Physiol. Res. 60: 467-474, 2011

N-acetylcysteine Treatment Prevents the Up-Regulation of MnSOD in Chronically Hypoxic Rat Hearts

P. BALKOVÁ[1,3], M. HLAVÁČKOVÁ[1], M. MILEROVÁ[2,3], J. NECKÁŘ[2,3], F. KOLÁŘ[2,3], F. NOVÁK[1,3], O. NOVÁKOVÁ[1]

[1]Department of Cell Biology, Faculty of Science, Charles University in Prague, [2]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, [3]Centre for Cardiovascular Research, Prague, Czech Republic

Received June 9, 2010
Accepted October 11, 2010
On-line March 14, 2011

Summary

Chronic intermittent hypoxia (CIH) is associated with increased production of reactive oxygen species that contributes to the adaptive mechanism underlying the improved myocardial ischemic tolerance. The aim was to find out whether the antioxidative enzyme manganese superoxide dismutase (MnSOD) can play a role in CIH-induced cardioprotection. Adult male Wistar rats were exposed to intermittent hypobaric hypoxia (7000 m, 8 h/day, 25 exposures) (n=14) or kept at normoxia (n=14). Half of the animals from each group received *N*-acetylcysteine (NAC, 100 mg/kg) daily before the hypoxic exposure. The activity and expression of MnSOD were increased by 66 % and 23 %, respectively, in the mitochondrial fraction of CIH hearts as compared with the normoxic group; these effects were suppressed by NAC treatment. The negative correlation between MnSOD activity and myocardial infarct size suggests that MnSOD can contribute to the improved ischemic tolerance of CIH hearts.

Key words

Chronic hypoxia • MnSOD • Ischemia/reperfusion • Myocardial infarction • Cardioprotection

Corresponding author

O. Nováková, Charles University, Faculty of Science, Dept. of Cell Biology, Viničná 7, 128 00 Prague 2, Czech Republic. Fax: +420 221 951 758. E-mail: olnov@natur.cuni.cz

Introduction

It has been shown that reactive oxygen species (ROS) formed under stress conditions, such as exercise, hyperthermia or various forms of preconditioning, may enhance the tolerance of the heart to ischemia-reperfusion injury by stimulation of cellular antioxidant defence systems that maintain optimal redox balance (Hoshida *et al*. 2002). The main source of ROS in myocardium is the respiratory chain of the inner mitochondrial membrane where manganese superoxide dismutase (MnSOD; SOD2), the key antioxidative enzyme, is also localized (Starkov *et al.* 2008). Several signalling pathways involving ROS as a second messenger in conjunction with the induction of MnSOD gene expression have been identified (Rogers *et al*. 2001, Hussain *et al*. 2004). MnSOD is an inducible homotetrameric protein, synthesized in the cytoplasm as a precursor and imported post-translationally in the mature form into the mitochondrial matrix (Wispe *et al*. 1989). MnSOD catalyzes the dismutation of two superoxide radicals, generating hydrogen peroxide and molecular oxygen (McCord *et al.* 1969). This enzyme is most likely of crucial importance for cell viability as mice lacking MnSOD die from dilated cardiomyopathy within the first days after the birth (Li *et al*. 1995).

Adaptation to chronic intermittent hypoxia (CIH) is known to protect the heart against acute ischemia/reperfusion injury. The mechanism underlying induction of the long-lasting protected phenotype of CIH adapted hearts is not precisely understood (Kolář *et*

al. 2004). It has been proposed that ROS generated during the adaptation period are important components of a signalling pathway leading to this form of cardioprotection as chronic treatment of rats with an antioxidant eliminated the infarct size-limiting effect of CIH (Kolář *et al*. 2007). The main goal of our study was, therefore, to find out whether CIH affects the activity and expression of MnSOD in rat heart mitochondria and whether these changes depend on ROS production. Levels of pyruvate dehydrogenase (PDH) in mitochondrial and cytosolic fractions were measured as a marker of mitochondrial integrity. We showed that CIH led to the up-regulation and activation of MnSOD, and these effects were absent in animals chronically treated with the antioxidant N-acetylcysteine (NAC) during the adaptation period. Moreover, the negative correlation between MnSOD activity and myocardial infarct size suggests that this enzyme can contribute to the improved ischemic tolerance of CIH-adapted hearts.

