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The Effect of Captopril on Nitric Oxide Formation and on Generation of Radical Forms of Mitochondrial Respiratory Chain Compounds in Ischemic Rat Heart

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Summary

The increase of radical forms of mitochondrial respiratory chain compounds (MRCC) is an indicator of an increased risk of the formation of oxygen radicals. Using electron paramagnetic resonance (EPR), we found an increase of signals corresponding to ubisemichinone radical (·QH) and ironsulfur proteins radical forms (·FeS) of these respiratory chain compounds during ischemia in the isolated perfused rat heart (·QH increased from 1.51 to 3.08, $\cdot FeS_1$ from 1.14 to 2.65 arbitrary units). During the 5-min reperfusion, the signals returned to normoxic levels. In isolated mitochondria exposed to anoxia and reoxygenation the radical forms of ·QH and $\cdot FeS_2$ changed in a similar manner as in the intact heart. A combination of *in vivo* captopril treatment and *in vitro* L-arginine administration significantly decreased the levels of MRCC radicals in the isolated myocardium (·QH from 2.61 to 1.72 and $\cdot FeS_1$ from 1.82 to 0.46 under normoxia; ·QH from 4.35 to 2.66 and $\cdot FeS_1$ from 1.93 to 1.35 during ischemia). This decrease in MRCC radical forms was associated with increased NO levels in the perfusate, determined as NO_2^- / NO_3^-, as well as tissue NO levels determined using EPR as the dinitrosyl iron complex (DNIC). These results provide new information about the cardioprotective effects of ACE inhibitors and L-arginine.

Key words

ACE inhibitors • L-arginine • Mitochondrial radicals • Nitric oxide • Ischemia-reperfusion injury • Heart ischemia

Introduction

Angiotensin-converting enzyme (ACE) inhibitors, which have both an antihypertensive and a cardioprotective action, are commonly used in the treatment of hypertension and most forms of heart failure (Brunner *et al*. 1979, Kiowski *et al*. 1991, Konstam *et al*. 1992). The beneficial effects of ACE inhibitors were thought to be primarily due to the inhibition of angiotensin II formation. However, a number of clinical and experimental studies have indicated that an additional mechanism linked to the inhibition of bradykinin degradation is also important for the cardioprotective effect of ACE inhibitors (Hecker *et al*. 1993). Bradykinin

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accumulation on the endothelial surface increases NO production (Linz *et al*. 1995, Hartman 1995). Numerous studies have demonstrated that the stimulation of vascular endothelial NO production – apart from the well documented vasodilation – results in reduced oxygen consumption (Geng *et al*. 1992). This fact seems to contribute to the enhanced myocardial resistance to injury due to oxygen deficiency and to the beneficial effect on post-ischemic changes. Shen *et al*. (1995) observed that addition of bradykinin and S-nitroso-N-acetyl-penicillamin (SNAP) reduces oxygen consumption by skeletal muscle slices in normal dogs. Loke *et al.* (1999) reported a similar effect of bradykinin and the ACE inhibitor ramiprilat on slices of the human heart explanted at the time of transplantation. Poderoso *et al.* (1998) observed a 50 % decrease in myocardial oxygen consumption when the isolated rat heart was perfused with bradykinin (1.5-7.5 μmol/l).

The reduction in oxygen consumption by NO-modulated cells has been explained by the inhibitory action of NO on the mitochondria because incubation of rat skeletal muscle mitochondria with the nitric oxide donor S-nitrosoglutathione reversibly inhibited oxygen utilization (Cleeter *et al.* 1994).

Mitochondria are one of the main sources of formation of reactive oxygen species (ROS) in myocytes. During the course of electron transfer from the substrate to oxygen, mitochondrial respiratory chain compounds (MRCC) turn transiently into radical forms thus becoming a potential source of electrons for the generation of ROS. Turrens *et al*. (1985) demonstrated that ubisemiquinone (·QH), a reduced form of ubiquinone, is responsible for $\cdot O_2$ formation by mitochondria. Ironsulfur proteins of complex I and II which appear in radical form during electron transfer to ubiquinone, are also a potential source for generation of toxic reactive oxygen species (Fig. 1). The question arises whether the increased NO production is associated with decreased formation of radicals by MRCC.

