The Role of NO in Ischemia/Reperfusion Injury in Isolated Rat Heart

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Abstract. Nitric oxide (NO) is an important regulator of myocardial function and vascular tone under physiological conditions. However, its role in the pathological situations, such as myocardial ischemia is not unequivocal, and both positive and negative effects have been demonstrated in different experimental settings including human pathology. The aim of the study was to investigate the role of NO in the rat hearts adapted and non-adapted to ischemia. Isolated Langendorff-perfused hearts were subjected to test ischemic (TI) challenge induced by 25 min global ischemia followed by 35 min reperfusion. Short-term adaptation to ischemia (ischemic preconditioning, IP) was evoked by 2 cycles of 5 min ischemia and 5 min reperfusion, before TI. Recovery of function at the end of reperfusion and reperfusion-induced arrhythmias served as the end-points of injury. Coronary flow (CF), left ventricular developed pressure (LVDP), and dP/dt_{max} (index of contraction) were measured at the end of stabilization and throughout the remainder of the protocol until the end of reperfusion. The role of NO was investigated by subjecting the hearts to 15 min perfusion with NO synthase (NOS) inhibitor L-NAME (100 μmol/l), prior to sustained ischemia. At the end of reperfusion, LVDP in the controls recovered to 29.0 ± 3.9% of baseline value, whereas preconditioned hearts showed a significantly increased recovery (LVDP 66.4 ± 5.7%, $p < 0.05$). Recovery of both CF and dP/dt_{max} after TI was also significantly higher in the adapted hearts (101.5 ± 5.8% and 83.64 ± 3.92%) as compared with the controls (71.9 ± 6.3% and 35.7 ± 4.87%, respectively, $p < 0.05$). NOS inhibition improved contractile recovery in the non-adapted group (LVDP 53.8 ± 3.1%; dP/dt_{max} 67.5 ± 5.92%) and increased CF to 82.4 ± 5.2%. In contrast, in the adapted group, it abolished the protective effect of IP (LVDP 31.8 ± 3.1%; CF 70.3 ± 3.4% and dP/dt_{max} 43.25 ± 2.19%). Control group exhibited 100% occurrence of ventricular tachycardia (VT), 57% incidence of ventricular fibrillation (VF) – 21% of them was sustained VF (SVF); application of L-NAME attenuated reperfusion arrhythmias (VT 70%, VF 20%, SVF 0%). Adaptation by IP also reduced arrhythmias, however, L-NAME in the preconditioned hearts increased the incidence of arrhythmias (VT 100%, VF 58%, SVF 17%). In conclusion: our results indicate that administration of L-NAME might be cardioprotective in the normal hearts exposed to ischemia/reperfusion

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(I/R) alone, suggesting that NO contributes to low ischemic tolerance in the non-adapted hearts. On the other hand, blockade of cardioprotective effect of IP by L-NAME points out to a dual role of NO in the heart: a negative role in the non-adapted myocardium subjected to I/R, and a positive one, due to its involvement in the mechanisms of protection triggered by short-term cardiac adaptation by preconditioning.

Key words: Ischemia — Reperfusion — Preconditioning — Heart — Rat

Introduction

Ischemic preconditioning (IP) is a phenomenon in which single or multiple brief periods of cardiac ischemia result in an increased resistance to a subsequent more prolonged period of ischemia (Murry et al. 1986) manifested by a reduction of necrotic changes, improved cardiac function and suppression of malignant arrhythmias. In most species, this phenomenon has two phases: an acute or early phase, in which the cardioprotective effect lasts for 1–3 h and a delayed phase or second window of protection, which reappears approximately 24 h after the acute phase and may last for up to 72 h (Baxter and Yellon 1997). This short-lasting adaptive phenomenon is described in all species including humans, undergoing multiple aortic cross-clamping during coronary artery bypass grafting (CABG) surgery or balloon inflations during percutaneous coronary angioplasty (PTCA), as well as in patients with preceding episodes of angina prior to myocardial infarction offers an extremely powerful protection exceeding the effectiveness of any pharmacological intervention (Yellon et al. 1993; Solomon et al. 2004). Numerous mechanisms have been reported to be involved in the phenomenon of IP. They include: opening of collateral vessels (Deutsch et al. 1990), inhibition of mitochondrial ATPase (Ambrosio et al. 1994), release of endogenous mediators such as adenosine (Liu et al. 1991), activation of 5'-nucleotidase (Kitakaze et al. 1993), release of catecholamines coupled with changes in G-proteins (Ravingerová et al. 1995), formation of cyclooxygenase products, particularly of prostaglandin (Vegh et al. 1992), induction of endogenous myocardial protective substances and among them bradykinin (Parratt et al. 1995). Endogenous substances released from the heart in the early phase of ischemia are suggested to activate multiple cascades of intracellular signalization, from membrane receptors *via* postreceptor signal transduction pathways, up to the final end-effector systems. Experimental studies of the mechanisms of IP lead to the observations that pharmacological modulations at different levels of signal transduction may mimick cardioprotective effects of IP (Nakano et al. 2000a) and thus provide a safer way of inducing the IP-like cardioprotection in humans without the harmful consequences of ischemia.

