Physiol. Res. 50: 143-152, 2001

Effect of ACE Inhibitor Captopril and L-Arginine on the Metabolism and on Ischemia-Reperfusion Injury of the Isolated Rat Heart

J. DIVIŠOVÁ, H. VAVŘÍNKOVÁ, M. TUTTEROVÁ, L. KAZDOVÁ, E. MESCHIŠVILI

Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Received March 10, 2000
Accepted July 20, 2000

Summary

We investigated the effects of *in vivo* treatment with the angiotensin-converting enzyme inhibitor (ACE-I) captopril and/or of *in vitro* administration of L-arginine on the metabolism and ischemia-reperfusion injury of the isolated perfused rat myocardium. Captopril (50 mg/l in drinking water, 4 weeks) raised the myocardial content of glycogen. After 25-min global ischemia, captopril treatment, compared with the controls, resulted in lower rates of lactate dehydrogenase release during reperfusion (8.58±1.12 vs. 13.39±1.88 U/heart/30 min, $p<0.05$), lower myocardial lactate contents (11.34±0.93 vs. 21.22±4.28 μmol/g d.w., $p<0.05$) and higher coronary flow recovery (by 25 %), and prevented the decrease of NO release into the perfusate during reperfusion. In control hearts L-arginine added to the perfusate (1 mmol/l) 10 min before ischemia had no effect on the parameters evaluated under our experimental conditions, presumably because of sufficient saturation of the myocardium with L-arginine. In the hearts of captopril-treated rats, L-arginine further increased NO production during reperfusion and the cGMP content before ischemia. Our results have shown that long-term captopril treatment increases the energy potential and has a beneficial effect on tolerance of the isolated heart to ischemia. L-arginine added into the perfusate potentiates the effect of captopril on the NO signaling pathway.

Key words

ACE inhibitors • L-arginine • Nitric oxide • Cyclic GMP • Isolated rat heart • Ischemia-reperfusion injury

Introduction

Angiotensin-converting enzyme inhibitors (ACE-I) are well-known as antihypertensive agents. In addition, they exert several cardioprotective effects such as prevention and regression of left ventricular hypertrophy or reduction of infarct size in both clinical and experimental studies, and may represent an important therapeutic approach in the treatment of congestive heart failure (Awan *et al.* 1981, Lund-Johansen and Omvik 1993, Pecháňová *et al.* 1997, Šimko and Šimko 1999). However, the mechanism of the beneficial effect of ACE-I is not fully understood.

Fax+4202 24920590
E-mail physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

ACE-I have been shown to interfere not only with the renin-angiotensin system through inhibition of angiotensin II production, but also with the kallikrein-kinin system through attenuation of endogenous bradykinin degradation by inhibiting kininase II, an enzyme identical with angiotensin-converting enzyme (Baumgarten *et al.* 1993, Hartman 1995). Bradykinin binding to B_2 kinin receptors has been demonstrated to activate several intracellular messenger cascades (Linz *et al.* 1995), one of them being the nitric oxide synthase (NOS) system, which produces the short-lived signaling compound nitric oxide (NO) (Wiemer *et al.* 1991). By activating soluble guanylate cyclase NO raises the levels of cyclic guanosine 3′,5′-monophosphate (cGMP) in target cells (Wiemer *et al.* 1991).

Most studies dealing with the effects of ACE-I on the isolated myocardium have focused on functional parameters (for review see Heusch *et al.* 1997), but only a few on metabolism, in particular on energy reserves. Moreover, ACE-I are often used in non-physiological doses *in vitro*, thereby eliminating the possibility of the evaluation of long-term effects. Therefore, we have chosen chronic treatment with low captopril doses and studied its effects on myocardial metabolism, namely ATP and glycogen content, under basal conditions and during ischemia in normotensive rats. As NO and cGMP is a part of the mode of captopril action that may participate in the myocardial metabolic status, we sought to enhance NO production directly by administering L-arginine, the substrate for NO synthesis, into the perfusate.

Controversial data regarding the effect of L-arginine on the isolated myocardium have been reported (Wang *et al.* 1997, Takeuchi *et al.* 1995). However, no information is available on the effect of L-arginine on energy metabolism. We therefore assessed myocardial metabolic parameters under basal conditions and also in the heart with ischemic injury after administration of L-arginine *in vitro* without previous ACE-I treatment.

The aims of the present study were (1) to examine the effects of long-term captopril treatment on myocardial metabolism and ischemic tolerance in normal rats, (2) to test whether the effects of captopril can be further enhanced by stimulation of NO production induced by concomitant *in vitro* L-arginine administration, and (3) to clarify the controversial effect of L-arginine alone on myocardial metabolism and ischemia-reperfusion injury.

Methods

Animals

The study was carried out on adult male Wistar rats weighing 330-420 g. The animals were fed standard chow and water *ad libitum*. Half of them were administered the ACE-I captopril (Sigma, St. Louis, USA) in a dose of about 2.5 mg/kg body weight per day (in drinking water, 50 mg/l, free access) for 4 weeks prior to decapitation. At the beginning of the experiments and prior to decapitation, blood pressure was measured by tail-cuff plethysmography in control animals and captopril-treated groups.

