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Protective effects of N-(2-mercaptopropionyl)-glycine against ischemia–reperfusion injury in hypertrophied hearts

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ABSTRACT

The beneficial effects of N-(2-mercaptopropionyl)-glycine (MPG) against ischemia–reperfusion injury in normotensive animals have been previously studied. Our objective was to test the action of MPG during ischemia and reperfusion in hearts from spontaneously hypertensive rats (SHR). Isolated hearts from SHR and age-matched normotensive rats Wistar Kyoto (WKY) were subjected to 50-min global ischemia (GI) and 2-hour reperfusion (R). In other hearts MPG 2 mM was administered during 10 min before GI and the first 10 min of R. Infarct size (IS) was assessed by TTC staining technique and expressed as percentage of risk area. Postischemic recovery of myocardial function was assessed. Reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and SOD cytosolic activity — as estimators of oxidative stress and MnSOD cytosolic activity — as an index of (mPTP) opening were determined. In isolated mitochondria H_2O_2-induced mPTP opening was also measured. The treatment with MPG decreased infarct size, preserved GSH levels and decreased SOD and MnSOD cytosolic activities, TBARS concentration, and H_2O_2 induced-mPTP opening in both rat strains. Our results show that in both hypertrophied and normal hearts an attenuation of mPTP opening via reduction of oxidative stress appears to be the predominant mechanism involved in the cardioprotection against reperfusion injury MPG-mediated.

Introduction

The reactive oxygen species (ROS) have a dual role. At low levels ROS act as messengers in waterfalls of signaling (Allen and Tresini, 2000; Herrlich and Böhmer, 2000; Otani, 2004) but at high concentrations are deleterious (Sastre et al., 2000). Accumulated evidence has shown that ROS production is a key event in reperfusion injury (Ambrosio et al., 1991; Bolli et al., 1989b; Papaharalambus and Griendling, 2007; Zweier, 1988). In these conditions, ROS generation exceeds the antioxidant capacity of myocardium and oxidative stress occurs (Ferrari et al., 1992; Griendling and FitzGerald, 2003). Thus, different interventions that diminish the ROS production or increase the antioxidant systems attenuate the alterations produced by ischemia–reperfusion (Asimakis et al., 2002; Bandyopadhyay et al., 2004; Kutala et al., 2006; Marczin et al., 2003; Ozer et al., 2005).

Cardiovascular disease is a leading cause of death, and hypertension is a critical risk factor for cardiovascular events. Cardiac hypertrophy has been linked to the development of a variety of cardiovascular diseases, including myocardial ischemia, arrhythmias, and sudden cardiac death. As multiple mechanisms likely contribute to the development of hypertension, including angiotensin, oxidative stress and hemodynamic changes, multi-targeted therapeutic interventions will likely be required for effective management of hypertension. Previous results provide evidence about the protective role of antioxidants against reperfusion injury in hypertrophied hearts (Abebe et al., 2010; Potenza et al., 2007; Sahna et al., 2008).

Thiols, by virtue of their ability to be reversibly oxidized, are recognized as key components involved in the maintenance of redox balance. Previous experiments performed by us (Fantinelli et al., 2006) and others (Andreadou et al., 2011; Beyersdorf et al., 1989; Koerner et al., 1991; Mitsos et al., 1986; Tanonaka et al., 2003) using normotensive animals show that N-(2-mercaptopropionyl)-glycine (MPG), a synthetic thiol compound with ROS scavenging properties, exerts beneficial effects against irreversible and reversible reperfusion injuries. Opposite results showing the lack of cardioprotection of MPG were also reported (Miki et al., 1999; Venturini et al., 1998). However, the action of MPG in hypertrophied hearts from spontaneously hypertensive rats (SHR) has not yet been examined. The fact that MPG is potentially available for oral prophylactic therapy and is FDA approved for human clinical use (Trinchieri et al., 2004), justifies further analysis for their effects.

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On the other hand, recent studies propose that injurious role of ROS is associated to the formation of mitochondrial permeability transition pore (mPTP), key event in the cellular death (Halestrap et al., 2004). The opening of mPTP leads to the loss of internal mitochondrial membrane impermeability and to a rapid release of matrix components.

Therefore, the aim of our work was to determine the effects of MPG on infarct size, postischemic myocardial function, oxidative stress and mPTP opening produced by ischemia–reperfusion in hypertrophied hearts from SHR.

Material and methods

Isolated heart preparation

Experiments were conducted in 5-month-old SHR and age-matched normotensive male rats (Wistar Kyoto, WKY), which were originally derived from Charles River Breeding Farms, Wilmington, Mass. All animals were identically housed under controlled lighting and temperature conditions with free access to standard rat chow and tap water. The experiments were 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 in 1996) and to the guidelines laid down by the Animal Welfare Committee of La Plata School of Medicine.