Nitric oxide (NO) plays multiple roles in the cardiovascular system mediating a number of physiological and pathophysiological processes**.** In smooth muscle cells, NO activates guanylatcyclase by hem-dependent mechanism resulting in increased concentration of guanosine 3',5'-cyclic monophosphate (cGMP) that leads

to a decreased intracellular concentration of Ca^{2+} and subsequent relaxation of the vessels (Ignarro et al. 1986). Reduced basal availability of NO and impairment of endothelial NO-dependent mechanisms due to dysfunction of the normally protective endothelium may be involved in the pathogenesis of several cardiovascular diseases including atherosclerosis, hypertension, heart failure, coronary heart disease, arterial thrombotic disorders, and stroke (Ignarro et al. 2002). In cardiomyocytes, NO/cGMP pathway is involved in the inhibition of Ca^{2+} influx by cGMP-dependent phosphorylation of L-type Ca^{2+} channels (Méry et al. 1991), antagonism of the effects of β-adrenergic stimulation (Balligand et al. 1995) and decrease in myocardial contractility and heart rate (Balligand et al. 1993), as well as in reduction in myocardial oxygen consumption (Lohmann et al. 1991) and opening of sarcolemmal K_{ATP} channels (Shinbo and Iijima 1997). Reduced Ca^{2+} current may alleviate Ca^{2+} overload associated with acute myocardial ischemia as one of the major mechanisms of ischemic injury (Bolli and Marban 1999).

Some findings recognize that NO can be also cytotoxic, and its abnormal production and action participate in arterial and cardiac pathologies, such as chronic heart failure, but the cause of the abnormalities and the role of NO in the pathogenesis of heart failure are yet to be clarified (Dusting 1996). It has also been hypothesized that the toxicity of NO is more likely resulted from its reaction with superoxide anion to produce a potent oxidant peroxynitrite that can exert cytotoxicity *via* its reaction with numerous molecular targets and appears to be potentially injurious to myocardial tissue (Lecour et al. 2001; Ferdinandy and Schulz 2003). On the other hand, it might be also involved in the mechanisms of cardiac adaptation as a signaling molecule (Bolli 2001). The role of NO in the cardioprotection conferred by IP was characterized mostly for the mechanisms of stunning in the delayed phase of IP (Bolli et al. 1997a; Bolli and Marban 1999) and its antiarrhythmic effect (Vegh et al. 1992), however, the role of NO in the classical IP is not clearly established. The aim of the study was to investigate the role of NO in ischemia/reperfusion (I/R) injury by inhibition of NO synthesis (NOS) with L^G-nitro-N-arginine methyl ester (L-NAME) and following its effect on postischemic contractile dysfunction and reperfusion-induced arrhythmias in the rat hearts adapted and non-adapted to ischemia.

Materials and Methods

Animals

Adult Wistar rats (250–300 g body weight), fed a standard diet and tap water ad libitum, were used. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (publication No. 85–23, revised 1996).

Perfusion technique

Rats were anesthetized by sodium pentobarbitone (60 mg/kg, i.p.). Hearts were rapidly excised, placed in ice-cold perfusion buffer, cannulated *via* the aorta and

perfused in the Langendorff mode at a constant perfusion pressure of 70 mmHg and temperature of 37 ℃. The perfusion solution was a modified Krebs-Henseleit buffer gassed with 95 % O_2 and 5 % CO_2 (pH 7.4) containing (in mmol/l): 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 filtered through a 5 μm porosity filter (Millipore) to remove contaminants.