Materials and Methods

Animal model

Adult male Wistar rats were exposed to intermittent hypobaric hypoxia of 7000 m for 8 h/day, 5 days a week, as described earlier (Kolář *et al*. 2007). Barometric pressure (P_B) was lowered stepwise, so that the final level equivalent to the altitude of 7000 m (P_B=306.8 mm Hg; PO_2=63.8 mm Hg) was reached after 13 exposures; the total number of exposures was 25. The control group of animals was kept at normoxia for the same period of time. One-half of the rats from each group received NAC (dissolved in saline and neutralized with sodium hydroxide) subcutaneously in a dose of 100 mg/kg daily before each hypoxic exposure and the remaining rats received the same volume of saline (2 ml/kg) in the corresponding manner. Rats were fed standard laboratory diet and had free access to water. All rats were employed on the next day after the last hypoxic exposure and sacrificed by decapitation. Hearts were rapidly excised, washed in cold saline and left ventricular (LV) walls with the septum were dissected, weighed and used for analysis. The study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The experimental protocol was approved by the Animal Care and Use Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic.

Table 1. Expression of common housekeeping proteins.

	Normoxia	**Hypoxia**	
Actin			42 kDa
	45.9±2.5	54.3±2.6*	
Calsequestrin			55 kDa
	43.9±3.2	56.0±3.2*	
GAPDH			36 kDa
	35.8±4.4	64.1±4.4*	
β-tubulin			50 kDa
	43.4±3.0	56.8±3.2*	

Representative Western blots are shown. Values are means ± S.E.M. from 6 hearts in each group (arbitrary units). *P<0.05 vs. normoxic group.

Isolation of mitochondrial and cytosolic fractions

LV with septum were chopped by scissors in ice-cold medium (10 vol of medium to 1 vol of tissue) consisting of (in mM) 20 Tris-HCl, 250 sucrose, 2.5 EGTA, 5 EDTA, 100 NaF, 5 dithiothreitol, 0.3 phenylmethylsulfonyl fluoride, 0.2 leupeptin, and 0.02 aprotinin (pH 7.4) and then homogenized by using a Potter-Elvehjem homogenizer with a loose-fit Teflon pestle. The homogenate was centrifuged at 800 × *g* for 20 min. The supernatant was centrifuged at 8,000 × *g* for 10 min. The mitochondrial pellet was washed (1 ml) twice and finally resuspended in the homogenization buffer. Supernatant was then centrifuged at 100,000 × *g* for 60 min to obtain the cytosolic fraction. All steps were performed at 4 °C. Samples for the measurement of MnSOD activity were sonicated using three 10-s bursts. Mitochondrial fraction for immunoblot analyses was extracted with 1 % Triton X-100 on ice for 60 min, the resulting detergent-treated supernatant was used. Triton X-100 was also added to the cytosolic fraction to reach the final concentration of 1 %. Protein content was determined according to Lowry modified by Peterson (Peterson *et al*. 1977). Neither hypoxia nor NAC influenced the protein yields in mitochondrial and cytosolic fractions (data not shown).

SDS-PAGE and Western blot analysis

Mitochondrial extracts and cytosolic fractions were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis on 15 % bis-acrylamide gel at

Table 2. Body weight and heart weight parameters.

Parameter	Normoxia	Normoxia + NAC	Hypoxia	Hypoxia + NAC
n	10	10	10	9
BW, g	380 ± 6	369 ± 7	340 ± 7*	332 ± 12*
RVW, mg	174 ± 6	180 ± 8	270 ± 11*	247 ± 17*
LVW+SW, mg	601 ± 13	582 ± 18	677 ± 29*	632 ± 26*
RVW/BW, mg/g	0.459 ± 0.019	0.487 ± 0.019	0.795 ± 0.028*	0.743 ± 0.034*
LVW+SW/BW, mg/g	1.584 ± 0.021	1.576 ± 0.033	1.994 ± 0.070*	1.908 ± 0.055*

n, number of animals; NAC, N-acetylcysteine-treated groups; BW, body weight; RVW, right ventricular weight; LVW, left ventricular weight; SW, septum weight; RVW/BW, relative RV weight; LVW+SW/BW, relative LV+S weight; *P<0.05 versus corresponding normoxic group.

30 mA/gel for 90 min on a Mini-Protean III apparatus (Bio-Rad, Hercules, CA); the resolved proteins were transferred to a nitrocellulose membrane (Amersham Biosciences, Germany). After blocking with 5 % dry low-fat milk in Tris-buffered saline with Tween 20 (TTBS) for 60 min at room temperature, membranes were washed and probed with rabbit anti-MnSOD polyclonal antibody (Stressgen Bioreagents, Victoria, Canada) and mouse monoclonal antibody to PDH E1β subunit (Molecular Probes, Oregon, USA). The membranes were washed again and incubated with the secondary swine anti-rabbit and anti-mouse (Sevapharma, Prague, Czech Republic; 1:4,000 in TTBS) antibodies, respectively, labelled with horseradish peroxidase for 60 min at room temperature. To ensure the specificity of immunoreactive protein, MnSOD standard (Stressgen Bioreagents, Victoria, Canada) were used. The samples from each experimental group were run on the same gel and quantified on the same membrane. The analysis of each heart sample was repeated at least three times and normalized to total protein.