Perfusion of isolated hearts

After decapitation under ether anesthesia, the hearts were removed and rapidly placed into ice-cool saline. Once the heart had stopped beating, the aorta was connected to a perfusion system. Each heart was perfused retrogradely under constant pressure (60 mm Hg) with oxygenated (equilibrated with 95 % O_2 + 5 % CO_2) non-recirculating Krebs-Henseleit bicarbonate solution (KRB), at pH 7.4 and 37 °C according to Langendorff (1895). The perfusate contained (in mmol/l): 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25.0 $NaHCO_3$, 0.5 Na-EDTA, 5.5 glucose. The stabilization period lasted 20 min and was followed by a 10-min period during which the hearts were perfused with either KRB or with KRB containing L-arginine (Sigma, St. Louis, USA) at a final concentration of 1 mmol/l. This was followed by 25-min global (no-flow) ischemia and 30-min retrograde reperfusion. The hearts were maintained at 37 °C in a heated-water jacketed chamber throughout the perfusion period. Coronary flow was measured by timed coronary effluent collection. Coronary effluent was used for further analysis. Lactate dehydrogenase was assessed as total amount of enzyme released into the perfusate during 30 min of reperfusion, lactate release was determined at minutes 1 and 2 of reperfusion and NO production in the perfusate was evaluated 1 min before ischemia and at 5 min of reperfusion. The whole hearts prior to ischemia, at the end of ischemia, and after 30-min reperfusion were frozen in liquid nitrogen using Wollenberger forceps,

then weighed and the ventricles were used for further analysis.

Chemical analysis of coronary effluent

Lactate dehydrogenase (L-lactate: NAD^+-oxidoreductase EC 1.1.1.27) in the medium was determined by a standard enzymatic procedure based on the reduction of NAD to NADH (Mosinger *et al.* 1978) as a test of myocardial injury. Lactate was determined by spectrophotometric assay using lactate dehydrogenase (Hohorst 1963). NO production in the perfusate was evaluated as the concentrations of stable NO metabolites NO_2^-/NO_3^- measured using the Griess reagent (Green *et al.* 1982) with minor modifications. Briefly, samples were incubated for 1 hour at room temperature with nitrate reductase (0.1 U/100 µl, *Aspergillus* species, Boehringer Mannheim GmbH, Germany) in the substrate buffer (pH 7.8) containing imidazole, NADPH, and FAD to convert all NO_3^- to NO_2^-. Total NO_2^- ($NO_2^- + NO_3^-$) was then analyzed by means of Griess reagent and absorbances were measured at 543 nm using a UV/VIS spectrophotometer (Secomam, France). Concentrations of NO_2^- in the samples were determined from the standard curve of $NaNO_2$. Nitrate recovery was 97 %.

Heart tissue biochemistry

Frozen heart ventricles were powdered in liquid nitrogen and ATP was determined in a neutralized perchloric acid extract by a spectrophotometric assay using hexokinase and glucose-6-phosphate-dehydrogenase (Lamprecht and Trautschold 1963). In the same extract, lactate was determined by a spectrophotometric assay using lactate dehydrogenase (Hohorst 1963) and cGMP was assessed using a radioimmunoassay kit (Immunotech, France). Glycogen was estimated enzymatically in the chloroform-methanol sediment after acid hydrolysis as glucose using the glucose oxidase method (Huggett and Nixon 1957).

Statistical analysis

Results are given as means ± S.E.M. Differences between the groups were evaluated by ANOVA and Tukey-Kramer multiple comparisons test. Differences were considered statistically significant at the level of $p<0.05$.

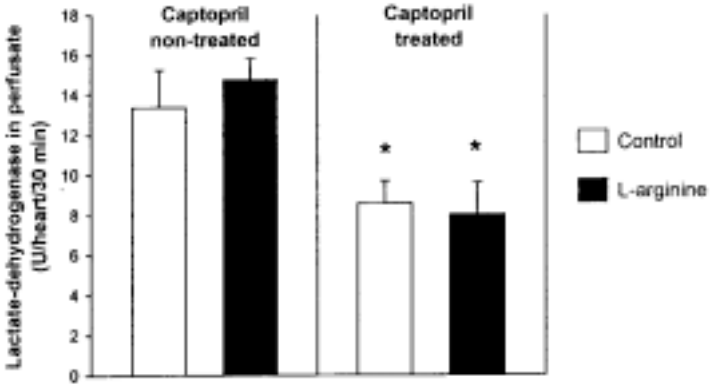

Fig. 1. *Effect of captopril in vivo and/or L-arginine in vitro (1 mM) on the lactate dehydrogenase released into coronary effluent during 30-min postischemic reperfusion. Statistical significance: * p<0.05 vs. non-treated groups. Data are means ± S.E.M., n = 5-7 hearts per group.*

Results

The dose of captopril used resulted in a decrease in blood pressure (Captopril: 123±1 (n=20) vs. Controls: 148±2 mm Hg (n=16), p<0.0001), but did not affect the absolute heart weight (Captopril: 1.589±0.032 (n=11) vs. Controls: 1.552±0.059 g (n=12), N.S.) or the heart weight/body weight (HW/BW) ratio (Captopril: 0.0039±0.0001 vs. Controls: 0.0041±0.0001, N.S.).