Beginning at 12 weeks of age, systolic blood pressure (SBP) was measured weekly in all animals by the standard tail-cuff method (Buñag, 1973) following the modifications detailed in a recent paper by Fritz and Rinaldi (2008). Left ventricular hypertrophy (LVH) was evaluated by the ratio between heart weight (HW) and body weight (BW).

All rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg body wt). The heart was rapidly excised and perfused by the non-recirculating Langendorff technique with Ringer's solution containing (in mmol/L): 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.35 $CaCl_2$, 20 $NaCO_3H$ and 11.1 dextrose. The buffer was saturated with a mixture of 95% O_2–5% CO_2, had a pH of 7.4, and was maintained at 37 °C. The conductive tissue in the atrial septum was damaged with a fine needle to achieve atrioventricular block, and the right ventricle was paced at 280 ± 10 beats/min. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve; the opposite end of the tube was then connected to a Statham P23XL pressure transducer. The balloon was filled with water to provide an end-diastolic pressure (LVEDP) of 8–12 mm Hg and this volume remained unchanged for the rest of the experiment. Coronary perfusion pressure (CPP) was monitored at the point of cannulation of the aorta and adjusted to approximately 70 mm Hg. Coronary flow (CF), controlled with a peristaltic pump, was 11 ± 2 mL/min. Left ventricular pressure (LVP) and CPP data were acquired by using an analog-to-digital converter and acquisition software (Chart V4.2.3 ADInstruments).

Experimental protocols

After 10 min of stabilization, hearts from WKY rats and SHR were assigned to the following experimental protocols:

1) Control hearts (C, n = 6 for each rat strain): Hearts were perfused for 3 h without any treatment.
2) Ischemic control hearts (IC, n = 7 for each rat strain): Hearts were subjected to 50 min of normothermic global ischemia followed by 2 h of reperfusion. Global ischemia was induced by stopping the perfusate inflow line and the heart was placed in a saline bath held at 37 °C.
3) MPG (n = 7 for each rat strain): Hearts were treated 10 min before ischemia and during the first 10 min of reperfusion with N-(2-mercaptopropionyl)-glycine (MPG). The administration time for MPG was chosen to attenuate the ROS production during ischemia and reperfusion. The dose was selected according to previous experiments performed in our laboratory (Fantinelli et al., 2006).

Additional experiments were performed (n = 6 for each protocol and for each rat strain) to assess the biochemical parameters.

Infarct size determination

Infarct size was assessed by the widely validated triphenyltetrazolium chloride (TTC) staining technique (Fishbein et al., 1981). At the end of reperfusion, atrial and right ventricular tissues were excised and left ventricle (VI) was frozen. The freeze VI was cut into six transverse slices, which were incubated for 5 min at 37 °C in a 1% solution of triphenyltetrazolium chloride (TTC). To measure myocardial infarction, the slices were weighed and scanned. The infarcted (pale) and viable ischemic/reperfused (red) areas were measured by computed planimetry (Scion Image 1.62; Scion Corp., Frederick, Maryland, USA). Infarct weights were calculated as $(A1\times W1)+(A2\times W2)+(A3\times W3)+(A4\times W4)+(A5\times W5)+(A6\times W6)$, where A is the infarct area for the slice and W is the weight of the respective section. Infarct size was expressed as a percentage of the total area (area at risk, AAR) (Suzuki et al., 2001).

Systolic and diastolic function

Myocardial contractility was assessed by the left ventricular developed pressure (LVDP), obtained by subtracting LVEDP to LVP peak, and maximal velocity of contraction ($+dP/dt_{max}$). The diastolic function was evaluated through LVEDP.

Assessment of coronary resistance (CR)

CR was calculated as a quotient between CPP and CF and expressed as difference between the values obtained at the end of reperfusion period and that observed in the preischemic period.

Preparation of tissue homogenate

At the end of reperfusion a portion of VI was homogenized in 5 vol. of 25 mM PO_4KH_2–140 mM ClK at pH = 7.4 with a Polytron homogenizer. Aliquots of the homogenate were used to assess reduced glutathione content (GSH) and lipid peroxidation. The remaining homogenate was centrifuged at 12,000 × *g* for 5 min at 4 °C and the supernatant stored at −70 °C until superoxide dismutase (SOD) activity was assayed.

Assessment of reduced glutathione (GSH)

GSH was determined by Ellman's method (Sedlak and Lindsay, 1968). This method was based on the reaction of GSH with 5,5′ dithiobis(2-nitrobenzoic acid) to give a compound that absorbs at 412 nm. GSH levels were expressed as μg/mg of protein.

Assessment of lipid peroxidation

The concentration of thiobarbituric acid reactive substances (TBARS) was determined in the supernatant following the Buege and Aust method (Buege and Aust, 1978). Absorbance at 535 nm was measured and TBARS expressed in nmol/g of tissue using an extinction coefficient of $1.56\times105\ M^{-1}\ cm^{-1}$.