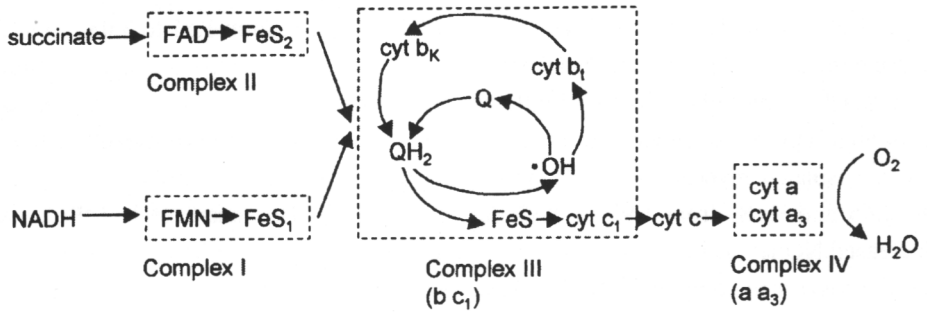

Fig. 1. *Scheme of the electron flow through the mitochondrial respiratory chain. ·FeS – ironsulfur proteins; cyt. – cytochromes; Q – ubiquinone; QH_2 – ubiquinol; ·QH – ubisemiquinone radical.*

Using electron paramagnetic resonance (EPR), we measured changes of the radical forms of MRCC in the isolated perfused rat myocardium as markers of reactions to long-term administration of the ACE inhibitor captopril, which possibly potentiates NO formation. In some groups, we intended to enhance NO production by addition of L-arginine – the substrate for NO synthesis – into the perfusion medium. The aim of the study was to demonstrate whether the effect of these compounds could be associated with the reduced generation of MRCC radicals.

Methods

Animals

All experiments using laboratory animals were performed according to the Guide for the Care and Use of Laboratory Animals published by US National Institute of Health (NIH publication No 85-223, revised 1985). Experiments were 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 inhibitor captopril (Sigma, St. Louis, USA) in drinking water (50 mg/l) 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 animals of the control and captopril-treated groups.

Perfusion of isolated hearts

After decapitation under ether anesthesia, the heart was removed and rapidly placed into ice-cold saline. When the heart stopped beating, the aorta was connected to a perfusion system. The hearts were retrogradely perfused (60 mm Hg) with Krebs-Heinseleit bicarbonate solution (KRB), pH 7.4, 37 °C equilibrated with 95% O_2 + 5 % CO_2. The KRB solution 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, and 5.5 glucose. The 20-min stabilization period was followed by a 10-min period when the hearts were perfused with either KRB or KRB containing L-arginine (Sigma, St. Louis, USA) at a final concentration of 1 mmol/l. After this period, a 25-min global ischemia (no-flow) was introduced and then 5-min retrograde reperfusion followed. During the 5th minute of reperfusion, the coronary flow recovered satisfactorily and the measurement of NO was possible not only in the myocardium, but also in the coronary effluent. Throughout the perfusion, the hearts were maintained at 37 °C in a water-jacketed chamber. Hearts were frozen in liquid nitrogen using Wollenberger forceps under normoxic conditions (N), in the equilibrated stabilized state prior to ischemia, at the end of ischemia (I) and after 5 min of reperfusion (R) following ischemia. The hearts were kept in liquid nitrogen until EPR analysis of MRCC radical forms and NO contents was performed.

Isolated mitochondria

Mitochondria were isolated from normal non-perfused rat heart in 250 mmol/l sucrose, 1 mmol/l EDTA, 5 mmol/l Tris/HCl buffer, pH 7.4 (Stieglerová *et al.* 2000) and suspended in the same medium at protein concentration 19.3 mg/ml. Three samples were prepared from the mitochondrial suspension. Normoxic sample (N): 200 µl of mitochondrial suspension was mixed with 200 µl of substrate and incubated under light air stream for 5 min. Ischemic sample (I): 200 µl of mitochondrial suspension was mixed with 200 µl of substrate and incubated under nitrogen for 10 min in a closed (airtight) cup. Reoxygenated sample (R): 200 µl of mitochondrial suspension was mixed with 200 µl of substrate and incubated for 10 min in absence of oxygen and then the suspension of mitochondria was mildly bubbled with air for 5 min. All incubations were performed at 25 ºC. At the end of incubation, the samples were frozen and stored in liquid nitrogen until EPR analyses.