For comparative quantification of the amount of monitored protein in Western blot analysis, an appropriate housekeeping protein as an internal control is usually used. We examined the effect of CIH on expression of four common housekeeping proteins: actin (Sigma-Aldrich, St. Louis, USA), calsequestrin (Abcam, Cambridge, USA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Abcam, Cambridge, USA), and β-tubulin (Abcam, Cambridge, USA) in heart homogenate. Data in Table 1 show that the expression of all these proteins increased significantly after the adaptation to CIH. On that ground, we used the total protein concentration as the most suitable referential value, because it was not altered under our experimental conditions. Obviously, the choice of a housekeeping protein as an internal standard in chronic experiments should always be carefully validated.

Measurement of MnSOD activity

Total superoxide dismutase (SOD) activity was determined by the modified nitroblue tetrazolium method (Elstner *et al*. 1983, Spitz *et al*. 1989) based on the generation of superoxide radicals from xanthine oxidase reaction that react further with nitroblue tetrazolium dye. Reduction of nitroblue tetrazolium by superoxide anion was measured spectrophotometrically at 540 nm (28 °C). Chloroform-ethanol extracts were then used to determine the SOD activity. The assay contained the following reagents: 0.1 mM phosphate buffer (pH 7.8), 4 mg/ml bovine serum albumin, 2 mg/ml nitroblue tetrazolium, and 1 mM xanthin. MnSOD activity was measured in the presence of 5 mM NaCN, the selective inhibitor of copper-zinc SOD.

Statistical analysis

The results are expressed as means ± S.E.M. Two-way ANOVA and subsequent Student-Newman-Keuls test were used for comparison of differences in parametric variables between the groups. Differences were assumed as statistically significant when $P<0.05$.

Results

Weight parameters

Adaptation of rats to CIH led to body growth retardation, pronounced hypertrophy of the right ventricle and moderate hypertrophy of the left ventricle. Treatment with NAC had no effect on body and heart weight parameters (Table 2).