Parameters of ischemia-reperfusion injury

Ischemia-reperfusion injury of the heart was evaluated both by measuring the release of lactate dehydrogenase into the coronary effluent during reperfusion, by determining the myocardial lactate content before ischemia, at the end of ischemia and at the end of reperfusion and by measuring coronary flow recovery. The total lactate dehydrogenase released into the perfusate during reperfusion is shown in Figure 1. Four-week captopril administration decreased lactate dehydrogenase release to almost 64% of the control group level (Captopril: 8.58±1.12 vs. Controls: 13.39±1.88 U/heart/ 30 min, p<0.05). Addition of L-arginine into the perfusate had no effect on the above parameter (14.77±1.05 U/heart/30 min), even when

combined with long-term captopril administration (8.01±1.61 U/heart/ 30 min).

After global ischemia, the myocardial lactate content rose (Fig. 2) to the same level in all experimental groups (Controls: 148.6±9.5, L-arginine: 132.0±4.9, Captopril: 147.0±5.9, Captopril + L-arginine: 169.0±3.0 μmol/g d.w., N.S.). At the end of reperfusion, the lactate content was significantly lower in captopril-treated groups than in those non-treated (Captopril: 11.3±0.9 vs. Controls: 21.5±3.0, Captopril + L-arginine: 9.3±1.0 vs. L-arginine: 23.3±4.7 μmol/g d.w., $p<0.05$). Lactate is supposed to have been washed out more effectively in the captopril-treated groups. This explanation is supported by the measurement of lactate released from the heart into the coronary effluent in the first two minutes of reperfusion (not shown) that was amplified in captopril-treated rats (Captopril: 34.9±0.9 vs. Controls: 29.7±1.2 μmol/2 min, $p<0.01$). Moreover, the beneficial effect of captopril is manifested by improved coronary flow recovery, which could also contribute to the enhanced lactate washout. At minute 5, 15 and 30 of reperfusion, coronary flow recovered to 121.0±7.7, 110.0±6.0 and 107.5±5.7% in the captopril-treated group vs. 94.2±8.5, 86.4±8.5 and 81.9±7.8% in the controls ($p<0.05$). Preischemic infusion of L-arginine had no effect on postischemic coronary flow recovery, even when combined with captopril. Baseline values of coronary flow (not shown) did not differ significantly either after captopril treatment or after addition of L-arginine into the perfusate (Controls: 14.4±0.6, L-arginine: 13.8±0.5, Captopril: 12.5±0.5, Captopril + L-arginine: 13.4±0.8 ml/min, N.S.).

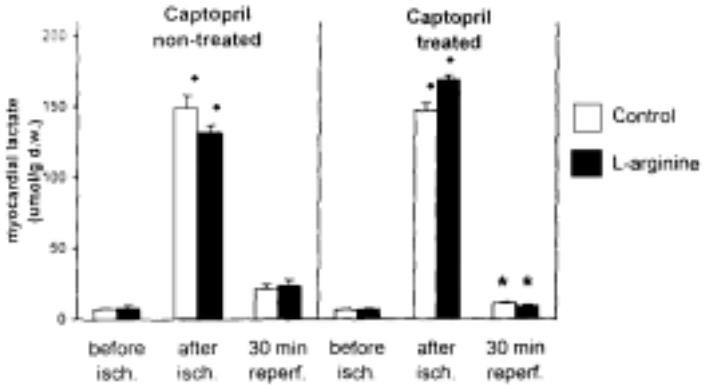

Fig. 2. *Effect of captopril in vivo and/or L-arginine in vitro (1 mM) on myocardial lactate content before ischemia and after 25-min ischemia (isch) and after 30-min reperfusion (reperf). Statistical significance: * $p<0.05$ vs. non-treated groups; ♦ $p<0.0001$ vs. preischemic levels; Data are means ± S.E.M., n = 3-8 hearts per group.*

Table 1. Effect of captopril *in vivo* and/or L-arginine *in vitro* (1 mM) on myocardial ATP and glycogen contents.