Measurement of SOD and MnSOD cytosolic activity

Superoxide dismutase (SOD) activity was measured by means of the nitroblue tetrazolium (NBT) method (Beauchamp and Fridovich,

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1971). Briefly, the supernatant was added to the reaction mixture of NBT with xanthine–xanthine oxidase, and SOD (SOD) activity measured colorimetrically as inhibition of blue formazan formation in the reaction mixture. For measuring MnSOD activity, 5 mmol/l KCN was added to inhibit Cu–ZnSOD activity.

Protein determination

The protein concentration was evaluated by the Bradford method (Bradford, 1976) using bovine serum albumin as a standard.

Isolation of rat heart mitochondria

The hearts were immediately removed from SHR and WKY rats and mitochondria from left ventricle (LV) were isolated as described by Mela and Seitz (1979). Briefly, LV were washed and homogenized in ice-cold isolation solution (IS) consisting of 75 mM sucrose, 225 mM mannitol, and 0.01 mM EGTA neutralized with Trizma buffer at pH 7.4. After the tissue pieces were settled, the entire supernatant was discarded and fresh IS (5 ml) was added, and the mixture was transferred to a hand homogenizer. Proteinase (0.8 mg, bacterial, type XXIV, Sigma, formerly called Nagarse) was added just before starting the homogenization procedure. The whole homogenization procedure took no longer than 14 min in two steps of 7 min each (with 5 ml addition of fresh IS each). The homogenate was carefully transferred after each step to a polycarbonate centrifuge tube. After 5 min of 480 *g* of centrifugation to discard unbroken tissue and debris, the supernatant was centrifuged at 7700 *g* for 10 min to sediment the mitochondria. The mitochondrial pellet was washed twice with IS and the last one with suspension solution (IS without EGTA) at 7700 g for 5 min each.

H_2O_2-induced mPTP opening

The ability of the mitochondria to resist swelling was assessed by incubating isolated mitochondria in a buffer containing (in mmol/L): 120 KCl, 20 MOPS, 10 Tris HCl and 5 KH_2PO_4 (Baines et al., 2003), adjusted to pH = 7.4. After 5-min preincubation, the mitochondria energized with the addition of 6 mM succinate were induced to swell with 3 mM H_2O_2 (Takayasu et al., 2007). If the mPTP is open in the presence of H_2O_2, solutes will be free to enter the inner matrix, causing the mitochondria to swell. These changes are observed as decreases of light scattering and followed using a temperature-controlled Hitachi F4500 spectrofluorometer operating with continuous stirring at excitation and emission wavelengths of 520 nm (Facundo et al., 2007). Light scattering decrease (LSD) was calculated for each sample by taking the difference of scattered light between before and after the addition of H_2O_2, in the presence and absence of MPG. In order to relate mPTP opening to decreased light scattering, we added cyclosporine (CsA) 1 μM to inhibit mPTP or to abolish any observed reduction. Thus, the H_2O_2-induced swelling was inhibited by Cs A (data not shown).

Statistical analysis

Data are presented as mean ± SE and repeated measures of two-way analysis of variance (ANOVA) with Newman–Keuls test were used for multiple comparisons among groups. A p value <0.05 was considered significant.

Results

At 5 months of age SHR have higher values of SBP (205 ± 5 mm Hg) and LVH (4.82 ± 0.15) in comparison to WKY (SBP = 122 ± 5 mm Hg; LVH = 1.42 ± 0.04). Fifty minutes of global ischemia followed by 2-hour reperfusion in rat hearts caused an infarct size of ~60% in SHR and ~40% in WKY. A significant reduction of infarct size in both rat strains was obtained when 2 mM MPG was added to the perfusate during 10 min before ischemia and during the first 10 min of reperfusion (Fig. 1).

Fig. 2 shows the effects of MPG treatment on systolic and diastolic myocardial functions. At the end of 3 h of perfusion the contractility of hearts from SHR and WKY rats decreased ~10% in comparison to baseline values. After ischemia and reperfusion contractility decreased more than 90% of the pre-ischemic value in hearts of both rat strains. MPG treatment significantly improved post-ischemic recovery of myocardial function only in SHR reaching an LVDP value of ~6% of the pre-ischemic value. A similar pattern was observed when $+dP/dt_{max}$ values were analyzed. Diastolic stiffness was evaluated by measuring LVEDP which was initially settled at ~10 mm Hg at the end of the stabilization period in the different experimental groups. In non-ischemic hearts after 3 h of perfusion LVEDP reached values similar to baseline (13 ± 3 mm Hg for SHR and 10 ± 1 mm Hg for WKY). LVEDP significantly increased in ischemic control hearts of both rat strains. At the end of reperfusion period LVEDP of SHR hearts reached values significantly higher than WKY. This increase was significantly attenuated by MPG treatment only in SHR hearts. Analysis of the data obtained in WKY rats shows that MPG did not exert protective effect on postischemic recovery of myocardial function.