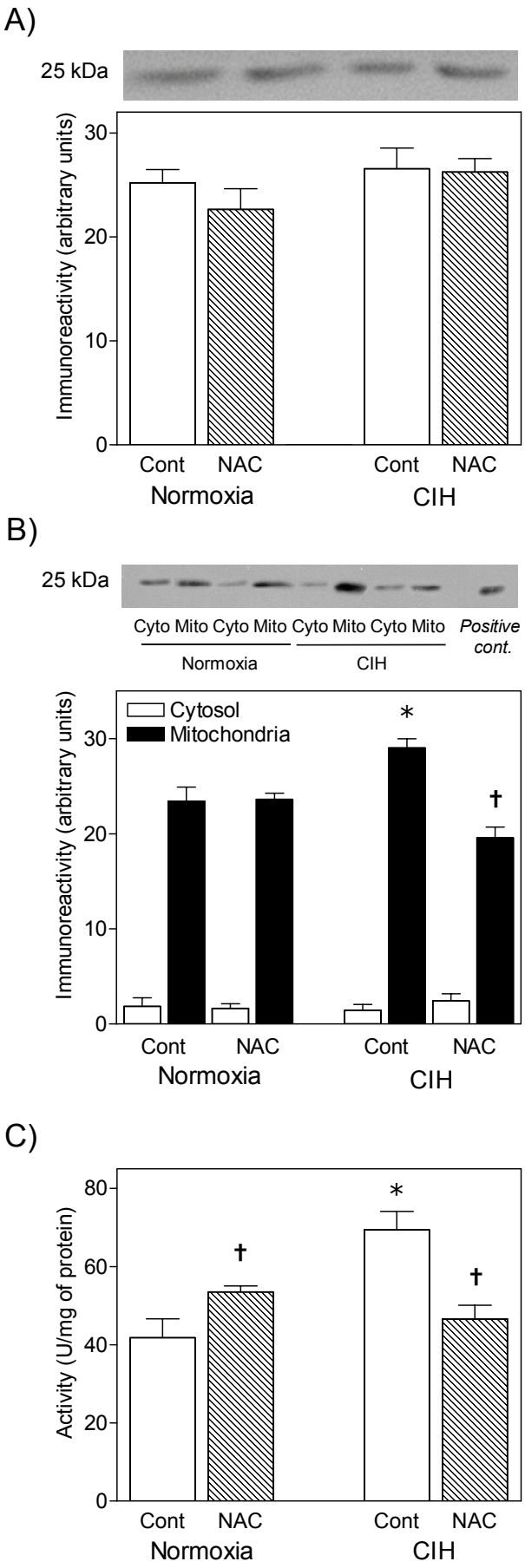

Fig. 1. Level of MnSOD in homogenate (**A**), mitochondrial and cytosolic fractions (**B**) and enzyme activity in the mitochondrial fraction (**C**) from the myocardium of control (Cont) and *N*-acetylcysteine-treated (NAC) rats adapted to CIH and of normoxic animals. Sample loading was normalized to equal protein concentration in individual samples (homogenate 5 μg, cytosol 15 μg, mitochondria 5 μg). Representative Western blots are shown. For details see *Materials and Methods*. Values are means ± S.E.M. from 6 hearts in each group. **P*<0.05 vs. corresponding normoxic group, †*P*<0.05 vs. corresponding untreated group.

Activity and expression of MnSOD

Neither CIH nor NAC treatment influenced the protein abundance of MnSOD in myocardial homogenate (Fig. 1A). On the other hand, both the activity and protein abundance of the enzyme were increased in the mitochondrial fraction of CIH hearts compared with the normoxic values (Fig. 1B, C). These effects of CIH were prevented by NAC. Interestingly, NAC treatment increased the MnSOD activity but not the expression in the mitochondrial fraction from normoxic tissue.

The low levels of MnSOD and PDH in cytosolic fractions from all experimental groups testify to good mitochondrial integrity. The protein abundance of PDH, as a mitochondrial marker, was decreased in homogenates of CIH hearts (Fig. 2A), but its abundance in the mitochondrial and cytosolic fractions was affected by neither CIH nor NAC treatment (Fig. 2B).

Correlation between MnSOD activity and infarct size

Relationship between the mean values of MnSOD activity in myocardial mitochondrial fraction and the mean infarct size normalized to the area at risk is presented in Fig. 3. Regression analysis demonstrated a negative linear relationship between MnSOD activity and infarct size with a correlation coefficient 0.93. Infarct size data were taken from our previous study (Kolář *et al*. 2007) using the same protocols of CIH and NAC treatment.

Discussion

In this study, we demonstrated that adaptation of adult rats to CIH significantly increased the activity and protein abundance of MnSOD in the mitochondrial fraction of LV myocardium but did not influence the expression of MnSOD in myocardial homogenate. This result is in line with our recent study which did not find any effect of CIH on the activity of MnSOD in myocardial homogenate (Kolář *et al*. 2007). The absence