An epicardial electrogram (EG) was registered by means of two stainless steel electrodes attached to the aortic cannula and the apex of the heart and continuously recorded (Mingograph ELEMA-Siemens, Solna, Sweden). Heart rate was calculated from the EG. After recording the baseline values, the hearts were electrically stimulated at 300 beats/min throughout the remainder of the protocol with the exception of initial 5 min of reperfusion for the assessment of reperfusion-induced arrhythmias. Coronary flow (CF) was measured by a timed collection of coronary effluent. 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 enddiastolic pressure of 5–7 mmHg) and connected to a pressure transducer (P23 Db model; Gould Statham Instruments, USA). Left ventricular developed pressure (LVDP, systolic minus diastolic pressure), left ventricular enddiastolic pressure (LVEDP), maximal rates of pressure development and fall ($+\mathrm{d}P/\mathrm{d}t_{\max}$ and $-\mathrm{d}P/\mathrm{d}t_{\max}$) as the indexes of contraction and relaxation, as well as the heart rate and CF were used to assess cardiac function. Its recovery after I/R was expressed as percentage of preischemic baseline values.

Arrhythmias were measured from the EG and evaluated in accordance with The Lambeth Conventions (Walker et al. 1988). In this study we analyzed the incidences of ventricular tachycardia (VT) and fibrillation (VF) as well as their duration. VT was defined as a run of four or more consecutive ectopic beats. VF lasting more than 2 min was considered as sustained VF (SVF).

Experimental protocols

After 15-min equilibration, all hearts were randomly assigned to the following protocols shown in Fig. 1:

1. Control test ischemia (TI) ($n = 14$)

After 15 min initial stabilisation, the hearts underwent 25 min global ischemia followed by 35 min reperfusion.

2. C+L-NAME ($n = 10$)

Hearts from this group underwent the same procedures as the hearts from the control group, but with an additional 15 min perfusion with perfusion buffer containing L-NAME (100 μmol/l), before TI.

3. IP ($n = 14$)

Hearts from this group were exposed to two cycles of ischemia and reperfusion, 5 min each, before TI.

4. IP+L-NAME ($n = 12$)

Hearts from this group underwent the same procedures as in protocol 3, but 5 min

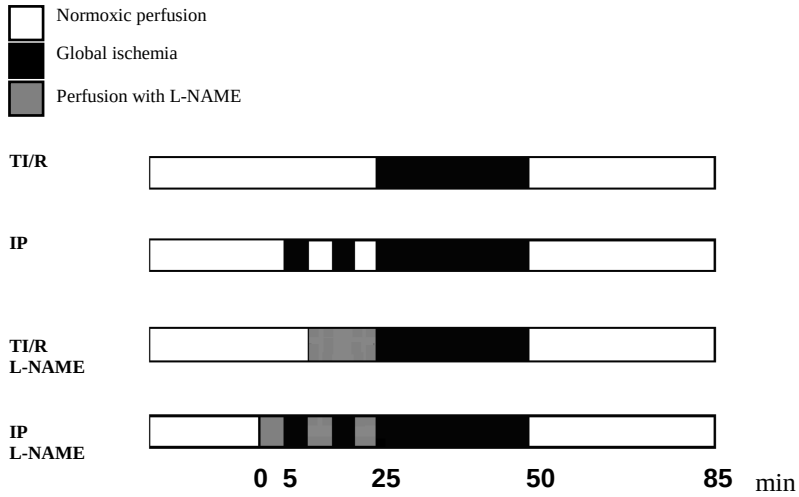

Figure 1. Experimental protocols. TI, test ischemia; R, reperfusion; IP, ischemic preconditioning; L-NAME, L^G-nitro-N-arginine methyl ester.

before and during IP, the hearts were perfused with perfusion buffer containing L-NAME (100 μmol/l).

Statistics

Data were expressed as mean ± SEM. The one-way analysis of variance and a subsequent Student–Newman–Keuls test were used for comparison of differences among groups. Incidences of VT and VF were compared using Fisher's exact test. Differences were considered significant when $p < 0.05$.

Results

Preischemic values of all haemodynamic parameters of hearts from all four groups are summarized in Table 1. There were no significant differences between all groups in any haemodynamic parameter. These values represent 100 % for comparison with postischemic changes in all experimental groups.

Coronary flow

The recovery of CF of all experimental groups during postischemic reperfusion is shown in Fig. 2. Renewal of CF after ischemia and reperfusion was significantly higher in the preconditioning-adapted hearts (101.5 ± 5.8 %) when compared with the value of this parameter in the control group (71.9 ± 6.3 %; $p < 0.05$). Application of L-NAME significantly decreased recovery of CF in the adapted hearts (70.3 ± 3.4 %; $p < 0.05$ *vs.* non-treated adapted hearts), whereas in the non-adapted controls L-NAME administration moderately increased CF recovery (82.4 ± 5.2 %) against the value of this parameter in the control non-treated group.