At the end of perfusion period CR reached values not different to baseline (7.3 ± 0.4 and 5.9 ± 0.5 mm Hg/ml × min^{-1} for SHR and

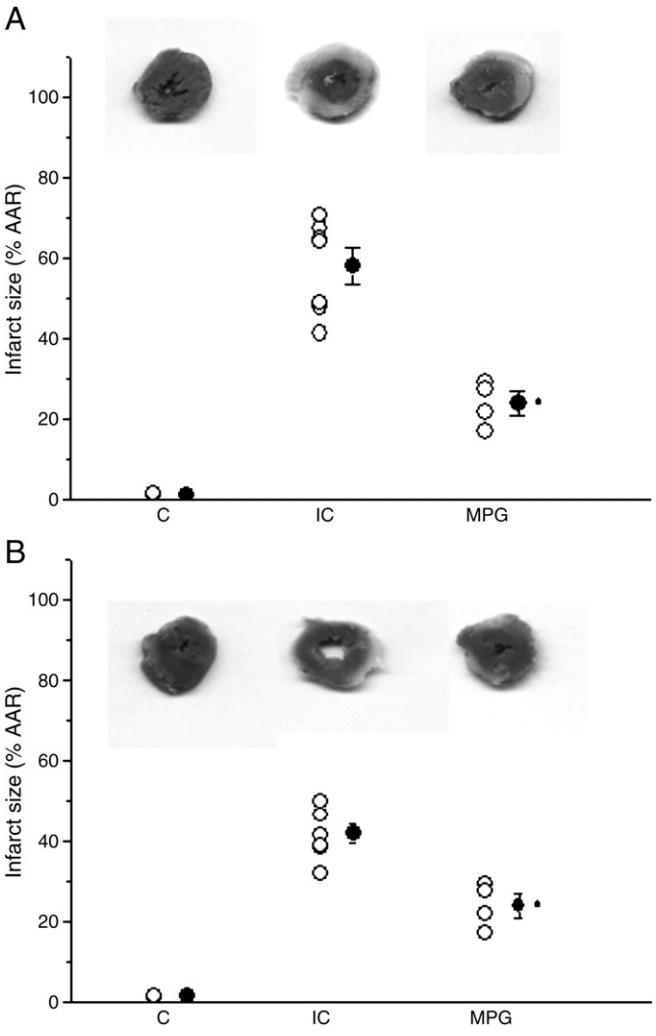

Fig. 1. Infarct size, expressed as percentage of area at risk (AAR), in control (C), ischemic control (IC) and MPG groups. A panel: SHR; B panel: WKY. Observe that the infarct size of SHR was significantly higher than WKY and that MPG diminished the infarct size in both rat strains. *p<0.05 with respect to IC.

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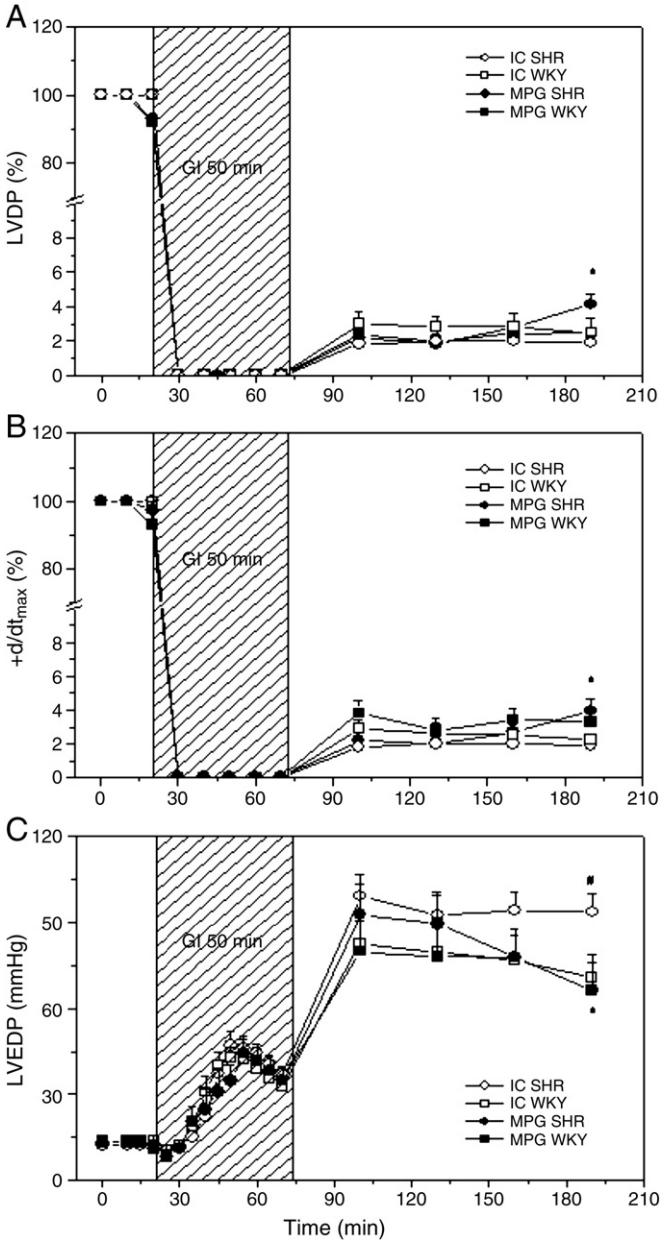