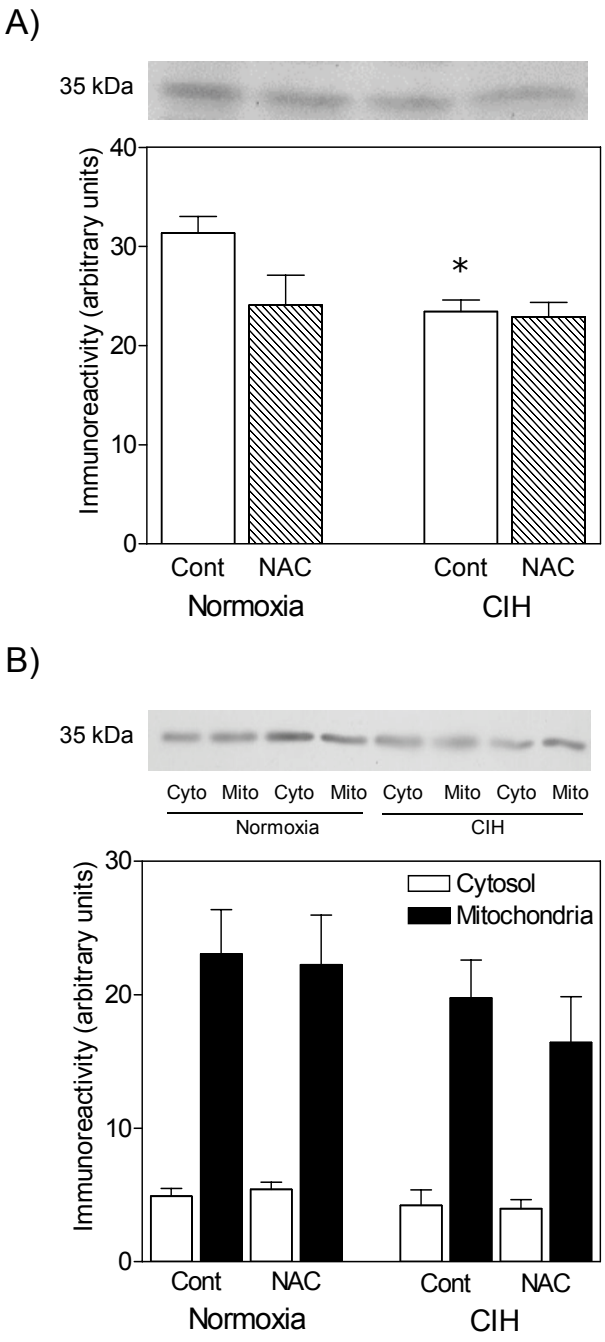

Fig. 2. Level of PDH in homogenate (**A**) and in cytosolic and mitochondrial fractions (**B**) from the myocardium of control (Cont) and *N*-acetylcysteine-treated (NAC) rats adapted to CIH and of normoxic animals. Sample loading was normalized to equal protein concentration in individual samples (homogenate 10 μg, cytosol 15 μg, mitochondria 8 μg). Representative Western blots are shown. Values are means ± S.E.M. from 6 hearts in each group. **P*<0.05 vs. corresponding normoxic group, †*P*<0.05 vs. corresponding untreated group.

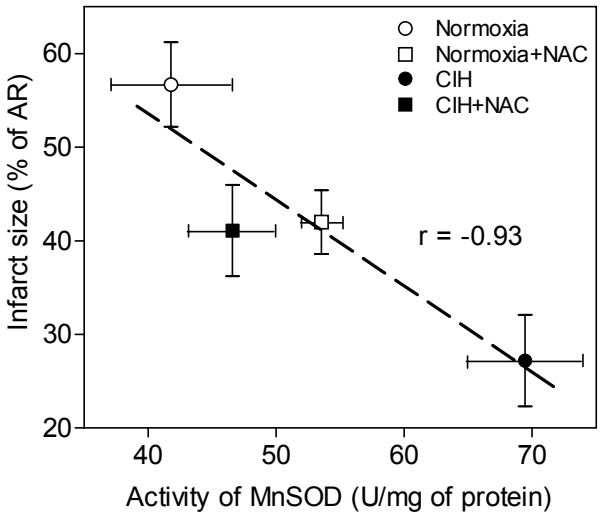

Fig. 3. Relationship between the mean values of MnSOD activity in myocardial mitochondrial fraction and the mean infarct size normalized to the area at risk (AR) in control and *N*-acetylcysteine-treated (NAC) rats adapted to CIH and in normoxic animals. Infarct size data were taken from our previous study (Kolář *et al.* 2007). Myocardial infarction was induced by a 20-min coronary artery occlusion and 3-h reperfusion in open-chest pump-ventilated animals and delineated by staining with triphenyltetrazolium chloride and potassium permanganate. r – correlation coefficient

of any effect on MnSOD in homogenate despite the significant up-regulation of the enzyme demonstrated in mitochondria can be possibly explained by a decrease in total mitochondrial mass caused by severe CIH. A decreased PDH level in the myocardial homogenate from CIH rats, shown in the present study, supports this view. Accordingly, Nouette-Gaulain *et al.* (2005) reported that CIH caused a global decrease in all OXPHOS complex activities in rat LV myocardium. Moreover, we showed previously that CIH reduced concentration of a mitochondrial inner membrane lipid marker cardiolipin in rat LV homogenate (Ježková *et al.* 2002).

The effects of CIH on MnSOD activity and protein abundance in the mitochondrial fraction were prevented by antioxidant NAC treatment during the adaptation period. These results conform to our recent observation that NAC attenuated the ROS-dependent cardioprotection induced by CIH (Kolář *et al.* 2007). However, at variance with our preliminary conclusion, the close negative correlation between MnSOD activity and myocardial infarct size suggests that this enzyme could possibly play a role in the improved mitochondrial ROS-buffering capacity and ischemic tolerance of chronically hypoxic hearts. Increased MnSOD activity was demonstrated also in the hearts of rats adapted to moderate hypoxia during early postnatal life (Zhu *et al.* 2004).

It is generally accepted that MnSOD represents the first line of cell defence against mitochondria-derived ROS and is involved in cardioprotection induced by

various stimuli (Hoshida *et al*. 2002). The MnSOD-inducing pathway in myocardial preconditioning involves ROS signalling as demonstrated in a number of studies (Lecour *et al*. 2006, Chen *et al.* 2008). The activity and expression of MnSOD were increased in a ROS-dependent manner in various forms of delayed preconditioning, and its important protective role against ischemia/reperfusion injury has been well established (Yamashita *et al.* 1999). It has been also reported that heat stress-induced preconditioning increased the expression of MnSOD mRNA in rats (Das *et al*. 1995) and total SOD activity in pig hearts (Liu *et al.* 1992). MnSOD elevation in the myocardium is supposed to be involved in the mechanism of cardioprotection induced by exercise (Hamilton *et al*. 2001). In agreement with these studies, Chen *et al*. (1998) demonstrated that overexpression of MnSOD in transgenic mice limited the infarct size.