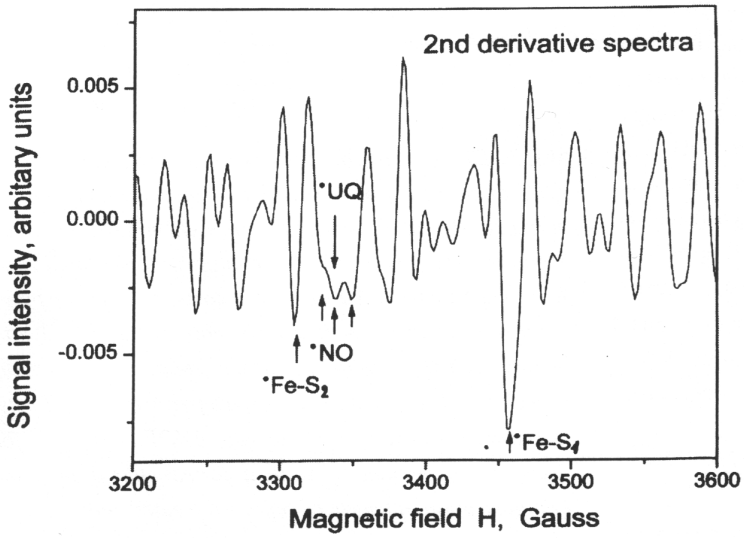

Fig. 2. *Recording of the 2nd derivative of EPR signals of some radical forms of the mitochondrial respiratory chain compounds (·FeS_2, ·QH, ·FeS_1) and NO measured as dinitrosyl-iron complex (DNIC) in the myocardium.*

Measurement of the radical form of some compounds of the mitochondrial respiratory chain and NO radicals, using EPR spectroscopy

Frozen tissue samples were removed from liquid nitrogen and divided into several pieces under gaseous nitrogen. These fragments were transferred into a quartz cuvette, and frozen to 77 K. This procedure prevents artificial formation of free radicals (Baker *et al.* 1989). Frozen samples of mitochondria were transferred under

nitrogen into a quartz cuvette and electron paramagnetic resonance spectra (EPR) were measured at 77 K.

In the case of whole heart, the EPR spectra were obtained using an EPR-220 instrument operating in the X band with a magnetic field modulation of 100 kHz. The magnetic field was measured with a Radiopon ^{1}H-NMR magnetometer, and the microwave frequency was established using a C3-54 frequency counter (Fig. 2). The registered spectra were evaluated using GenPlot software. The standard for calibration was Mn(II)ZnS (Galenus, Berlin, Germany).

During mitochondrial respiration, the respiratory chain compounds are partly converted into radical forms, whose signals can be detected using EPR. We employed this method to measure the following three defined types of compounds both in isolated mitochondria and in myocardial samples: a) ·FeS_1 NADH dehydrogenase radical (g = 1.935), b) ·QH ubisemiquinone radical (g = 1.967), c) ·FeS_2 succinate dehydrogenase radical (g = 2.018). The factor "g" characterizes the type of the radical.

NO radicals were measured directly in myocardial samples using the EPR signal of the dinitrosyl iron complex (DNIC) (g=2.03). Its physical and chemical properties and relation to NO originating in tissues has been described by Vanin and Kleschyov (1998) (Fig. 2).

NO production in the perfusate was evaluated as the concentrations of stable NO metabolites NO_2^-/NO_3^- measured by the Griess reagent (Green *et al.* 1982) with minor modifications. In brief, samples were incubated for one hour at room temperature with nitrate reductase (0.1 U/100 µl: Aspergillus species, Boehringer, Mannheim, Germany) in substrate buffer (pH 7.8) containing imidazole, NADPH and FAD to convert all NO_3^- to NO_2^-. Total NO_2^- was then analyzed using the Griess reagent and absorbency was measured at 543 nm using a UV/VIS spectrophotometer (Secomam, France). Concentrations of NO_2^- in the samples were determined from a standard curve of $NaNO_2$ obtained from $NaNO_3$ (0-100 µmol) using the above described enzymatic conversion. Nitrate recovery was 97 %.

Statistical analysis

Statistically significant differences between the groups were evaluated using ANOVA. The four-way ANOVA was performed in 24 rats for evaluation of the effect of captopril and L-arginine on MRCC radicals formation in myocardium during normoxia, ischemia and reperfusion (Fig. 3) and three-way ANOVA for assessing the effect on NO radical contents (Fig. 4, bottom).

Results

Generation of the radical forms of some respiratory chain compounds in the isolated myocardium

Signals of radicals forms of some MRCC were determined in the isolated myocardium, under normoxic conditions (N), after 25-min ischemia (I) and after ischemia followed by 5-min reperfusion (R). We found an increase of ·QH and ·FeS_1 during ischemia and a decrease during reperfusion (Fig. 3). Signals of the radical form of ·FeS_2 protein of succinate dehydrogenase did not change in the perfused myocardium.