Fig. 2. Time course of left ventricular developed pressure (LVDP, A panel), maximal velocity of contraction ($+dP/dt_{max}$, B panel) and left ventricular end diastolic pressure (LVEDP, C panel) during ischemia and reperfusion in ischemic control (IC) and MPG groups for SHR and WKY rats. LVDP and $+dP/dt_{max}$ were expressed as percentage of preischemic values and LVEDP in mm Hg. MPG improved the myocardial function only in SHR hearts. *p<0.05 with respect to IC; #p<0.05 SHR vs. WKY.

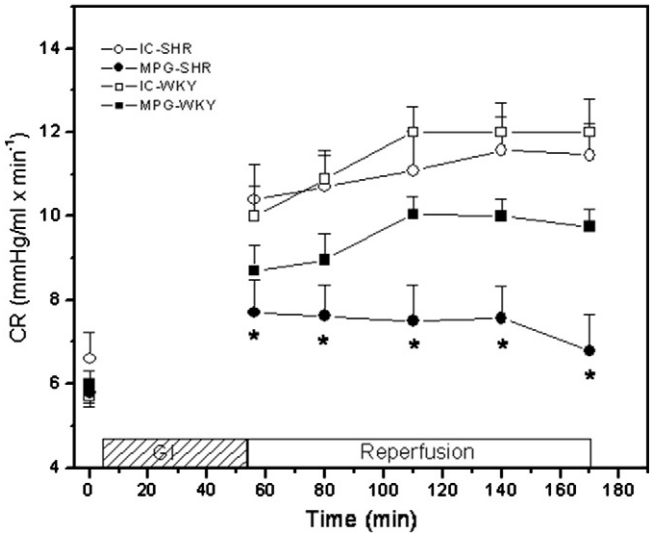

Fig. 3. Changes of coronary resistance (CR), expressed in mm Hg/ml × min^{-1} during the reperfusion period in ischemic control (IC) and MPG treated hearts from SHR and WKY. Note that the increase of CR produced during reperfusion was significantly attenuated by MPG only in SHR hearts. * p<0.05 with respect to IC.

WKY, respectively). The changes of CR during reperfusion in hearts from both rat strains untreated and treated with MPG are shown in Fig. 3. Similar increases of CR during reperfusion were detected in ischemic control hearts from SHR and WKY rats. However, the treatment with MPG attenuated these increases only in SHR. These results show that MPG exerted protective effect against postischemic vascular damage solely in hypertrophied hearts.

Given that the decrease of endogenous antioxidant systems could be the cause of oxidative stress we assessed the effects of MPG on GSH content and SOD activity. Firstly Fig. 4 shows that control hearts of SHR possess lower GSH content than WKY. These levels were significantly diminished after ischemia and reperfusion in both rat strains showing SHR hearts the lowest values. The treatment with MPG preserved the GSH content in both rat strains but these values were similar to those observed in non-ischemic hearts only in SHR (Fig. 4, A panel). Moreover, the SOD cytosolic activity increased in ischemic control hearts from both rat strains but a significant diminution by MPG was only detected in SHR hearts (Fig. 4, B panel). Lipid peroxidation – assessed by TBARS – increased in ischemic control hearts of both rat strains showing SHR hearts the highest values. MPG treatment produced a decrease in TBARS in hearts from SHR and WKY rats reaching values not different to those observed in non-ischemic hearts (Fig. 4, C panel). Altogether these data are indicating the presence of a higher oxidative stress in SHR compared to WKY rats which was attenuated by MPG treatment.

As shown in Fig. 5 a significant level of MnSOD cytosolic activity was detected in non-ischemic hearts from SHR indicating – taking into account the suggestions reported by Jin et al. (2005) – that a degree of mPTP opening is present even in basal conditions. This was not observed in WKY. When hearts were submitted to ischemia and reperfusion the MnSOD cytosolic activity increased in hearts from both rat strains. These increases were significantly attenuated by MPG treatment.

Fig. 6 shows the effects of MPG on H_2O_2-induced mPTP opening in isolated mitochondria of hearts from SHR and WKY rats. Addition of 3 mM H_2O_2 caused a significant decrease in the absorbance which was significantly attenuated by MPG treatment in both rat strains.

Discussion

This study shows that MPG protects hypertrophied hearts from SHR against ischemia–reperfusion injury decreasing the infarct size, improving the postischemic recovery of myocardial and vascular function and attenuating the oxidative stress. Similar beneficial effects after MPG treatment were also observed in hearts from normotensive WKY rats.