Whereas numerous stimuli have been proposed to participate in the MnSOD up-regulation (Kiningham *et al*. 2004, Li *et al*. 1998, Oberley *et al*. 1987), no specific ROS-dependent transduction pathway leading to nuclear MnSOD gene induction has been described in the myocardium. Storz *et al*. (2005) suggested that the induction of the MnSOD gene with the subsequent increase in mitochondrial MnSOD expression in HeLa cells required transcription factor NF-κB initiated by ROS-activated protein kinase D. Other investigators demonstrated a dramatic elevation of mRNA and protein level of MnSOD induced by tumor necrosis factor-α and interleukin-1β in human skin fibroblasts and in mouse kidney, thymus, spleen and bone marrow tissues (Masuda *et al*. 1988, Wong *et al.* 1988). Interestingly, chronic hypoxia increased the level of pro-inflammatory cytokines in fetal guinea pig hearts (Oh *et al*. 2008) and in rat carotid bodies (Lam *et al*. 2008). PKC has been proposed as a further important player in the signaling pathway leading to MnSOD induction because PKC inhibitors prevented the up-regulation of MnSOD in the human adenocarcinoma cell line (Das *et al*. 1998). Accordingly, we observed that the induction of PKC-δ during the adaptation of rats to CIH was ROS-dependent because it was eliminated by NAC treatment (Kolář *et al.* 2007).

In the present study, NAC treatment completely prevented the increase in MnSOD activity and expression in mitochondria induced by CIH. It has also been reported that NAC abrogated the elevation of heat-stress protein 32 as another protective intracellular stress-responsive protein induced by the hypoxic stimulus (Borger *et al*. 1998). Heat-stress proteins in connection with MnSOD play a role in delayed cardiac preconditioning (Bolli *et al.* 2000). Suzuki *et al.* (2002) suggested that the rise in MnSOD activity is connected with the heat-stress protein 72-induced cardioprotection. In addition, heat-stress protein 72 acts as chaperone in the MnSOD maturation and its incorporation into mitochondria (Hoshida *et al*. 2002, Voos *et al.* 1993).

Interestingly, NAC treatment increased the activity of MnSOD in normoxic hearts without affecting its expression. Using the same experimental protocol, we observed a smaller infarct size in NAC-treated normoxic rats compared with untreated ones (Kolář *et al*. 2007). Menon *et al.* (2007) demonstrated that increased MnSOD activity was due to antioxidant properties of NAC. In addition, the restoration of MnSOD activity by NAC reduced oxidative stress and attenuated the development of myocardial dysfunction in diabetic rats (Xia *et al*. 2006). It appears likely that NAC acts as indirect antioxidant protecting MnSOD activity by preventing nitration of critical tyrosine residues in its active site (Barreiro *et al*. 2006, Navarro-Antolin *et al.* 2007, MacMillan-Crow *et al*. 1999).

It is known that MnSOD eliminates ROS more effectively in intact mitochondria than in those that are damaged (Raha *et al*. 2000). We therefore analyzed additional mitochondrial marker, PDH in mitochondrial and cytosolic fractions to confirm the integrity of isolated mitochondria and their vulnerability to disruption during the isolation procedure. Low levels of MnSOD and PDH in the cytosolic fraction from all experimental groups indicate good preservation of mitochondria during their isolation in this study. Neither CIH nor NAC treatment affected the sensitivity of the mitochondrial inner membrane to disruption.

In conclusion, the up-regulation and activation of mitochondrial MnSOD in close correlation with the reduction of myocardial infarct size suggest that this enzyme can contribute to the mechanism of CIH-induced tolerance against ischemia/reperfusion injury. Blunting of these effects by treatment with NAC supports the view that ROS-dependent signaling during hypoxic adaptation plays an important role in this form of cardioprotection.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

Supported by the research project MSM 0021620858 and grants GACR 305/07/0875 and 305/07/1008.

References

BARREIRO E, SANCHEZ D, GALDIZ JB, HUSSAIN SN, GEA J: N-acetylcysteine increases manganese superoxide dismutase activity in septic rat diaphragms. *Eur Respir J* **26**: 1032-1039, 2006.

BOLLI R: The late phase of preconditioning. *Circ Res* **87**: 972-983, 2000.

BORGER DR, ESSIG DA: Induction of HSP 32 gene in hypoxic cardiomyocytes is attenuated by treatment with N-acetyl-L-cysteine. *Am J Physiol* **274**: H965-H973, 1998.

CHEN CH, LIU K, CHAN JY: Anesthetic preconditioning confers acute cardioprotection via up-regulation of manganese superoxide dismutase and preservation of mitochondrial respiratory enzyme activity. *Shock* **29**: 300-308, 2008.