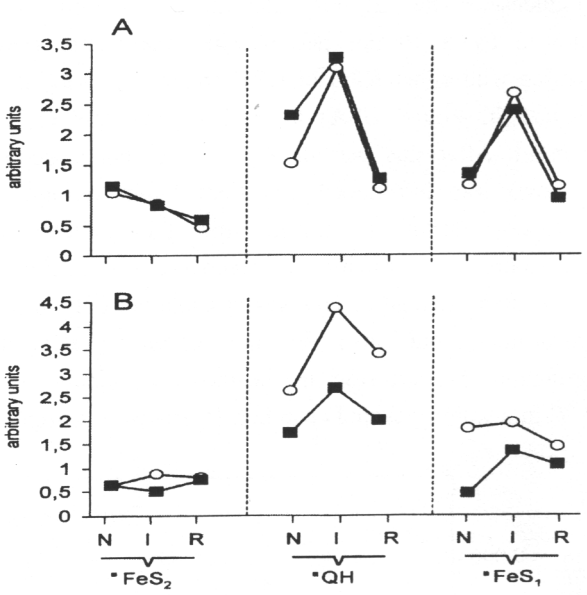

Fig. 3. *Changes in the amount of radical forms ·FeS_2, ·QH and ·FeS_1 in the isolated myocardium during normoxia (N), at the end of 25-min ischemia (I), and after 5 min of reperfusion (R).* ***A*** *= captopril non-treated rats,* ***B*** *= captopril-treated rats. ○ hearts of rats perfused without L-arginine, ■ hearts of rats perfused, 10 min before ischemia, with perfusate containing 1 mmol/l L-arginine. The measurements were performed in 24 rats. Based on F statistic from four-way ANOVA, $p<0.005$ for decrease in amount of radical forms after L-arginine administration in hearts of captopril treated rats.*

In the hearts of captopril non-treated rats (Fig. 3A), 25-min ischemia resulted in substantially increased contents of the radicals ·QH (1.51 to 3.08 arbitrary units) and ·FeS_1 (1.14 to 2.65 arbitrary units). Addition of L-arginine into perfusion medium 10 min

prior to ischemia did not change the contents of the ·QH and ·FeS_1 (·QH: 3.08 to 3.25, ·FeS_1: 2.65 to 2.37 arbitrary units). At the end of 5-min reperfusion, the signals of the radical forms of ·QH and ·FeS_1 decreased to levels close to those seen under normoxia. L-arginine was without any effect.

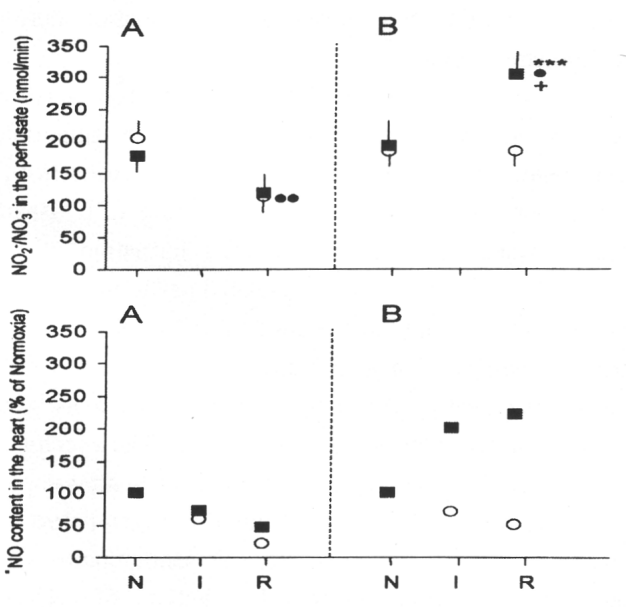

Fig. 4. (top): *NO_2^- / NO_3^- contents in perfusate. Effect of captopril in vivo and/or L-arginine in vitro (1 mM) on NO release into the coronary effluent before ischemia and after 5 min of postischemic reperfusion.* ***A*** *= captopril non-treated rats,* ***B*** *= captopril treated rats. . ○ hearts of rats perfused without L-arginine, ■ hearts of rats perfused, 10 min before ischemia, with perfusate containing 1 mmol/l L-arginine, *** p<0.001 vs. captopril non-treated groups; ● p<0.02, ●● p<0.01 vs. corresponding pre-ischemic levels; + p<0.01 vs. captopril group without L-arginine. Data are means ± S.E.M., n = 4-7 hearts per group.* **(bottom):** *NO radical contents measured as dinitrosyl-iron complex (DNIC) in the isolated myocardium. Under normoxia (N), at the end of 25-min ischemia (I) and after 5 min of reperfusion (R), expressed as the percentage of normoxic values. Based on F statistic from three-way ANOVA, p<0.01 for increase in amount of NO radical after L-arginine administration in hearts of captopril-treated rats.*