In physiological conditions, ROS are principally formed in mitochondria and are eliminated by the endogenous antioxidant systems (Andreyev et al., 2005). In pathological conditions, such as ischemia and reperfusion, the ROS production exceeds the capacity of antioxidant systems resulting in oxidative damage (Ambrosio et al., 1991; Bolli et al., 1989b; Ferrari et al., 1992; Papaharalambus and Griendling, 2007; Zweier, 1988). The cardioprotective role of some antioxidants including thiol compounds against reperfusion injury in normotensive groups has been previously reported (Asimakis et al., 2002; Bandyopadhyay et al., 2004; Bolli et al., 1989a; Dhalla

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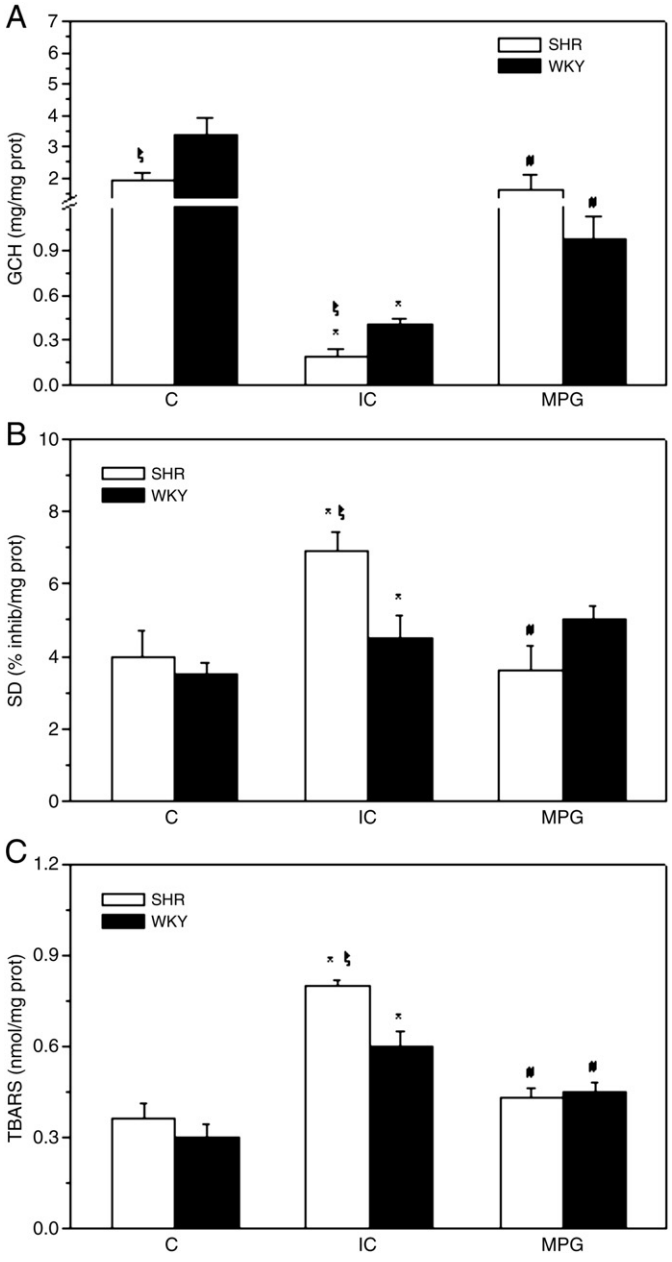

Fig. 4. Reduced glutathione content (GSH, A panel), expressed in μg/mg prot, superoxide dismutase (SOD, B panel), expressed as percentage of inhibition/mg prot and thiobarbituric acid reactive substance concentration (TBARS, C panel), expressed in nmol/mg prot in control (C), ischemic control (IC) and MPG groups for SHR and WKY. MPG treatment produced a preservation of GSH and lesser lipid peroxidation in comparison to IC in both rat strains. A decrease of SOD cytosolic activity by MPG was only detected in SHR hearts. *p<0.05 with respect to C; # p<0.05 with respect to IC; ξp<0.05.

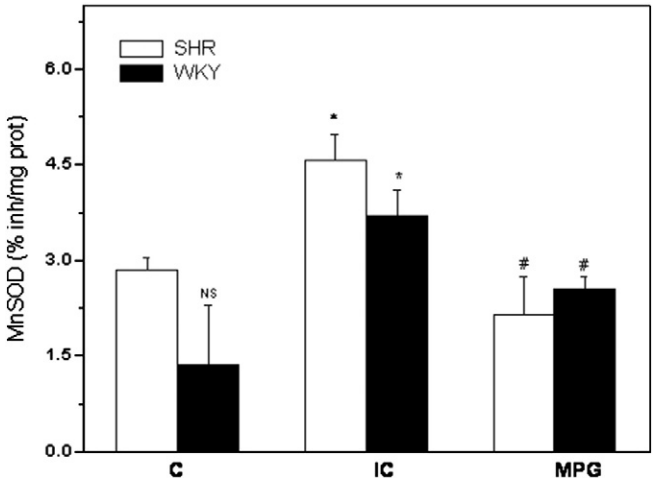

Fig. 5. Cytosolic activity of MnSOD (expressed as percentage of inhibition/mg prot) – as an index of mPTP opening – in control (C), ischemic control (IC) and MPG in hearts from SHR and WKY. Note that the cytosolic activity of MnSOD was not statistically significant in C hearts from WKY and that MPG attenuated the increase detected in IC hearts in both rats strains. *p<0.05 with respect to C; #p<0.05 with respect to IC.