Table 1. Preischemic values of haemodynamic parameters of isolated rat hearts

Group	n	HR (beats /min)	CF (ml/min)	$+\mathrm{d}P/\mathrm{d}t_{\max}$ (mmHg/s)	$-\mathrm{d}P/\mathrm{d}t_{\max}$ (mmHg/s)	LVEDP (mmHg)	LVDP (mmHg)
Control	14	209±10	12.2±1.1	2229±117	1725±99	6.8±2.0	68.6±6.4
C+L-NAME	10	242±14	12.2±0.5	2452±240	1860±110	4.4±2.1	60.4±7.1
IP	14	212±9	11.4±1.9	2166±104	1505±87	5.7±1.8	64.4±3.8
IP+L-NAME	12	233±12	12.3±0.4	2579±210	1717±94	4.7±0.9	78.8±8.4

C+L-NAME, controls with L-NAME; IP, ischemic preconditioning; IP+L-NAME, IP with L-NAME; HR, heart rate; CF, coronary flow; LVDP, left ventricular developed pressure; LVEDP, left ventricular enddiastolic pressure; $\pm\mathrm{d}P/\mathrm{d}t_{\max}$, rates of pressure development and decline, respectively; n, number of hearts in each group. Data are means ± SEM.

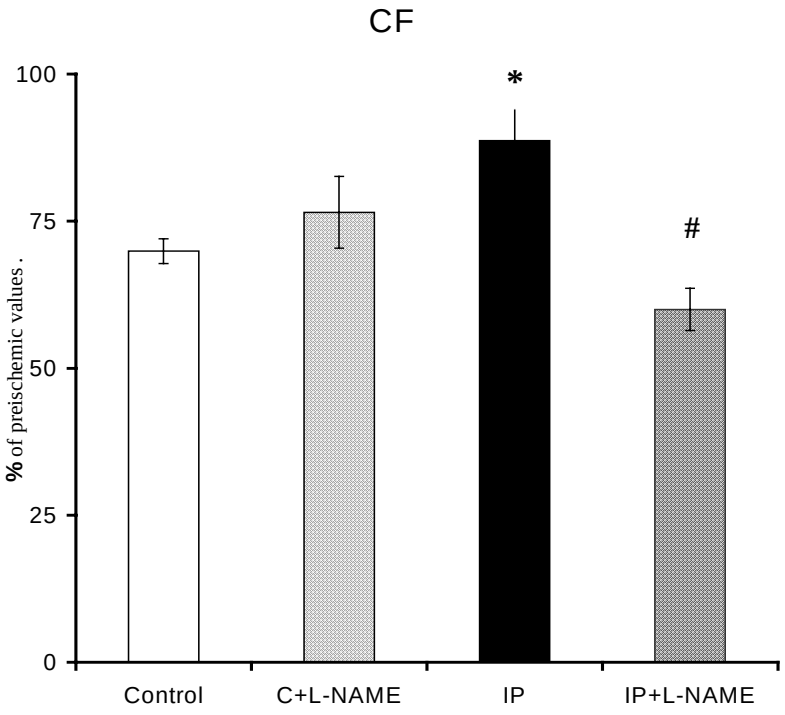

Figure 2. Effect of L^{G}-nitro-N-arginine methyl ester (L-NAME) on the recovery of coronary flow (CF) after myocardial ischemia and reperfusion and IP in rat myocardium. Data are means ± SEM expressed in % of baseline values. Abbreviations: C+L-NAME, controls with L-NAME; IP, ischemic preconditioning; IP+L-NAME, IP with L-NAME, * $p < 0.05$ *vs.* Control; # $p < 0.05$ *vs.* IP.