The addition of L-arginine to the perfusate in hearts of captopril-treated rats (Fig. 3B) reduced the ischemia-induced increase of ·QH and all values of ·FeS_1 in normoxic, ischemic and reperfused hearts were lower compared to the hearts without addition of L-arginine during perfusion. Under normoxia, the content of ·QH without and with L-arginine was 2.61 vs. 1.72, respectively, those of ·FeS_1 1.82 vs. 0.46; during ischemia, ·QH without and with L-arginine was 4.35 vs. 2.66, and ·FeS_1 1.93 vs. 1.35, respectively. Lower levels of the radical forms were still evident after 5 min of reperfusion: ·QH 3.39 vs. 1.98 and ·FeS_1 1.43 vs. 1.06, respectively.

A significant decrease in the amount of radical forms was only observed after the administration of L-arginine in hearts of captopril-treated rats (p=0.0034). The decrease induced by L-arginine may be associated with enhanced NO production as noted both in perfusate (Fig. 4, top) and in the myocardium (Fig. 4, bottom).

Content of NO in the perfusate and the isolated perfused myocardium

The amount of NO released into the coronary effluent (Fig. 4, top) in the equilibrated stabilized state before ischemia did not differ significantly in any investigated group. There was a significant decrease of NO release in the hearts of rats not receiving captopril after 5 min of postischemic reperfusion (Fig. 4A, top). The addition of L-arginine into the perfusate did not increase NO release. In captopril-treated groups without arginine (Fig. 4B, top, open circles), there was no reduction in the NO release between normoxia and postischemic reperfusion. The addition of arginine (Fig. 4B, top, full squares) increased significantly the perfusate NO content (304±28.1 nmol/min) compared with NO content after ischemia in group A (118±8.9 nmol/min) or NO content in group B without L-arginine addition (184±23 nmol/min).

The content of the myocardial NO complex, as determined using EPR (Fig. 4 bottom), was lower by an order of magnitude than the perfusate NO complex content and ranged from 0.2 to 0.8 nmol/g d.w. The results shown in Figure 4 are expressed as percentage values measured under normoxia (N). In the captopril non-treated group (A), the myocardial NO content decreased during ischemia and reperfusion both in the absence (100, 59, 21 %) and presence of L-arginine in perfusate (100, 72, 47 %). In the captopril-treated group (B), myocardial NO content decreased after ischemia and reperfusion only in the absence of L-arginine in the perfusate (100, 71, 51 %). L-arginine considerably enhanced NO production both at the end of ischemia and after 5 min of reperfusion (100, 200, 221%, p<0.01).

Effect of ischemia and reoxygenation on radical formation by isolated mitochondria

Under normoxia (N), isolated mitochondria produce a certain amount of radical forms, which are associated with electron transfer in the respiratory chain, and which can be detected using EPR (Fig. 2). In our experimental setting, the respective normoxic values were expressed as arbitrary units – 10.8 for ·FeS_2, 13.7 for ·QH and 14.5 for ·FeS_1. During incubation of mitochondria without air (I), we found enhanced signals of ·FeS_2 of succinate dehydrogenase and ·QH of ubiquinone from 10.8 to 14.9 and 13.7 to 24.5 arbitrary units, respectively. In the samples of mitochondria where ischemia was followed by reoxygenation, the contents of radical forms of ·FeS_2 and ·QH decreased to preischemic levels (9.7 and 15.2 arbitrary units).

Signals of the ·FeS_1 complex are consistent with the radical part of the molecule of NADH dehydrogenase (14.5 arbitrary units). During ischemia, the signals decreased but did not rise after reoxygenation (7.9 and 8.2 arbitrary units). In our experiments, isolated mitochondria lacked the supply of substrate connecting them to the metabolic pathway involving NADH dehydrogenase. For this reason, no changes were found in the signals of a reduced form of NADH dehydrogenase similar to those seen with ·FeS_2 and ·QH (Fig. 5).