et al., 2000; Kutala et al., 2006; Marczin et al., 2003; Mitsos et al., 1986; Ozer et al., 2005; Vento et al., 2003). However, in hypertrophied hearts even though oxidative stress is involved in the genesis of hypertension (Álvarez et al., 2008; Beal, 2002; Sawyer et al., 2002) the effects of antioxidants in situations of ischemia and reperfusion have not been sufficiently examined. In rats with abdominal aorta coarctation the addition of SOD and catalase to the line of perfusion attenuated the postischemic damage (Kirshenbaum and Singal, 1993). In a previous study we demonstrated that acute treatment with a non-alcoholic extract of red wine induces cardioprotection against ischemia–reperfusion injury to hearts from SHR (Fantinelli and Mosca, 2007). A similar result was obtained by Potenza et al. (2009) using polyphenols derived from green tea. A decrease of infarct size after MPG treatment was also recently showed in a study performed in spontaneously hypertensive stroke-prone rats (Yano et al., 2011). Concerning to myocardial

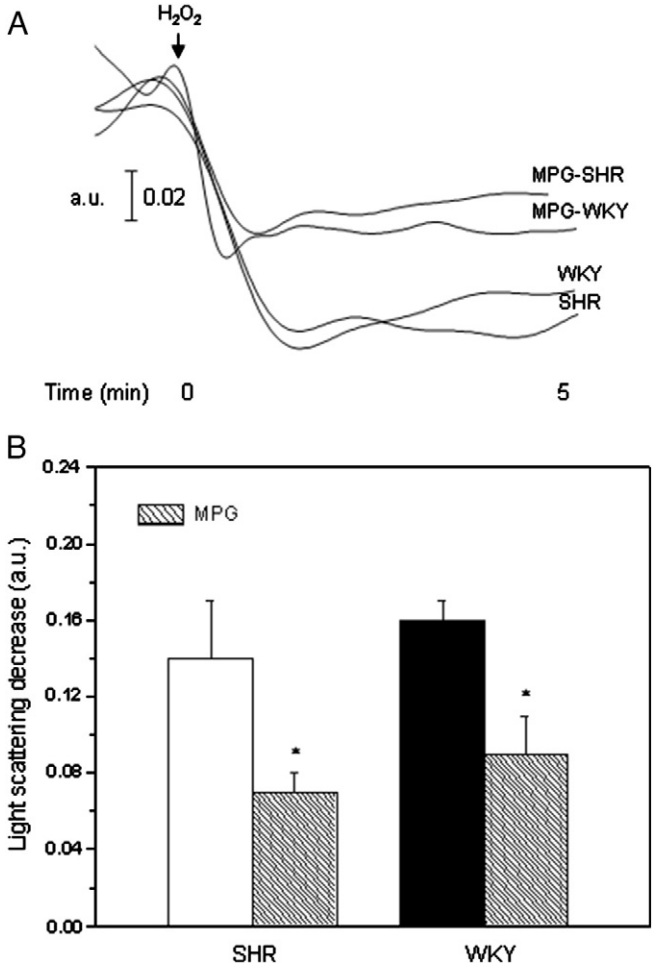

Fig. 6. A: Typical traces produced by 3 mM H_2O_2 in addition to mitochondrial suspensions of SHR and WKY rats. B: Mean values of the light scattering decreases (LSD) after H_2O_2 addition, expressed in arbitrary units (a.u.). Observe that LSD of both rat strains was similar and it was significantly attenuated by MPG. *p<0.05 vs. without any treatment.

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function we showed that MPG promoted a small but significant improvement of systolic postischemic recovery in hearts from SHR. This effect could be consequent to the attenuation of diastolic stiffness produced by the drug and/or to the presence of a high proportion of stunned myocardium in hearts of hypertensive rats. Therefore the absence of protective action of MPG on diastolic function and/or a low degree of stunned myocardium could be the cause of the lack of beneficial effect of the drug on postischemic contractility in WKY rats.

Simultaneously, hearts from both rat strains treated with MPG showed a decrease in myocardial TBARS content indicating that the beneficial actions of the drug are associated to diminution of injurious role of ROS. However, this effect of MPG was not found in previous studies performed in cardiomyopathic hamster heart and Dahl salt-sensitive hypertensive rats (Kyoi et al., 2006; Matsuhisa et al., 2008). These contradictory results could be attributed to differences in species and experimental design which can determine the appearance of harmful or beneficial role of ROS. Taking into account a previous report (Amersi et al., 2002) showing that thiol donors are extremely efficient in minimizing ROS-mediated signaling, the beneficial role of MPG demonstrated in this study would be linked to the decrease of ROS as aggressive agents.