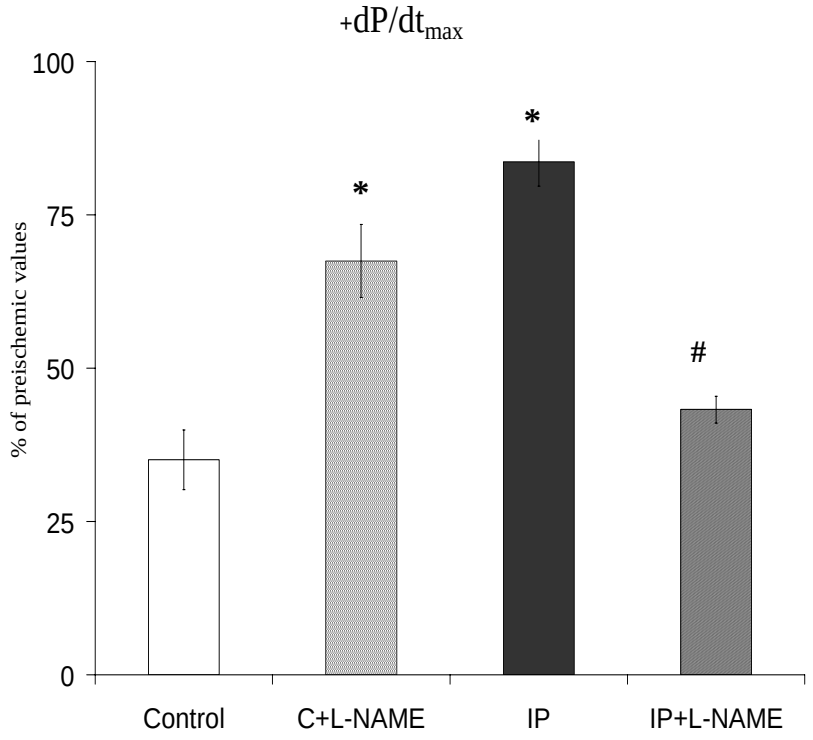

Figure 3. Effect of L-NAME on the recovery of maximal rate of contraction ($+\mathrm{d}P/\mathrm{d}t_{\max}$) after myocardial ischemia and reperfusion. Data are means ± SEM expressed in % of baseline values. (Abbreviations as in Fig. 2.) * $p < 0.05$ *vs.* Control; # $p < 0.05$ *vs.* IP.

Recovery of contractile function

IP significantly improved recovery of $+\mathrm{d}P/\mathrm{d}t_{\max}$ (1878 ± 179 mmHg/s) after ischemia and reperfusion in comparison with the control group (999 ± 128 mmHg/s; $p < 0.05$). After administration of L-NAME, postischemic values of $+\mathrm{d}P/\mathrm{d}t_{\max}$ were significantly increased in the non-adapted hearts (1374 ± 219 mmHg/s; $p < 0.05$) as compared with the non-treated controls. In contrast, in the adapted group, treatment with L-NAME reduced the recovery of $+\mathrm{d}P/\mathrm{d}t_{\max}$ (1266 ± 89 mmHg/s) in comparison with the non-treated adapted hearts ($p < 0.05$). Changes in $-\mathrm{d}P/\mathrm{d}t_{\max}$ were similar to $+\mathrm{d}P/\mathrm{d}t_{\max}$ after all interventions (not shown). Fig. 3 summarizes maximal recovery of contraction after TI in all groups.

Recovery of LVDP and LVEDP

The changes of LVDP and LVEDP during I/R in the control non-adapted and adapted hearts are shown in Figs. 4 and 5. Values of LVDP during the whole postischemic reperfusion were significantly higher in the adapted hearts in compar-