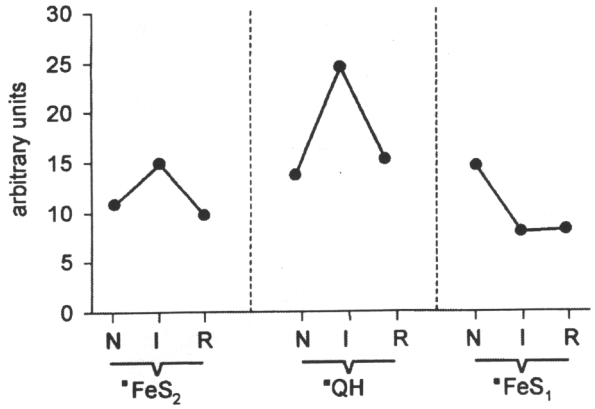

Fig. 5. *The amount of radical forms of some respiratory chain compounds in isolated mitochondria. ·FeS_2 = succinyl dehydrogenase, ·QH = ubisemiquinone, ·FeS_1 = NADH dehydrogenase. Mitochondria in oxidized state (N = normoxia), mitochondria after 10-min of oxygen-free incubation (I = anoxia), mitochondria after 10-min incubation in anoxia followed by 5-min incubation in open caps accessible to air (R = reoxygenation). Protein concentration in incubation medium was 9.65 mg/ml. Succinate (5 mmol/l) was present in all samples as substrate.*

Discussion

The amount of radical forms of MRCC in the myocardium increased during ischemia, thus implying a risk for the generation of reactive oxygen species during reperfusion. The present study shows that a combination of captopril and L-arginine decreased formation of radical forms of MRCC and enhanced NO formation during postischemic period.

Mitochondria are the main source of oxygen radicals in the heart and might be an important contributor to tissue damage following postischemic reperfusion (Ambrosio *et al.* 1993). Using EPR, Baker and Kalyanaraman (1989) described formation of the radical forms of MRCC in the isolated perfused rat heart. These authors identified the conditions for excluding the artifact findings of oxygen radical signals, which readily form when treating the samples. Our experiments were carried out according to their procedure. The spectra of the radical forms of MRCC of perfused heart samples measured at the temperature of liquid nitrogen (–196 ºC) consist of three components: ubisemiquinon ·QH, reduced iron-sulfur center I (·FeS_1) of NADH dehydrogenase, and reduced iron-sulfur center II (·FeS_2) of succinate dehydrogenase. The EPR spectra of most other iron-sulfur centers of MRCC can be detected only at liquid helium temperatures.

Association between the formation of radical forms of MRCC and myocardial damage was demonstrated by Vavřínková *et al*. (1994) and Ide *et al.* (1999). Therefore, we studied the extent to which the activation of NO formation can decrease the generation of radical forms of some compounds of the mitochondrial respiratory chain. In our experiments, we tested the effect of long-term administration of the ACE inhibitor captopril on the generation of the radical form of some compounds of the mitochondrial respiratory chain during normoxia, anoxia and reperfusion. In our previous experiments, we have shown that long-term captopril treatment increases the energy potential and has beneficial effects on tolerance of the isolated heart to ischemia (Divišová *et al.* 2001). In some experiments, NO formation was enhanced by the addition of L-arginine as substrate for NO synthesis.

To obtain a more precise idea about changes in the content of reduced forms of MRCC under ischemia and reperfusion, we performed also measurements of the state of MRCC in isolated perfused heart.

EPR measurements allow to monitor changes in the mitochondrial state with only minimal interference into the integrity of myocardial tissue, thus minimizing the development of artifacts. It can be assumed that the state of mitochondria, as seen during EPR-based analysis, is consistent with the actual state of the perfused myocardium. At the end of ischemia with no oxygen supply to the heart, we observed elevated contents of the radical forms ·QH and ·FeS_1. This finding suggests that mitochondria, under these circumstances, are a potential source of the enhanced formation of toxic oxygen radicals thus contributing to post-ischemia myocardial injury during reperfusion (Ide *et al.* 1999). We did not find any change in ·FeS_2 signals in myocardial samples, which would indicate that substrates connected with complex II function are less utilized for cell function under these experimental conditions.

The addition of L-arginine into the perfusate did not affect the formation of MRCC radical forms in the hearts of captopril non-treated rats. By contrast, in captopril-treated rats, L-arginine was associated with a significant reduction in the content of radical forms ·QH and ·FeS_1, implying a lower risk for the generation of oxygen radicals during reperfusion.