CHEN Z, SIU B, HO YS, VINCENT R, CHUA CC, HAMDY RC, CHUA BH: Overexpression of MnSOD protects against myocardial ischemia/reperfusion injury in transgenic mice. *Mol Cell Cardiol* **30**: 2281-2289, 1998.

DAS DK, MAULIK N, MORARU II: Gene expression in acute myocardial stress. Induction by hypoxia, ischemia, reperfusion, hyperthermia and oxidative stress. *J Mol Cell Cardiol* **27**: 181-193, 1995.

DAS KC, GUO XL, WHITE CW: Protein kinase C-δ dependent induction of manganese superoxide dismutase gene expression by microtubule-active anticancer drugs. *J Biol Chem* **273**: 34639-34645, 1998.

ELSTNER EF, YOUNGMAN RJ, OBWALD W: Superoxide dismutase. In: *Methods of Enzymatic Analysis*, vol. III. BERGMEYER HU (ed), Verlag Chemie, Weinheim, 1983, pp 293-302.

HAMILTON KL, POWERS SK, SUGIURA T, KIM S, LENNON S, TUMER N, MEHTA JL: Short-term exercise training can improve myocardial tolerance to I/R without elevation in heat shock proteins. *Am J Physiol Heart Circ Physiol* **281**: H1346-H1352, 2001.

HOSHIDA S, YAMASHITA N, OTSU K, HORI M: Repeated physiologic stresses provide persistent cardioprotection against ischemia-reperfusion injury in rats. *J Am Coll Cardiol* **40**: 826-831, 2002.

HUSSAIN SP, AMSTAD P, HE P, ROBLES A, LUPOLD S, KANEKO I, ICHIMIYA M, SENGUPTA S, MECHANIC L, OKAMURA S, HOFSETH LJ, MOAKE M, NAGASHIMA M, FORRESTER KS, HARRIS CC: p53-induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. *Cancer Res* **64**: 2350-2356, 2004.

JEŽKOVÁ J, NOVÁKOVÁ O, KOLÁŘ F, TVRZICKÁ E, NECKÁŘ J, NOVÁK F: Chronic hypoxia alters fatty acid composition of phospholipids in right and left ventricular myocardium. *Mol Cell Biochem* **232**: 49-56, 2002.

KININGHAM KK, DAOSUKHO C, CLAIR DK: IκBα (inhibitory κBα) identified as labile repressor of MnSOD (manganese superoxide dismutase) expression. *Biochem J* **384**: 543-549, 2004.

KOLÁŘ F, OŠŤÁDAL B: Molecular mechanisms of cardiac protection by adaptation to chronic hypoxia. *Physiol Res* **53** (Suppl 1): S3-S13, 2004.

KOLÁŘ F, JEŽKOVÁ J, BALKOVÁ P, BŘEH J, NECKÁŘ J, NOVÁK F, NOVÁKOVÁ O, TOMÁŠOVÁ H, SRBOVÁ M, OŠŤÁDAL B, WILHELM J, HERGET J: Role of oxidative stress in PKC-δ upregulation and cardioprotection induced by chronic intermittent hypoxia. *Am J Physiol Heart Circ Physiol* **292**: H224-H230, 2007.

LAM SY, TIPOE GL, LIONG EC, FUNG ML: Chronic hypoxia upregulates the expression and function of proinflammatory cytokines in the rat carotid body. *Histochem Cell Biol* **130**: 549-559, 2008.

LECOUR S, OWIRA P, OPIE LH: Ceramide-induced preconditioning involves reactive oxygen species. *Life Sci* **78**: 1702-1706, 2006.

LI JJ, OBERLEY LW, FAN M, COLBURN NH: Inhibition of AP-1 and NF-κB by manganese-containing superoxide dismutase in human breast cancer cells. *FASEB J* **12**: 1713-1723, 1998.

LI Y, HUANG TT, CARLSON EJ, MELOV S, URSELL PC, OLSON JL, NOBLE LJ, YOSHIMURA MP, BERGER C, CHAN PH, WALLACE DC, EPSTEIN CJ: Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* **11**: 376-381, 1995.

LIU X, ENGELMAN RM, MORARU II, ROUSOU JA, FLACK JE 3rd, DEATON DW, MAULIK N, DAS DK: Heat shock. A new approach for myocardial preservation in cardiac surgery. *Circulation* **86**: 358-363, 1992.

MACMILLAN-CROW LA, THOMPSON JA: Tyrosine modifications and inactivation of active site manganese superoxide dismutase mutant (Y34F) by peroxynitrite. *Arch Biochem Biophys* **366**: 82-88, 1999.