	Controls	**L-arginine**	**Captopril**	**Captopril + L-arginine**
Preischemia				
ATP	19.0±0.5 (10)	21.4±0.8 (10)	21.0±0.7 (7)	21.3±1.0 (6)
Glycogen	91.7±4.1 (7)	107.6±2.3 (6)	119.0±6.1 (6)**	112.0±5.9 (6)*
After 30-min reperfusion				
ATP	8.0±1.0 (5)	8.7±1.6 (5)	7.7±0.8 (7)	7.0±0.6 (8)
Glycogen	31.2±3.5 (5)	27.0± .9 (5)	25.6±1.4 (7)	25.5±2.7 (6)

*Data (in μmol/g d.w.) are given as means ± S.E.M., number of hearts per group is given in parentheses. Significantly different from the control group: * $p<0.05$, ** $p<0.01$.*

ATP and glycogen contents

As is shown in Table 1, long-term captopril administration increased the myocardial glycogen content and also tended to increase the myocardial ATP content (significant only when the captopril group was compared with controls using the t-test, $p = 0.034$). L-arginine

administered into the perfusate had no significant effects. The differences observed in the heart before ischemia were no longer present at the end of reperfusion.

Release of nitric oxide and myocardial cGMP content

The amount of NO released into the coronary effluent in the stabilized state before ischemia did not differ significantly between the groups (Fig. 3). At minute 5 of postischemic reperfusion, there was a decrease of NO release in the hearts of rats not receiving captopril; L-arginine likewise did not lead to improved NO release. By contrast, NO release from the hearts of captopril-treated rats was not attenuated after ischemia, and the addition of L-arginine into the perfusate still enhanced NO production (Captopril: 184.2±23.0 nmol/min vs. Captopril + L-arginine: 304.0±28.1 nmol/min, $p<0.01$).

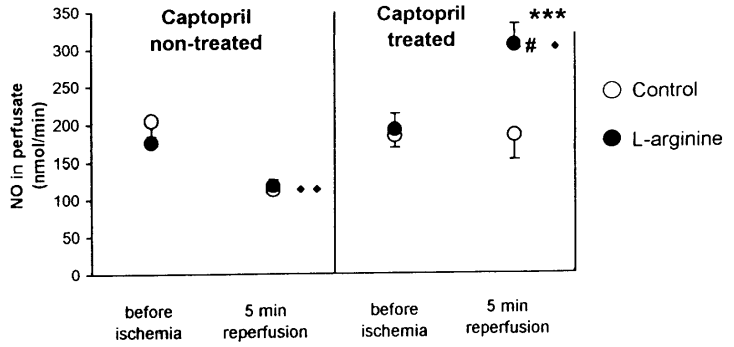

Fig. 3. *Effect of captopril in vivo and/or L-arginine in vitro (1 mM) on NO release into coronary effluent before ischemia and at minute 5 of postischemic reperfusion.* *** *$p<0.001$ vs. non-treated groups;* ♦ *$p<0.02$,* ♦♦ *$p<0.01$ vs preischemic levels;* # *$p<0.01$ vs. Captopril group without L-arginine. Data are means ± S.E.M., n = 4-7 hearts per group.*

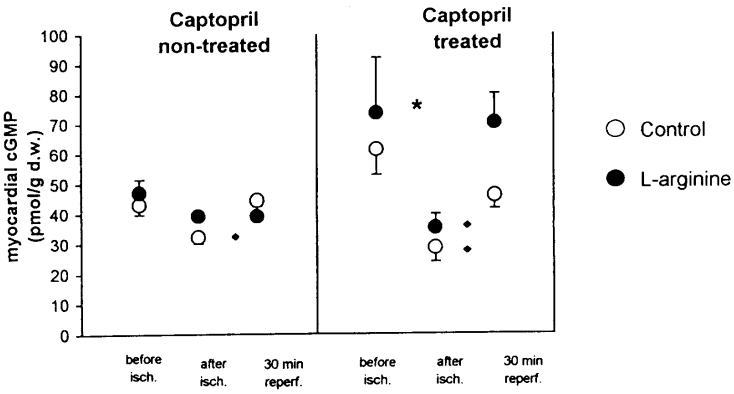

Fig. 4. *Effect of captopril in vivo and/or L-arginine in vitro (1 mM) on myocardial cGMP content before and after 25-min ischemia (isch) and after 30-min reperfusion (reperf).* * *$p<0.05$ vs. non-treated group;* ♦ *$p<0.05$ vs. preischemic levels. Data are means ± S.E.M., n = 2-8 hearts per group.*

As is shown in Figure 4, the cGMP content in the equilibrated heart before ischemia was slightly increased in the group of rats receiving captopril for 4 weeks (61.3±8.5 pmol/g d.w.), but it was significantly higher only when captopril treatment was combined with the addition of L-arginine into the perfusate (73.5±18.5 vs. Controls: 43.1±3.3 pmol/g d.w., $p<0.05$). The addition of L-arginine into the perfusate of the controls did not result in a significant increase. The 25-min ischemia was associated with a reduction in cGMP content in all the studied groups. It is evident that the effect of captopril promoting cGMP production was no longer present at the end of 30-min reperfusion (45.8±4.3 pmol/ g d.w.), whereas it persisted when captopril was combined with L-arginine (70.2±9.7 pmol/g d.w.). However, this point cannot be statistically evaluated because of an insufficient number of samples.