Our results suggest an association between the content of radical forms of MRCC and NO formation. In captopril-treated rats the hearts of which had been perfused with an L-arginine-supplemented solution, enhanced NO formation was observed, both in the perfusate (at the end of reperfusion) and in the myocardium (at the end of ischemia and at the end of reperfusion). The enhanced NO formation may be due to reduced bradykinin degradation (Hartman 1995) induced by captopril so that the activity of endothelial NOS may have been increased (Linz *et al.* 1992). NOS is a complex enzyme the integrity of which is degraded during ischemia. However, NOS is more stable if bound, in excessive amount, to the substrate L-arginine, (Huk *et al.* 1997) and its activation by captopril may become manifest.

We measured NO in myocardial tissue using EPR, but without employing spin-trapping agents. NO determination in living systems still poses a challenge. Using EPR, Vanin and his group (for review see Vanin and Kleschyov 1998) detected in the liver and other organs signals of the non-heme iron-NO complex DNIC (dinitrosyl iron complex) with endogenous thiol ligand (g=2.03). In their ensuing studies, Műlsch *et al.* (1993) observed that DNIC is formed *via* the L-arginine dependent pathway. The resulting NO binds to intracellular "readily available" non-heme iron. Indirect evidence suggested the existence of an intracellular pool for DNIC. While its exact value is still not clear, it is a directly measurable parameter of the intracellular content of NO. Using EPR, we measured the signals of this complex in myocardial samples and evaluated their relationship with the other parameters measured. Our results indicated that the signal of DNIC changes consistently with the contents of NO_2^- / NO_3^- in the myocardial perfusate depending on the state of the myocardium (normoxia, ischemia, reperfusion) and responds to addition of L-arginine into the perfusate. These results thus confirm our conclusion that NO measured in the heart as DNIC using EPR reflects the state of intracellular NO.

We demonstrated the formation of certain amounts of the radical forms of MRCC in isolated mitochondria under normoxic conditions. In the absence of oxygen, the content of most radical forms of MRCC increased. Electrons accumulate in mitochondria as their transfer by cytochrome c oxidase to oxygen is blocked. Accumulation of reduced forms, ·QH in particular, increases the risk of the formation of oxygen radicals at the onset of reperfusion. Following the incubation of mitochondria in the presence of oxygen, the contents of the radical forms of MRCC again decreased.

The content of radical forms of ·FeS_1 in isolated mitochondria did not tend to increase during either ischemia or reperfusion. NADH dehydrogenase expressed by these signals lacks substrate supply in isolated mitochondria and it cannot consequently be converted into a reduced radical form. In our experiments, we used succinate as substrate and this could be the reason why we found an increase of radical forms of ·FeS_2 of succinate dehydrogenase in ischemia in contrast to isolated heart. These results also indicate that the changes in the content of radical forms of various respiratory chain compounds may reflect the amount of the metabolized substrate.

The results of this study present further evidence that ischemia is associated with the accumulation of mitochondrial respiratory chain compounds in radical form. This situation may accelerate oxygen radical formation, especially during reperfusion, i.e. during reoxygenation, when the oxygen tension in the cell increases. Under circumstances where NO synthesis is

enhanced within physiological limits, i.e. in captopril-treated rats, with sufficient substrate for NOS by the addition of L-arginine during ischemia, the content of radical forms of MRCC decreases, thus reducing the risk of the generation of toxic oxygen radicals. These results may be helpful in explaining the mechanisms of the cardioprotective effect of ACE inhibitors in combination with L-arginine.

Acknowledgements

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References

AMBROSIO G, ZWEIER JL, DUILIO C, KUPPUSAMY P, SANTORO G, ELIA PP, TRITTO I, CIRILLO P, CONDORELLI M., CHIARIELLO M, FLAHERTY JT: Evidence that mitochondrial respiration is a source of potentially toxic oxygen free radicals in intact rabbit hearts subjected to ischemia and reflow. *J Biol Chem* **263:** 18532-18541, 1993.

BAKER JE, KALYANARAMAN B: Ischemia-induced changes in myocardial paramagnetic metabolites: implications for intracellular oxy-radical generation. *FEBS Lett* **244:** 311-314, 1989.

BRUNNER HR, GAVRAS H, WAEBER B, KERSHAW GR, TURINI GA, VUKOVICH RA, McKINSTRY DM: Oral angiotensin-converting enzyme inhibitor in long-term treatment of hypertensive patients. *Ann Intern Med* **90:** 19-23, 1979.

CLEETER MWJ, COOPER JM, DARLEY-USMAR VM, MONCADA S, SCHAPIRA AHV: Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. Implications for neurodegenerative diseases. *FEBS Lett* **345:** 50-54, 1994.