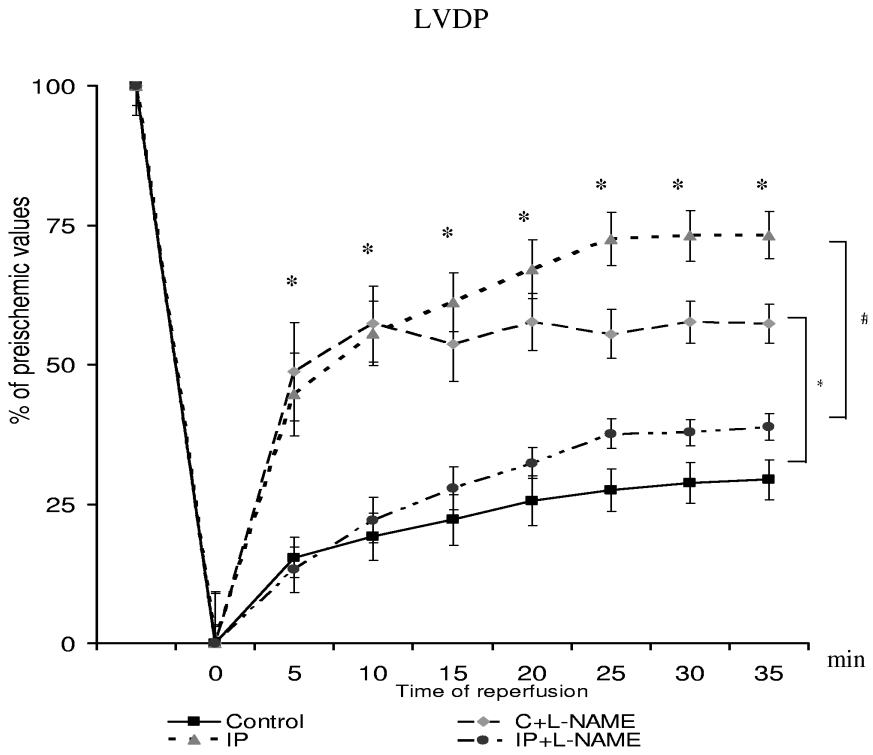

Figure 4. Postischemic changes of left ventricular developed pressure (LVDP) in the rat heart: effect of L-NAME in non-adapted hearts and in the preconditioned hearts. Data are means ± SEM expressed in % of baseline values. (Abbreviations as in Fig. 2.) * $p < 0.05$ *vs.* Control; # $p < 0.05$ *vs.* IP.

ison with controls (Fig. 4). Treatment with L-NAME significantly improved postischemic values of LVDP during the whole reperfusion in comparison with postischemic levels of LVDP in the control group. In contrast, in the adapted hearts, administration of L-NAME significantly decreased postischemic values of LVDP as compared with those in the preconditioned hearts without L-NAME. In addition, both administration of L-NAME and IP lowered the values of LVEDP (Fig. 5). Treatment with L-NAME resulted in significantly increased values of LVEDP in the adapted hearts in comparison with preconditioned hearts without treatment.

Arrhythmias

The incidence of VT was 100 % in the control group, whereas VF occurred in 57 % of hearts and was sustained in 21 % of them (Fig. 6). The incidence of VT was not significantly changed by any intervention although a tendency of its reduction was observed after L-NAME treatment and in the preconditioned hearts. On the other

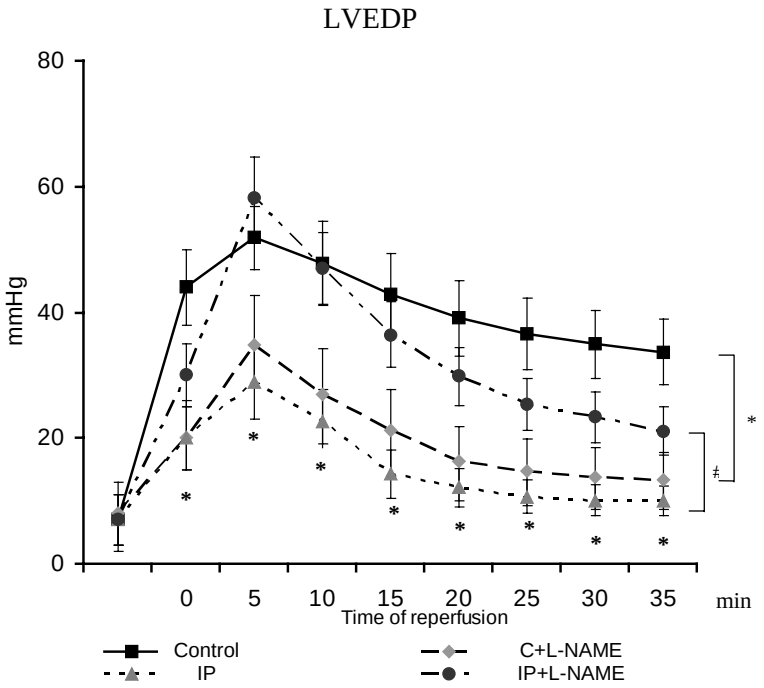

Figure 5. Postischemic changes of left ventricular enddiastolic pressure (LVEDP) in the rat heart: effect of L-NAME in non-adaptated hearts and in the preconditioned. Data are means ± SEM expressed in mmHg of baseline values. (Abbreviations as in Fig. 2.) * $p < 0.05$ *vs.* Control; # $p < 0.05$ *vs.* IP.