A diminution of endogenous antioxidant systems during ischemia and reperfusion has been previously documented (Haramaki et al., 1998; Leichtweis and Ji, 2001). In our experimental conditions, GSH content decreased and SOD cytosolic activity increased after ischemia and reperfusion in both rat strains showing SHR the greatest changes. MPG treatment modified those parameters. Thus, GSH content was higher and SOD cytosolic activity lower than the values observed in untreated hearts. These results are in concordance with previous studies realized in normotensive animals (Ambler et al., 2008; Amersi et al., 2002; Horwitz and Sherman, 2001) and provide evidence to that thiol group of MPG which is contributing to the maintenance of endogenous defense systems. The favorable changes in GSH and SOD were reflected in the lower lipid peroxidation detected in hearts treated with MPG in comparison to untreated hearts and are evidence of an attenuation of oxidative stress in the presence of the drug.

On the other hand, a balance between the production of NO and ROS controls the endothelial function (Cai and Harrison, 2000; Kodja and Harrison, 1999). When the NO production is normal its bioavailability may be reduced because of the oxidative inactivation by an excessive production of superoxide ($O_2^{-\bullet}$) in the vascular wall. During ischemia and reperfusion the ROS production increases and NO availability diminishes leading to myocardial and coronary microvascular dysfunction. Data from the present study show that ischemia–reperfusion produced an increase of coronary resistance in both rat strains which was attenuated by MPG treatment only in SHR hearts. This result could be attributed to a higher bioavailability of NO in presence of MPG leading to a normalization of endothelial function. In other words, the MPG scavenging activity unmasks the known vasodilator effect of NO (Schulz and Triggle, 1994). Taking into account recent data obtained by Potenza et al. (2009) the vasodilator effect mediated by MPG could partially explain the beneficial effects of the drug on the myocardium. Contrarily, in WKY rats MPG did not modify the coronary resistance suggesting that other factors rather than the attenuation of oxidative stress are involved in the protection of the drug against postischemic vascular damage. Through these data we can also hypothesize that the diminution of infarct size by MPG in WKY rats could be a direct effect on myocardium without any contribution of vascular component.

The shift of mitochondria redox to a more reduced state (e.g. greater GSH) after MPG would inhibit to the mPTP opening. Several protective mechanisms are intrinsic to mitochondria, including modulation of ROS generation and/or regulation of signaling pathways that impact on the PTP (Javadov et al., 2003). Overexpression of MnSOD, either in transgenic models or by adenoviral gene transfer, has provided direct evidence that MnSOD is a key component of cardioprotection during ischemia–reperfusion injury (Abunasra et al., 2001; Chen et al., 1998). In this study, we tested the hypothesis that the loss of internal mitochondrial membrane impermeability should lead to MnSOD release, a response that was previously studied in myocardial tissue by Jin et al. (2005). In our experimental design 50-min global ischemia followed by 2-hour reperfusion applied to hearts from SHR and WKY resulted in an increase in MnSOD cytosolic activity which was attenuated by treatment with MPG. These results indicate a lesser mPTP opening in treated than untreated hearts. Results obtained in the present study in isolated mitochondria provide evidence that the scavenging activity of MPG is implicated in the attenuation of mPTP opening reinforcing the idea of mitochondria as target of cardioprotective action of MPG. These data are consistent with previous observations in normotensive animals (Tanonaka et al., 2003) and with a recent study performed in spontaneously hypertensive stroke-prone rats (Yano et al., 2011).

Regarding metabolic aspects it is recognized that in all clinically relevant situations of ischemia fatty acid levels are often elevated causing more rapid or severe deterioration of the myocardium. Among the adverse effects of fatty acids an activation of Na^+/Ca^{2+} exchanger reverse mode has been previously reported (Riedel et al., 2006). Interestingly, fatty acids are also increased in hypertrophy (Finck et al., 2003). Therefore, in our experimental preparation using hypertrophied hearts would be possible to find an increase of that chemical species. Additionally, a recent paper showed an attenuation of intracellular Ca^{2+} overload MPG-mediated (Saini-Chohan and Dhalla, 2009). Taking into account these considerations a diminution of deleterious effects of fatty acids and/or Ca^{2+} concentration could be possible mechanisms implicated in the cardioprotective action of MPG detected in the present study.

Conclusions

We conclude that MPG diminishes the deleterious effects produced by ischemia and reperfusion in hypertrophied hearts from SHR which appear to be associated to a decrease of mPTP formation and/or opening via an oxidative stress reduction. Similar beneficial effects on myocardium but not in vascular tissue were obtained in age-matched normotensive WKY rats. Thus, the protective effects of MPG appear to be more pronounced in SHR hearts probably due to increased oxidative stress present in this rat strain. Taking into account that MPG was approved for human clinical use (Trinchieri et al., 2004), these results have significant therapeutic implications.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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