DIVIŠOVÁ J, VAVŘÍNKOVÁ H, TUTTEROVÁ M, KAZDOVÁ L, MESCHIŠVILI E: Effect of ACE inhibitor captopril and L-arginine on the metabolism and on ischemia reperfusion injury of the isolated rat heart. *Physiol Res* **50**: 143-152, 2001.

GENG Y-J, HANSSON GK, HOLME E: Interferon-γ and tumor necrosis factor synergize to induce nitric oxide production and inhibit mitochondrial respiration in vascular smooth muscle cells. *Circ Res* **71:** 1268-1276, 1992.

GREEN L, WAGNER D, GLOGOWSKI I, SKIPPER P, WISHNOK I, TANNENBAUM S: Analysis of nitrate, nitrite and [^{15}N] nitrite 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.

HECKER M, BARA AT, BUSSE R: Relaxation of isolated coronary arteries by angiotensin-converting enzyme inhibitors: role of endothelium-derived kinins. *J Vasc Res* **30:** 257-262, 1993.

HUK I, NANOBASHVILI J, NEUMAYER C, PUNZ A, MUELLER M, AFKHAMPOUR 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.

IDE T, TSUTSUI H, KINUGAWA S, UTSUMI H, KANG D, HATTORI N, UCHIDA K, ARIMURA K-I, EGASHIRA K, TAKESHITA A: Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. *Circ Res* **85:** 357-363, 1999.

KIOWSKI W, ZUBER M, ELSASSER S, ERNE P, PFISTERER M, BURKART F: Coronary vasodilatation and improved myocardial lactate metabolism after angiotensin converting enzyme inhibition with cilazapril in patients with congestive heart failure. *Am Heart J* **122:** 1382-1388, 1991.

KONSTAM MA, ROUSSEAU MF, KRONENBERG MW, UDELSON JE, MELIN J, STEWART D, DOLAN N, EDENS TR, AHN S, KINAN D, HOWE DM, KILCOYNE L, METHERALL J, BENEDICT C, YUSUF S, POULEUR H: Effects of the angiotensin converting enzyme inhibitor enalapril on the long-term progression of left ventricular dysfunction in patients with heart failure. *Circulation* **86:** 431-438, 1992.

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.

LOKE KE, LAYCOCK SK, MITAL S, WOLIN M, BERNSTEIN R, OZ M, ADDONIZIO L, KALEY G, HINTZE TH.: Nitric oxide modulates mitochondrial respiration in failing human heart. *Circulation* **100:** 1291-1297, 1999.

MÜLSCH A, MORDVINTCEV PI, HAUSCHILDT S, BUSSE R: Formation and release of dinitrosyl iron complexes by endothelial cells. *Biochem Biophys Res Commun* **196:** 1303-1308, 1993.

PODEROSO JJ, PERALTA JG, LISDERO CL, CARRERAS MC, RADISIC M, SCHÖPFER F, CADENAS E, BOVERIS A: Nitric oxide regulates oxygen uptake and hydrogen peroxide release by the isolated beating rat heart. *Am J Physiol* **274:** C112-C119, 1998.

SHEN W, HINTZE TH, WOLIN MS: Nitric oxide. An important signaling mechanism between vascular endothelium and parenchymal cells in the regulation of oxygen consumption. *Circulation* **92:** 3505-3512, 1995.

STIEGLEROVÁ A, DRAHOTA Z, OŠŤÁDAL B, HOUŠTĚK J: Optimal condition for determination of cytochrome c oxidase activity in the rat heart. *Physiol Res* **49:** 245-250, 2000.

TURRENS JF, ALEXANDRE A, LEHNINGER AL: Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. *Arch Biochem Biophys* **237:** 408-414, 1985.

TUTTEROVÁ M, VAVŘÍNKOVÁ H, DIVIŠOVÁ J, KAZDOVÁ L: Effects of captopril treatment on heart and kidney function and heart ischemic tolerance in female rats. *Physiol Res* **48**: S132, 1999.

VANIN AF, KLESCHYOV AL: EPR detection and biological implications of nitrosyl nonheme iron complexes. In: *Nitric Oxide in Transplant Rejection and Anti-Tumor Defense*. S LUKIEWICZ, JL ZWEIER (eds), Kluwer Academic Publishers, Boston, 1998, pp 49-82.

VAVŘÍNKOVÁ H, TUTTEROVÁ M, STOPKA P: Influence of dietary fish oil on the generation of free radicals during reperfusion of the isolated rat heart. *Proc 15th Eur Mtg Int Soc Heart Res* 1994, pp 649-652.

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**: S134, 1999.

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