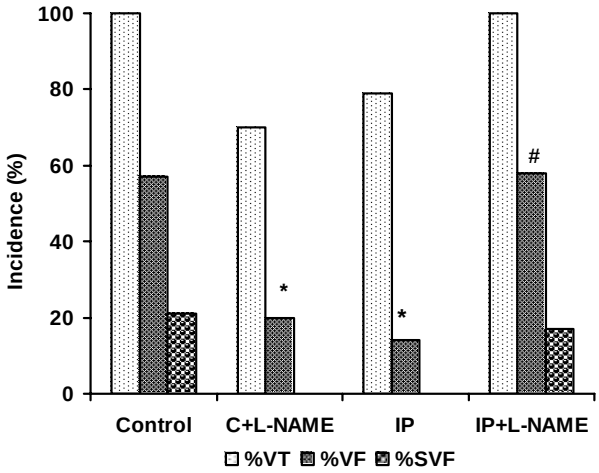

Figure 6. Incidence of ventricular tachycardia (VT), ventricular fibrillation (VF) and sustained VF (SVF) during postischemic reperfusion rat heart. Data are % of incidence. (Abbreviations as in Fig. 2.) * $p < 0.05$ *vs.* Control; # p < 0.05 *vs.* IP.

hand, the incidence of VF was markedly reduced in the above groups to 20 and 14 %, respectively, and SVF was completely suppressed. Treatment with L-NAME abolished this effect of IP and increased the incidence of VF and SVF to its value in the control groups (VF 58 %, SVF 17 %).

Discussion

Over the past decade, an enormous number of studies have focused on the role of NO in myocardial ischemia and IP. It is important to distinguish between the function of NO in unstressed (non-preconditioned) myocardium from its function in preconditioned myocardium, i.e., in the myocardium that has shifted to a defensive phenotype in response to stress (Bolli 2001). Generally, most studies proposed a positive role of NO in IP (Horimoto et al. 2000; Nakano et al. 2000b; Hill et al. 2001), but its role in the non-adapted myocardium is controversial. Vast majority of authors have concluded that NO has a protective effect in non-preconditioned hearts during I/R (Pernow et al. 1994; Sato et al. 1995; Mizumura et al. 1995), but other studies suggest that NO has a detrimental effect during I/R and limitation of its production may reduce the extent of myocardial injury (Matheis et al. 1992; Patel et al. 1993).

The aim of our study was to investigate the effect of NOS blockade with its inhibitor L-NAME on postischemic contractile dysfunction in the rat hearts non-adapted to ischemia and in the hearts adapted by preconditioning before sustained ischemic challenge. The results indicate that administration of L-NAME might be cardioprotective in the non-preconditioned hearts exposed to prolonged I/R alone. Thus, treatment of these hearts with L-NAME improved postischemic systolic function (increased values of LVDP and $+\mathrm{d}P/\mathrm{d}t_{\max}$), attenuated diastolic dysfunction (decreased values of LVEDP) and malignant ventricular arrhythmias (reduced incidence of VF and suppressed SVF), as well as increased the recovery of CF upon reperfusion. This is in agreement with a study of Zhang et al. (2001) that demonstrated that another NOS inhibitor, nitro-L-arginine (L-NNA), ameliorated myocardial stunning and decreased production of free radicals in the dog myocardium subjected to I/R. Similarly, Yasmin et al. (1997) observed improvement in left ventricular alterations after treatment with NOS inhibitor $\mathrm{N^G}$-monomethyl-L-arginine (L-NMMA). Liu et al. (1997) have also found that in rats, ventricular arrhythmias due to occlusion of left anterior descending (LAD) coronary artery were associated with 6.7-fold increase in the activity of inducible NOS (iNOS) in the ischemic region, enhanced immunoreactivity of nitrotyrosine (a marker of peroxynitrite formation), and superoxide and tissue NO levels elevated by 140 and 90 %, respectively, indicating that enhanced production of NO and radicals is involved in myocardial injury during I/R. In line with these findings, exogenous peroxynitrite at high (micromolar) concentrations increased the incidence of VF in the isolated rat heart subjected to I/R (Sedat et al. 1999). Further evidence of the negative role of NO (produced by iNOS) in cardiac dysfunction has been presented in the

study of Saito et al. (2002), when treatment with the selective iNOS inhibitor S-methylisothiourea (SMT) improved myocardial function and reduced infarct size in rats. Moreover, in iNOS-knockouted mice, both myocardial iNOS messenger ribonucleic acid (mRNA) expression and depression of contractility after LAD ligation were attenuated (Feng et al. 2001). Mechanisms of the deleterious effects of NO have been reported to be linked with acceleration of apoptosis (Kawaguchi et al. 1997), inhibition of mitochondrial respiration and stimulation of mitochondrial production of radicals (Brown and Borutaite 2002), however, others reported the opposite proapoptotic effect of inhibition of NOS in isolated rat hearts subjected to I/R (Weiland et al. 2000).