Discussion

In the present study, we investigated the effects of either long-term *in vivo* treatment with the ACE-I captopril or acute *in vitro* administration of L-arginine on metabolic parameters and ischemia-reperfusion injury in isolated hearts of normotensive rats. As the common denominator of both ACE-I treatment and L-arginine administration is the activation of the NO pathway in the heart, we also evaluated the effects of a combination of

both stimuli. While numerous studies have been designed to monitor the effects of ACE-I on the myocardium (Schölkens *et al.* 1988, Massoudy *et al.* 1994), only a few have assessed the action of long-term ACE-I administration *in vivo* on the metabolism and function of the isolated heart (Werrmann and Cohen 1994). We have chosen a model of the heart perfused according to Langendorff under constant pressure because it eliminated changes in NOS activity due to shear stress (Curtis and Ellwood 1998, Vavřínková *et al.* 1999).

The four-week treatment with captopril improved myocardial resistance to ischemia as was confirmed by the decreased lactate dehydrogenase release into the coronary effluent during postischemic reperfusion. Captopril treatment lowered the myocardial lactate content at the end of reperfusion. It is supposed that the reduced accumulation of lactate in the captopril-treated groups was a consequence of its facilitated washout due to improved coronary flow recovery. It was not possible to measure other functional parameters under our experimental conditions, but there is also convincing evidence of a beneficial effect of ACE-I on the functional parameters of ischemia-reperfusion injury (Werrmann and Cohen 1994, Massoudy *et al.* 1994). The beneficial effect of ACE-I on ischemia-reperfusion injury of the rat heart was also demonstrated after the addition of another ACE-I (ramipril) into the perfusate or after a single oral dose one hour prior to decapitation (Schölkens *et al.* 1988). The results of our study have demonstrated that the changes induced *in vivo* by captopril also persisted in the isolated myocardium. This finding could possibly be explained by protracted binding of captopril to the angiotensin-converting enzyme which is persisting even in the isolated heart (Brown *et al.* 1998). Apart from inhibiting angiotensin II production, this binding results in a reduced degradation of bradykinin which is released in the heart by the local kallikrein-kinin system (Nolly *et al.* 1994). Inhibition by ACE-I leads to increased NO formation through bradykinin-mediated NOS activation (Wiemer *et al.* 1991), as we demonstrated during postischemic myocardial reperfusion, where captopril prevented the decrease in NO production. Enhanced production of vasorelaxant NO may have contributed to improved coronary flow recovery during reperfusion and may also have been associated with increased supply of O_2 into the tissue and with the elimination of anaerobic metabolic products. A protective role may also be played by reduced O_2 consumption, demonstrated after ACE-I administration in the isolated heart (Zhang *et al.* 1997). A certain role in the improved myocardial ischemic tolerance may also have been played by the increased cGMP level prior to ischemia, that can reduce Ca^{2+} entry into cells (Méry *et al.* 1991) associated with ischemic injury.

In our experiments, captopril administration raised the glycogen content and tended to increase the ATP content in perfused hearts under basal conditions. This is consistent with the results published by Gohlke *et al.* (1994) who reported increased ATP and glycogen levels after chronic administration of the ACE-I perindopril in "stroke-prone" hypertensive rats. The increased glycogen content observed after captopril administration could be a consequence of enhanced glucose uptake. A number of studies have shown that ACE-I or bradykinin enhance tissue glucose uptake (Henriksen and Jacob 1995). Another mechanism by which the ACE-I could have raised the content of ATP and glycogen is their cleavage controlled by catecholamines. It is just the release of neurotransmitters from the nerve endings and their myocardial uptake which are affected by the ACE-I (Kawai *et al.* 1999). This is under the inhibitory control of endogenous NO (Schwarz *et al.* 1995).

NO formation may be enhanced by ACE-I as a result of increased bradykinin levels (Wiemer *et al.* 1991). In our study, long-term captopril administration prevented the postischemic decrease of NO release into the perfusate, which was noted in the control group. A similar reduction in NO release in the same phase of reperfusion was reported by Maulik *et al.* (1996). It has been suggested that this was due to a major decrease in NOS activity during ischemia (70-90% reduction) (Giraldez *et al.* 1997, Wang *et al.* 1997).

An important role in the NO signaling pathway is played by cGMP. Compared with the controls, the cGMP content in the heart before ischemia was elevated after the treatment with captopril, especially when potentiated by L-arginine administration. A rise in the cGMP content after long-term administration of ramiprilat was also detected in the aortas of SHR rats (Gohlke *et al.* 1993). In our experiments, the cGMP content declined at the end of 25-min ischemia. Similar results were reported by Maulik *et al.* (1996) and Mizuno *et al.* (1998). On the contrary, Depré and Hue (1994)

noted an increase in the cGMP content in isolated hearts after 10-min ischemia. The cGMP content in the heart, which is varying during ischemia, depends on the duration of ischemia and on the status of the myocardium. This might be related to the fact that cGMP can be affected by bradykinin activation, which occurs at the onset of ischemia, but declines as early as in minute 15 of ischemia (Baumgarten *et al.* 1993).