MASUDA A, LONGO DL, KOBAYASHI Y, APPELLA E, OPPENHEIM JJ, MATSUSHIMA K: Induction of mitochondrial manganese superoxide dismutase by interleukin 1. *FASEB J* **15**: 3087-3091, 1988.

McCORD JM, FRIDOVICH I: Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J Biol Chem* **244**: 6049-6055, 1969.

MENON SG, SARSOUR EH, KALEN AL, VENKATARAMAN S, HITCHLER MJ, DOMANN FE, OBERLEY LW, GOSWAMI PC: Superoxide signaling mediates N-acetyl-L-cysteine-induced G1 arrest: regulatory role of cyclin D1 and manganese superoxide dismutase. *Cancer Res* **67**: 6392-6399, 2007.

NAVARRO-ANTOLIN J, REDONDO-HORCAJO M, ZARAGOZA C, ALVAREZ-BARRIENTOS A, FERNANDEZ AP, LEON-GOMEZ E, RODRIGO J, LAMAS S: Role of peroxynitrite in endothelial damage mediated by Cyclosporine A. *Free Radic Biol Med* **42**: 394-403, 2007.

NOUETTE-GAULAIN K, MALGAT M, ROCHER C, SAVINEAU JP, MARTHAN R, MAZAT JP, SZTARK F: Time course of differential mitochondrial energy metabolism adaptation to chronic hypoxia in right and left ventricles. *Cardiovasc Res* **66**: 132-140, 2005.

OBERLEY LW, CLAIR DK, AUTOR AP, OBERLEY TD: Increase in manganese superoxide dismutase activity in the mouse heart after X-irradiation. *Arch Biochem Biophys* **254**: 69-80, 1987.

OH C, DONG Y, LIU H, THOMPSON LP: Intrauterine hypoxia upregulates proinflammatory cytokines and matrix metalloproteinases in fetal guinea pig hearts. *Am J Obstet* **199**: 1-6, 2008.

PETERSON GL: A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal Biochem* **83**: 346-356, 1977.

RAHA S, McEACHERN GE, MYINT AT, ROBINSON BH: Superoxides from mitochondrial complex III: the role of manganese superoxide dismutase. *Free Radic Biol Med* **29**: 170-180, 2000.

ROGERS RJ, MONNIER JM, NICK HS: Tumor necrosis factor α selectively induces MnSOD expression via mitochondria-to-nucleus signaling, whereas interleukin-1β utilizes an alternative pathway. *Biol Chem* **276**: 20419-20427, 2001.

SPITZ DR, OBERLEY LW: An assay for superoxide dismutase activity in mammalian tissue homogenates. *Anal Biochem* **179**: 8-18, 1989.

STARKOV AA: The role of mitochondria in reactive oxygen species metabolism and signaling. *Ann NY Acad Sci* **1147**: 37-52, 2008.

STORZ P, DÖPPLER H, TOKER A: Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species. *Mol Cell Biol* **19**: 8520-8530, 2005.

SUZUKI K, MURTUZA B, SAMMUT IA, LATIF N, JAYAKUMAR J, SMOLENSKI RT, KANEDA Y, SAWA Y, MATSUDA H, YACOUB MH: Heat shock protein 72 enhances manganese superoxide dismutase activity during myocardial ischemia-reperfusion injury, associated with mitochondrial protection and apoptosis reduction. *Circulation* **106**: I270-I276, 2002.

VOOS W, GAMBILL BD, GUIARD B, PFANNER N, CRAIG EA: Presequence and mature part of preproteins strongly influence the dependence of mitochondrial protein import on heat shock protein 70 in the matrix. *J Cell Biol* **123**: 119-126, 1993.

WISPE JR, CLARK JC, BURHANS MS, KROPP KE, KORFHAGEN TR, WHITSETT JA: Synthesis and processing of the precursor for human mangano-superoxide dismutase. *Biochim Biophys Acta* **994**: 30-36, 1989.

WONG GHW, GOEDDEL DV: Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. *Science* **242**: 941-944, 1988.

XIA Z, GUO Z, NAGAREDDY PR, YUEN V, YEUNG E, McNEILL JH: Antioxidant N-acetylcysteine restores myocardial Mn-SOD activity and attenuates myocardial dysfunction in diabetic rats. *Eur J Pharmacol* **544**: 118-125, 2006.

YAMASHITA N, HOSHIDA S, OTSU K, ASAHI M, KUZUYA T, HORI M: Exercise provides direct biphasic cardioprotection via manganese superoxide dismutase activation. *J Exp Med* **189**: 1699-1706, 1999.

ZHU WZ, DONG JW, DING HL, YANG HT, ZHOU ZN: Postnatal development in intermittent hypoxia enhances resistance to myocardial ischemia/reperfusion in male rats. *Eur J Appl Physiol* **91**: 716-722, 2004.