coomassie Blue G.

Statistical evaluation

Data were expressed as means ± SEM. The experimental data were statistically evaluated by Bonferroni probability value computed with the aid of one way analysis of variance (ANOVA) using GraphPAD InStat software version 1.13.

Results

The protein pattern of HS fraction obtained from blood sera of rats after 20-min *in situ* liver ischaemia followed by 30-min reperfusion is shown in Fig 2. It may be seen that the levels of several low molecular weight proteins increased. On the other hand, several high molecular weight proteins were lacking in fraction originated from blood after liver ischaemia as compared with nonischaemic samples.

The effect of *in situ* ischaemia of the liver prior Langendorf perfusion of heart on its haemodynamic parameters is documented in Fig. 3. Liver ischaemia caused

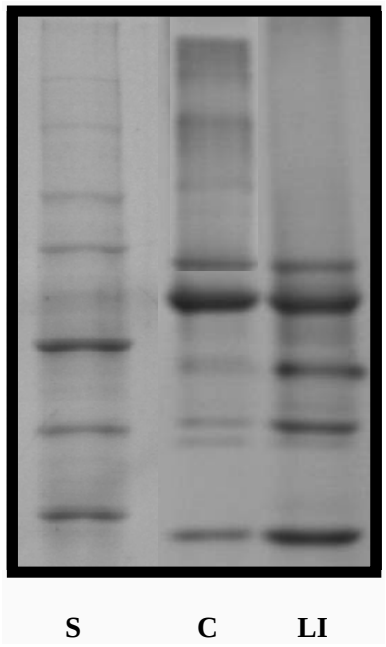

Figure 2. Protein pattern of HS fractions isolated from blood sera after *in situ* liver ischaemia. Proteins were separated by the means of SDS-PAGE in 5–15% gradient gels, visualized with Coomasie Blue staining. S, molecular weight standards; C, sha e-operated control rats; LI, rats after *in situ* liver ischaemia.

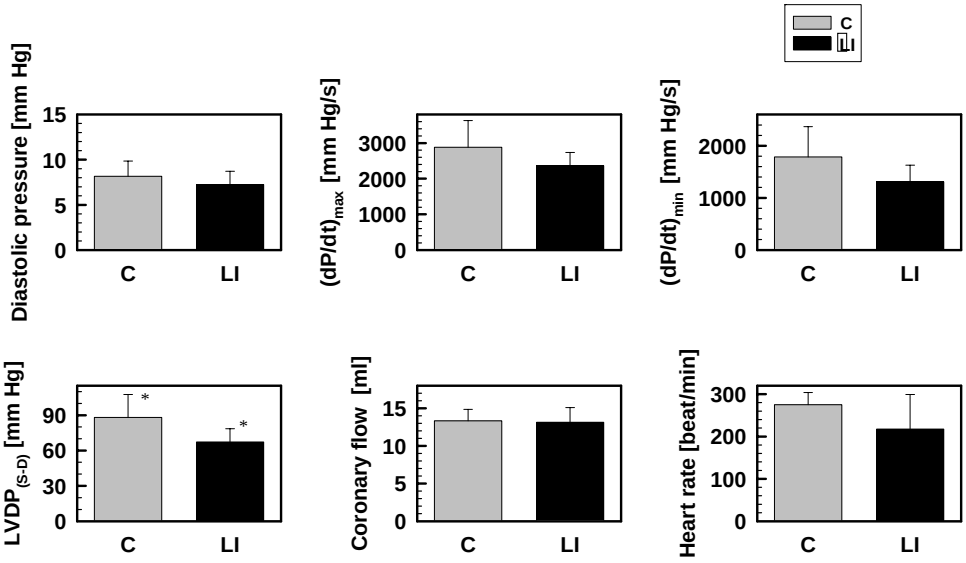

Figure 3. Haemodynamic parameters of Langendorf perfused rat hearts in 15th min of stabilization perfusion before ischaemia. Experimental data represents means ± SEM from five independent measurements. C, shame-operated control rats; LI, rats after *in situ* liver ischaemia; * significant difference in the level $p < 0.05$.