In our experiments, attention was also paid to the effects of L-arginine, the substrate for NO synthesis. It had no significant effect either on ATP and glycogen contents or on myocardial tolerance to ischemia. This finding is consistent with that reported by Masini *et al.* (1999). Neither did it increase NO formation or myocardial cGMP content before ischemia. Similarly, Takeuchi *et al.* (1995) did not find increased NO release following the addition of L-arginine into the perfusate. In our experiments, L-arginine did not enhance NO release and myocardial cGMP content even during reperfusion. By contrast, Maulik *et al.* (1996) described increased NO release and Mizuno *et al.* (1998) reported an elevated myocardial cGMP content during reperfusion in the presence of L-arginine. These controversial findings can presumably be explained by differences in the model used. Maulik *et al.* (1996) and Mizuno *et al.* (1998) used the working heart, whereby the coronary arteries are affected by shear stress, contrary to our model of non-working perfusion (Pinsky *et al.* 1997). The changes in NOS activity produced by shear stress (Curtis and Ellwood 1998, Vavřínková *et al.* 1999) were only negligible in our experiments, hence the consumption of L-arginine may have been lower than in the working models. The low consumption of L-arginine and its high cellular concentrations (200-800 μM) (Baydoun *et al.* 1990), which are several times above the K_m for NOS (35 μM; Mayer *et al.* 1991), explain why a further supply of L-arginine did not result in any additional changes.

Our results indicate that the situation is different in the hearts of captopril-treated rats. In this case, the addition of L-arginine to the perfusate increased NO release into the perfusate at minute 5 of reperfusion. In a similar situation, Vavřínková *et al.* (2001) found an increased NO content directly in the myocardium using electron-spin resonance. It can reasonably be assumed that NOS activity and hence also the consumption of L-arginine were higher in the hearts of rats treated by captopril. Consequently, there may have occurred a local deficiency of L-arginine, which may also take place at the site of NO synthesis *in vivo* (Huk *et al.* 1997). Some results indicated a beneficial effect of exogenous supply of L-arginine that can only be observed in a situation of its local deficiency (Gold *et al.* 1990). Another possible contribution of L-arginine to NO production enhancement is the fact that the NOS enzyme, bound to substrate in excess, is more resistant to its deactivation during ischemia (Huk *et al.* 1997). Last but not least, it should also be mentioned that at suboptimal concentrations of L-arginine the complex NOS enzyme catalyzes the generation of superoxide radicals instead of NO formation (Pou *et al.* 1992).

It can thus be summarized that the effect of long-term administration of the angiotensin-converting enzyme inhibitor captopril to normotensive rats persists *in vitro* and improves some metabolic parameters in the isolated rat heart, enhances its energy potential and increases its tolerance to ischemia. Addition of L-arginine to the perfusate had no effect on the parameters of myocardial ischemia-reperfusion injury except for potentiating the effect of captopril on NO release during reperfusion and the cGMP content before ischemia. Under basal conditions, the myocardium in our non-working heart model was presumably sufficiently saturated with L-arginine and its increased availability was not effective except when captopril had activated the NO pathway.

Acknowledgements

This study was supported by grant No. 4201-3 (Internal Grant Agency of the Ministry of Health of the Czech Republic) and by VZ/CEZ:L17/98:00023001.

References

AWAN NA, EVENSON MK, NEEDHAM KE, WIN A, MASON DT: Efficacy of oral angiotensin-converting enzyme inhibition with captopril therapy in severe chronic normotensive congestive heart failure. *Am Heart J* **101:** 21-31, 1981.

BAUMGARTEN CR, LINZ W, KUNKEL G, SCHÖLKENS BA, WIEMER G: Ramiprilat increases bradykinin outflow from isolated hearts of rat. *Br J Pharmacol* **108:** 293-295, 1993.

BAYDOUN AR, EMERY PW, PEARSON JD, MANN GE: Substrate-dependent regulation of intracellular amino acid concentrations in cultured bovine aortic endothelial cells. *Biochem Biophys Res Commun* **173:** 940-948, 1990.

BROWN NJ, VAUGHAN DE: Angiotensin-converting enzyme inhibitors. *Circulation* **97:** 1411-1420, 1998.

CARVALHO CRO, THIRONE ACP, GONTIJO JAR, VELLOSO LA, SAAD MJA: Effect of captopril, losartan, and bradykinin on early steps of insulin action. *Diabetes* **46:** 1950-1957, 1997.

CURTIS MJ, ELLWOOD AJ: The role of shear stress-independent release of nitric oxide in mediating drug-induced coronary vasodilation can be assessed in the constant pressure Langendorff preparation. *Med Sci Res* **26:** 79-81, 1998.

DEPRÉ C, HUE L: Cyclic GMP in the perfused rat heart. Effect of ischaemia, anoxia and nitric oxide synthase inhibitor. *FEBS Lett* **345:** 241-245, 1994.