On the other hand, in our study, protective effect of L-NAME was observed only in the non-adapted hearts, whereas in the hearts subjected to brief adaptation by IP, inhibition of NOS blocked protective effect of IP on the systolic and diastolic cardiac alterations, improved recovery of CF and reduction in the incidence of severe ventricular arrhythmias. These results suggest that limitation of NO production during brief episodes of ischemia preceding a prolonged ischemic insult also suppressed this important mechanism of cardioprotection that was triggered by preconditioning. Our results are in accordance with those demonstrated that in the rabbit myocardium, brief I/R enhanced production of NO (Xuan et al. 2000), and NOS inhibitor L-NNA prevented infarct size-limiting effect of IP (Williams et al. 1995). These findings indicate that NO produced during episodes of IP is an important player in the mechanisms of attenuation of injury during subsequent sustained ischemia. The latter raises a question of whether or not IP would be effective in some pathological conditions when NO production is impaired, e.g., due to endothelial dysfunction. However, whereas some authors have demonstrated that IP is a healthy heart phenomenon (Ferdinandy 2004) and a failure to precondition diseased animal (Juhasz et al. 2004) and human diabetic or failing heart (Ghosh et al. 2001), others reported that a potential for endogenous self-protective mechanisms to exert a beneficial action was preserved even in patients with severe heart failure (Kitakaze et al. 1999). Moreover, IP itself has been shown to ameliorate endothelial dysfunction (Pagliaro et al. 2003) and improve postischemic endothelium-dependent vascular response by a mechanism that involved enhanced NO generation (Beresewicz et al. 2004). The source of NO in the early phase of IP appears to be endothelial isoform of NO synthase (eNOS), since cardioprotection by IP was blocked by a non-selective L-NNA, but not by a selective inhibitor of iNOS SMT (Bolli et al. 1997b). NO generated during brief ischemia might play a signaling role producing peroxynitrite which in turn activates protein kinase Cε and triggers signaling cascades including tyrosine kinase and mitogen-activated protein kinases (MAPKs; Bolli 2001). In addition, peroxynitrite itself can exert cardioprotective effects and at low (nanomolar) concentrations effectively suppress the incidence of VF after I/R in the rat heart (Sedat et al. 1999) and induce vasorelaxation in the human coronary arteries (Ku et al. 1995) and isolated rat aortic rings (Lefer et al. 1997).

Many studies have demonstrated that basal endogenous NO is an important vasodilator and NOS inhibition could increase vascular tone and thus reduce CF

(Lieberthal et al. 1991; Nassem et al. 1995). In our study, blockade of NO production by administration of L-NAME significantly decreased CF in the adapted hearts after sustained ischemia. On the other hand, in the non-adapted hearts, L-NAME administration increased CF after I/R, suggesting different regulatory response of vascular tone to NO under different experimental conditions. The latter could be probably related to different amounts of NO and peroxynitrite generated during brief *vs.* prolonged I/R (Wang and Zweier 1996; Yasmin et al. 1997; Wang et al. 1999), as well as to involvement of different isoforms of NOS (eNOS and iNOS) activated during the timecourse of I/R (Banerjee et al. 1999; Bolli 2001). Opposite effect of L-NAME administration on the CF recovery in the non-preconditioned hearts might also confirm that during sustained ischemia, NO plays a detrimental role and contributes to myocardial injury upon reperfusion, possibly *via* impaired regulation of vascular tone due to development of endothelial dysfunction (Tsao and Lefer 1990). It can also be proposed that NO plays a positive role in IP by its vasodilatation effect that enables better postischemic recovery of the preconditioned hearts *via* an increased oxygen and substrate delivery.

In conclusion, our results suggest that NO plays a dual role in the rat heart and besides its deleterious effects and negative role in the normal non-adapted heart subjected to I/R alone, it might also play a positive role being involved in the mechanisms of protection triggered by cardiac adaptation by IP.

Acknowledgements. The experimental work has been supported by the grants VEGA Slovakia No. 2/5110/25, SP51/0280900/0280901, SP51/0280800/0280802 and APVT 51-027404. The authors wish to thank Mrs. Iveta Blažíčková and Mrs. Iveta Formánková for their expert technical assistance and Dr. Ivan Gabauer and Dr. Tomáš Jankovič for the assistance with the preparation of the manuscript.

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Final version accepted: October 27, 2005