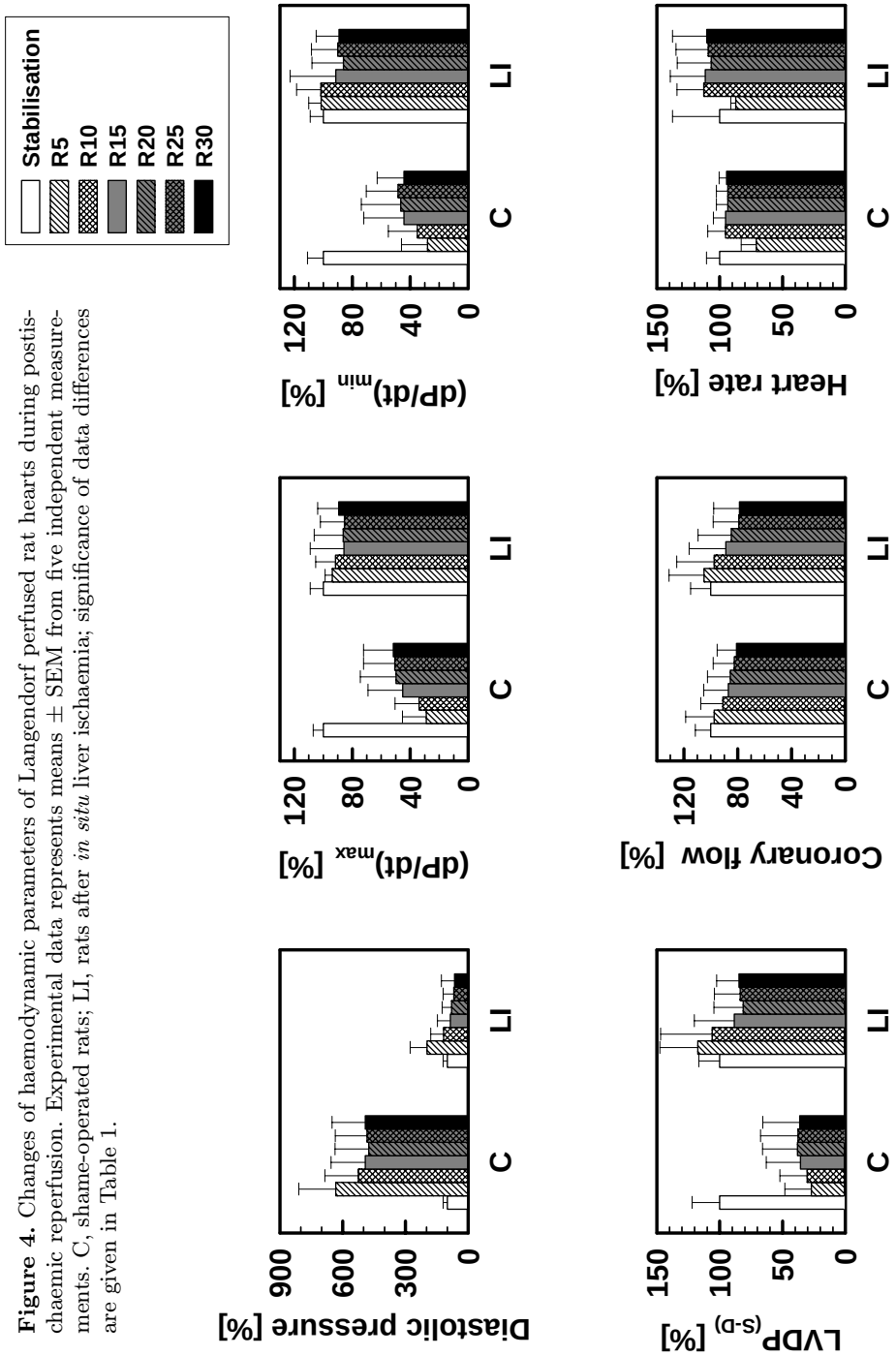

Figure 4. Changes of haemodynamic parameters of Langendorf perfused rat hearts during postischaemic reperfusion. Experimental data represents means ± SEM from five independent measurements. C, shame-operated rats; LI, rats after *in situ* liver ischaemia; significance of data differences are given in Table 1.

Table 1. Significances of ischaemia induced changes of haemodynamic parameters measured during postischaemic reperfusion of hearts originated from control rats (C) and rats treated by liver ischaemia (LI) in comparison to corresponding parameters measured during stabilisation (according to Bonferroni p value computed by ANOVA using GraphPAD I**NS**tat software version 1.13)