GIRALDEZ RR, PANDA A, XIA Y, SANDERS SP, ZWEIER JL: Decreased nitric-oxid synthase activity causes impaired endothelium-dependent relaxation in the postischemic heart. *J Biol Chem* **272:** 21420-21426, 1997.

GOHLKE P, LAMBERTY V, KUWER I, BARTENBACH S, SCHNELL A, LINZ W, SCHÖLKENS BA, WIEMER G, UNGER T: Long-term low-dose angiotensin converting enzyme inhibitor treatment increases vascular cyclic guanosine 3',5'-monophosphate. *Hypertension* **22:** 682-687, 1993.

GOHLKE P, KUWER I, BARTENBACH S, SCHNELL A, UNGER T: Effect of low-dose treatment with perindopril on cardiac function in stroke-prone spontaneously hypertensive rats: role of bradykinin. *J Cardiovasc Pharmacol* **24:** 462-469, 1994.

GOLD ME, WOOD KS, BYRNS RE, BUGA GM, IGNARRO LJ: L-arginine-dependent vascular smooth muscle relaxation and cGMP formation. *Am J Physiol* **259:** H1813-H1821, 1990.

GREEN L, WAGNER D, GLOGOWSKI I, SKIPPER P, WISHNOK I, TANNENBAUM S: Analysis of nitrate, nitrite and [^{15}N] nitrate in biological fluids. *Anal Biochem* **126:** 131-138, 1982.

HARTMAN JC: The role of bradykinin and nitric oxide in the cardioprotective action of ACE inhibitors. *Ann Thorac Surg* **60:** 789-792, 1995.

HENRIKSEN EJ, JACOB S: Effects of captopril on glucose transport activity in skeletal muscle of obese Zucker rats. *Metabolism* **44:** 267-272, 1995.

HEUSCH G, ROSE J, EHRING T: Cardioprotection by ACE inhibitors in myocardial ischaemia/reperfusion. *Drugs* **54** (Suppl 5)**:** 31-41, 1997.

HOHORST HJ: L-lactate determination with lactic dehydrogenase and DPN. In: *Methods of Enzymatic Analysis*. HU BERGMEYER (ed), Academic Press, New York, 1963, pp 266-270.

HUGGETT ASG, NIXON DA: Use of glucose oxidase, peroxidase, and o-dianisidine in determination of blood and urinary glucose. *Lancet* **1:** 368-370, 1957.

HUK I, NANOBASHVILI I, NEUMAYER C, PUNZ A, MUELLER M, AFKAMPOUR K, MITTLBOEK M, LOSERT U, POLTERAUER P, ROTH E, PATTON S, MALINSKI T: L-arginine treatment alters the kinetics of nitric oxide and superoxide release and reduces ischemia/reperfusion injury in skeletal muscle. *Circulation* **96:** 667-675,1997.

KAWAI H, FAN T, DONG E, SIDDIQUI RA, YATANI A, STEVENS SY, LIANG CS: ACE inhibition improves cardiac NE uptake and attenuates sympathetic nerve terminal abnormalities in heart failure. *Am J Physiol* **277:** H1609-H1617, 1999.

LAMPRECHT W, TRAUTSCHOLD J: Adenosine-5-triphosphate determination with hexokinase and glucose-6-dehydrogenase. In: *Methods of Enzymatic Analysis*. HU BERGMEYER (ed), Academic Press, New York, 1963, pp 543-551.

LANGENDORFF O: Untersuchungen am überlebenden Saugentiereherzen. *Pflügers Arch* **61:** 291-332, 1895.

LINZ W, WIEMER G, SCHÖLKENS BA: ACE inhibition induces NO formation in cultured bovine endothelial cells and protects isolated ischemic rat hearts. *J Mol Cell Cardiol* **24:** 909-919, 1992.

LINZ W, WIEMER G, GOHLKE P, UNGER T, SCHÖLKENS BA: Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacol Rev* **47:** 25-49, 1995.

LUND-JOHANSEN P, OMVIK P: Cardiac effects of ACE inhibition. *J Cardiovasc Pharmacol* **22** (Suppl 1): S36-S40, 1993.

MASINI E, SALVEMINI D, NDISANG JF, GAI P, BERNI L, MONCINI M, BIANCHI S, MANNAIONI PF: Cardioprotective activity of endogenous and exogenous nitric oxide on ischemia reperfusion injury in isolated guinea pig hearts. *Inflamm Res* **48:** 561-568, 1999.

MASSOUDY P, BECKER BF, GERLACH E: Bradykinin accounts for improved postischemic function and decreased glutathione release of guinea pig heart treated with the angiotensin-converting enzyme inhibitor ramiprilat. *J Cardiovasc Pharmacol* **23:** 632-639, 1994.

MAULIK N, ENGELMAN DT, WATANABE M, ENGALMAN RM, DAS DK: Nitric oxide - a retrograde messenger for carbon monoxide signaling in ischemic heart. *Mol Cell Biochem* **157:** 75-86, 1996.

MAYER B, JOHN M, BÖHME E: Partial purification and characterization of a Ca^{2+}/calmodulin-dependent endothelium-derived relaxing factor-forming enzyme from porcine cerebellum. *J Cardiovasc Pharmacol* **17** (Suppl 3): S46-S51, 1991.

MÉRY PF, LOHMANN SM, WALTER U, FISCHMEISTER R: Ca^{2+} current is regulated by cyclic GMP-dependent protein kinase in mammalian cardiac myocytes. *Proc Natl Acad Sci USA* **88:** 1197-1201, 1991.

MIZUNO T, WATANABE M, SAKAMOTO T, SUNAMORI M: L-arginine, a nitric oxide precursor, atenuates ischemia-reperfusion injury by inhibiting inositol-1,4,5-triphosphate. *J Thorac Cardiovasc Surg* **115:** 931-936, 1998.

MOSINGER B, STEJSKAL J, TUTTEROVÁ M, VAVŘÍNKOVÁ H: Myocardial lesions induced by natural catecholamines in vitro. *Eur J Cardiol* **6:** 389-402, 1978.

NOLLY H, CARBINI LA, SCICLI G, CARRETERO OA, SCICLI AG: A local kallikrein-kinin system is present in rat hearts. *Hypertension* **23:** 919-923, 1994.

PECHÁŇOVÁ O, BERNÁTOVÁ I, PELOUCH V, ŠIMKO F: Protein remodelling of the heart in NO-deficient hypertension: the effect of captopril. *J Mol Cell Cardiol* **29:** 3365-3374, 1997.

PINSKY DJ, PATTON S, MESAROS S, BROVKOVYCH V, KUBASZEWSKI E, GRUNFELD S, MALINSKI T: Mechanical transduction of nitric oxide synthesis in the beating heart. *Circ Res* **81:** 372-379, 1997.

POU S, POU WS, BREDT DS, SNYDER SH, ROSEN GM: Generation of superoxide by purified brain nitric oxide synthase. *J Biol Chem* **267:** 24173-24176, 1992.

SCHÖLKENS BA, LINZ W, KÖNIG W: Effects of the angiotensin converting enzyme inhibitor, ramipril, in isolated ischaemic rat heart are abolished by a bradykinin antagonist. *J Hypertens* **6** (Suppl 4): S25-S28, 1988.

SCHWARZ P, DIEM R, DUN NJ, FORSTERMANN U: Endogenous and exogenous nitric oxide inhibits norepinephrine release from rat heart sympathetic nerves. *Circ Res* **77:** 841-848, 1995.

ŠIMKO F, ŠIMKO J: Heart failure and angiotensin converting enzyme inhibition: problems and perspectives. *Physiol Res* **48:** 1-8, 1999.

TAKEUCHI K, MCGOWAN FX, DANH H, GLYNN P, SIMPLACEANU E, NIDO PJ: Direct detrimental effects of L-arginine upon ischemia-reperfusion injury to myocardium. *J Mol Cell Cardiol* **27:** 1405-1414, 1995.

VAVŘÍNKOVÁ H, TUTTEROVÁ M, DIVIŠOVÁ J, STOPKA P, KAZDOVÁ L: Role of L-arginine administration on metabolism and ischemic resistance of the rat heart. *Physiol Res* **48** (Suppl 1): S134, 1999.

VAVŘÍNKOVÁ H, TUTTEROVÁ M, STOPKA P, DIVIŠOVÁ J, KAZDOVÁ L, DRAHOTA Z: Effect of captopril on nitric oxide formation and on mitochondrial respiratory chain radicals generation in rat heart. *Physiol Res* **50:** (in press) 2001.

WANG QD, MORCOS E, WIKLUND P, PERNOW J: L-arginine enhances functional recovery and Ca^{2+}-dependent nitric oxide synthase activity after ischemia and reperfusion in the rat heart. *J Cardiovasc Pharmacol* **29:** 291-296, 1997.

WERRMANN JG, COHEN SM: Comparison of effects of angiotensin-converting enzyme inhibition with those of angiotensin II receptor antagonism on functional and metabolic recovery in postischemic working rat heart as studied by [^{31}P] nuclear magnetic resonance. *J Cardiovasc Pharmacol* **24:** 573-586, 1994.

WIEMER G, SCHÖLKENS BA, BECKER RHA, BUSSE R: Ramiprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium-derived bradykinin. *Hypertension* **18:** 558-563, 1991.

ZHANG X, XIE Y-W, NASJLETTI A, XU X, WOLIN MS, THOMAS H, HINTZE TH: ACE-inhibitors promote nitric oxide accumulation to modulate myocardial oxygen consumption. *Circulation* **95:** 176-182, 1997.

Reprint requests

J. Divišová, Institute for Clinical and Experimental Medicine, Vídeňská 1958/9, 140 21 Prague 4, Czech Republic. Fax: 02/6108 3490 E-mail: jadi@medicon.cz