	Haemodynamic parameter					
	Diastolic pressure	$(\mathrm{d}P/\mathrm{d}t)_{\max}$	$(\mathrm{d}P/\mathrm{d}t)_{\min}$	$\mathrm{LVDP}_{(\mathrm{S\text{-}D})}$	Coronary flow	Heart rate
C	S *vs.* R05 $p < 0.01$	S *vs.* R05 $p < 0.01$	S *vs.* R05 $p < 0.01$	S *vs.* R05 $p < 0.05$	S *vs.* R05 ns	S *vs.* R5 $p < 0.05$
	S *vs.* R10 $p < 0.05$	S *vs.* R10 $p < 0.01$	S *vs.* R10 $p < 0.01$	S *vs.* R10 $p < 0.05$	S *vs.* R10 ns	S *vs.* R10 ns
	S *vs.* R15 $p < 0.05$	S *vs.* R15 $p < 0.05$	S *vs.* R15 $p < 0.05$	S *vs.* R15 $p < 0.05$	S *vs.* R15 ns	S *vs.* R15 ns
	S *vs.* R20 $p < 0.05$	S *vs.* R20 $p < 0.05$	S *vs.* R20 $p < 0.05$	S *vs.* R20 $p < 0.05$	S *vs.* R20 ns	S *vs.* R20 ns
	S *vs.* R25 $p < 0.05$	S *vs.* R25 $p < 0.05$	S *vs.* R25 $p < 0.05$	S *vs.* R25 $p < 0.05$	S *vs.* R25 ns	S *vs.* R25 ns
	S *vs.* R30 $p < 0.05$	S *vs.* R30 $p < 0.05$	S *vs.* R30 $p < 0.05$	S *vs.* R30 $p < 0.05$	S *vs.* R30 ns	S *vs.* R30 ns
LI	S *vs.* R05 ns	S *vs.* R05 ns	S *vs.* R05 ns	S *vs.* R05 ns	S *vs.* R05 ns	S *vs.* R05 ns
	S *vs.* R10 ns	S *vs.* R10 ns	S *vs.* R10 ns	S *vs.* R10 ns	S *vs.* R10 ns	S *vs.* R10 ns
	S *vs.* R15 ns	S *vs.* R15 ns	S *vs.* R15 ns	S *vs.* R15 ns	S *vs.* R15 ns	S *vs.* R15 ns
	S *vs.* R20 ns	S *vs.* R20 ns	S *vs.* R20 ns	S *vs.* R20 ns	S *vs.* R20 ns	S *vs.* R20 ns
	S *vs.* R25 ns	S *vs.* R25 ns	S *vs.* R25 ns	S *vs.* R25 ns	S *vs.* R25 ns	S *vs.* R25 ns
	S *vs.* R30 ns	S *vs.* R30 ns	S *vs.* R30 ns	S *vs.* R30 ns	S *vs.* R30 ns	S *vs.* R30 ns

S, values were registered during stabilization period; R05–R30, values were registered in 5–30 min of postischaemic reperfusion; ns, non significant.

significant decrease in $\mathrm{LVDP}_{(\mathrm{S\text{-}D})}$ value and nonsignificant decreases in $[\mathrm{d}P/\mathrm{d}t]_{\min}$, $[\mathrm{d}P/\mathrm{d}t]_{\max}$ and heart rate. This indicated that *in situ* ischaemia and reperfusion of liver induced a moderate decrease in heart contractility and rate.

Heart ischaemia induced strong increases in diastolic pressure and decreases in $\mathrm{LVDP}_{(\mathrm{S\text{-}D})}$, $[\mathrm{d}P/\mathrm{d}t]_{\min}$ and $[\mathrm{d}P/\mathrm{d}t]_{\max}$, measured in 5–30 min of reperfusion as compared with corresponding data obtained from stabilisation period in hearts from animals without liver ischaemia and reperfusion (Fig. 4). The rates of the same hearts were decreased only in the 5th min of the reperfusion. All the above changes were significant at the level of probability 0.1 or 0.5 as it is documented in Table 1. In contrast, ischaemia of hearts did not induce any significant changes of heamodynamic parameters measured after 5–30 min of reperfusion in the heart from animals treated by ischaemia and reperfusion of liver (Fig. 4, Table 1). Coronary flow was nonsignificantly decreased after heart ischaemia independently of the fact if hearts originated from animals treated by liver ischaemia and reperfusion.

Thus, liver ischaemia has beneficial effect on heart function during ischaemia-

reperfusion injury that may be documented namely by improved heart pressure parameters and improved heart contractility.

Discussion

In our previous work we have shown that some proteins exerting protective effect against ischaemia-reperfusion injury of heart were released from isolated perfused rat liver after global ischaemia and reperfusion (Barteková et al. 2003). The current study showed that also liver ischaemia *in situ* can protect rat heart against ischaemia-reperfusion injury. Cardioprotective effect of liver ischaemia can be documented by improved pressure, contractility and contraction-relaxation cycles parameters. Especially stabilisation of diastolic pressure indicates that liver ischaemia can efficiently prevent diastolic dysfunction of isolated hearts after ischaemia. Improved contractility during postischaemic reperfusion of hearts originated from rat treated by liver ischaemia and reperfusion is characterized by stabilisation of $LVDP_{(S\text{-}D)}$ on the level similar to that observed prior heart ischaemia. These findings are consistent with improved physiological parameters mediated by ischaemic preconditioning and also mediated by administration of HS fraction released from dog heart after ischaemic preconditioning (Ziegelhöffer et al. 1995) or from perfused rat liver during postischaemic reperfusion (Barteková et al. 2003)

The SDS-PAGE analysis showed visibly changed protein pattern of HS fraction after *in situ* liver ischaemia. Particularly, the amounts of several low molecular weight proteins (especially about 10 kDa) were increased in blood of animals treated with liver ischaemia and reperfusion as compared with nontreated animals. An elevation of protein band with similar molecular weight was observed in HS fraction isolated from perfusate after ischaemia and reperfusion of isolated rat liver and this fraction exerts beneficial effect against ischaemia- reperfusion injury of heart (Barteková et al. 2003). Interestingly, we have observed an elevation of protein band with similar molecular weight in HS fraction (also with similar cardioprotective effect) isolated from dog blood after ischaemic preconditioning. All the above facts indicated that proteins with beneficial effect against ischaemia and reperfusion injury are released from several organs (heart, liver) in consequence to ischaemic insult. It could be assumed that the effects of these proteins are only a part of protective mechanisms, cooperating with many other biochaemical pathways in endogenous adaptation of organism to induce better resistance against ischaemia and reperfusion injury. The identification of these proteins will represent the topic of the future study.

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References

Barteková M., Styk J., Pancza D., Kukan M., Šeboková J., Breier A. (2003): Proteins released from liver after ischaemia induced an elevation of heart resistance against ischaemia-reperfusion injury: 1. Beneficial effect of protein fraction isolated from perfusate after ischaemia and reperfusion of liver. Gen. Physiol. Biophys. **22**, 567—577

Birnbaum Y., Hale S. L., Kloner R. A. (1997): Ischemic preconditioning at a distance: reduction of myocardial infarct size by partial reduction of blood supply combined with rapid stimulation of the gastrocnemius muscle in the rabbit. Circulation **96**, 1641—1646

Du Z. Y., Hicks M., Winlaw D., Spratt P., Macdonald P. (1996): Ischemic preconditioning enhances donor lung preservation in the rat. J. Heart Lung Transplant. **15**, 1258—1267

Gho B. C., Schoemaker R. G., Van den Doel M. A., Duncker D.J., Verdouw P.D. (1996): Myocardial protection by brief ischemia in noncardiac tissue. Circulation **94**, 2193—2200

Kume M., Yamamoto Y., Saad S., Gomi T., Kimoto S., Shimabukuro T., Yagi T., Nakagami M., Takada Y., Morimoto T., Yamaoka Y. (1996): Ischemic preconditioning of the liver in rats: implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. J. Lab. Clin. Med. **128**, 251—258

Marber M. S., Latchman D. S., Walker J. M., Yellon D. M. (1993): Cardiac stress protein elevation 24 hours after brief ischaemia or heat stress is associated with resistance to myocardial infarction. Circulation **88**, 1264—1272

Matsuyama K., Chiba Y., Ihaya A., Kimura T., Tanigawa A., Muraoka R. (1997): Effect of spinal cord preconditioning on paraplegia during cross-clamping of the thoracic aorta. Ann. Thorac. Surg. **63**, 1315—1320

Murry C. E., Jennings R. B., Reimer K. A. (1986): Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation **74**, 1124—1136

Pang C. Y., Neligan P., Xu H., He W., Zhong A., Hopper R., Forrest C. R. (1997): Role of ATP-sensitive K^+ channels in ischemic preconditioning of skeletal muscle against infarction. Am. J. Physiol. Heart Circ. Physiol. **273**, H44—51

Reimer K. A., Murry C. E., Jennings R. B. (1990): Cardiac adaptation to ischaemia. Ischaemic preconditioning increases myocardial tolerance to subsequent ischaemic episodes. Circulation **82**, 2266—2268

Sakamoto K., Urushidani T., Nagao T. (2000): Translocation of HSP27 to sarcomere induced by ischaemic preconditioning in isolated rat hearts. Biochem. Biophys. Res. Commun. **269**, 137—142

Schulz R., Cohen M. V., Behrends M., Downey J. M., Heusch G. (2001): Signal transduction of ischemic preconditioning. Cardiovasc. Res. **52**, 181—198

Zhou J. J., Pei J. M., Wang G. Y., Wu S., Wang W. P., Cho C. H., Wong T. M. (2001): Inducible HSP70 mediates delayed cardioprotection via U-50488H pretreatment in rat ventricular myocytes. Am. J. Physiol. Heart Circ. Physiol. **281**, H40—47

Ziegelhöffer A., Vrbjar N., Styk J., Breier A., Džurba A., Ravingerová T. (1995): Adaptation of the heart to ischemia by preconditioning: effects on energy equilibrium, properties of sarcolemmal ATPases and release of cardioprotective proteins. Mol. Cell. Biochem. **147**, 129—137

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