J Bioenerg Biomembr (2014) 46:347–355
DOI 10.1007/s10863-014-9551-2

Effects of methylglyoxal and pyridoxamine in rat brain mitochondria bioenergetics and oxidative status

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Received: 31 March 2014 / Accepted: 2 May 2014 / Published online: 17 May 2014
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Abstract Advanced glycation end products (AGEs) and methylglyoxal (MG), an important intermediate in AGEs synthesis, are thought to contribute to protein aging and to the pathogenesis of age-and diabetes-associated complications. This study was intended to investigate brain mitochondria bioenergetics and oxidative status of rats previously exposed to chronic treatment with MG and/or with pyridoxamine (PM), a glycation inhibitor. Brain mitochondrial fractions were obtained and several parameters were analyzed: respiratory chain [states 3 and 4 of respiration, respiratory control ratio (RCR), and ADP/O index] and phosphorylation system [transmembrane potential ($\Delta\Psi_m$), ADP-induced depolarization, repolarization lag phase, and ATP levels]; hydrogen peroxide (H_2O_2) production levels, mitochondrial aconitase activity, and malondialdehyde levels as well as non-enzymatic antioxidant defenses (vitamin E and glutathione levels) and enzymatic antioxidant defenses (glutathione disulfide reductase (GR), glutathione peroxidase (GPx), and manganese superoxide dismutase (MnSOD) activities). MG treatment induced a statistical significant decrease in RCR, aconitase and GR activities, and an increase in H_2O_2 production levels. The administration of PM did not counteract MG-induced effects and caused a significant decrease in $\Delta\Psi_m$. In mitochondria from control animals, PM caused an adaptive mechanism characterized by a decrease in aconitase and GR activities as well as an increase in both α-tocopherol levels and GPx and MnSOD activities. Altogether our results show that high levels of MG promote brain mitochondrial impairment and PM is not able to reverse MG-induced effects.

Keywords Brain mitochondria · Methylglyoxal · Oxidative stress · Pyridoxamine

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Introduction

Glycation and formation of advanced glycation endproducts (AGEs) result from an endogenous process that leads to post-translational modifications of proteins with evidence suggesting a role for AGEs in the development of degenerative conditions, including cataracts, diabetic complications and Alzheimer's disease. Among the many reactive carbonyl compounds and AGE precursors, methylglyoxal (MG) contributes significantly to intracellular AGEs formation (Edeas et al. 2010). MG is a highly toxic glycating agent constantly produced by degradation of triosephosphates, which readily damages proteins by reacting with amino and guanidino groups of lysine and arginine residues, respectively (Edeas et al. 2010; Rabbani and Thornalley 2008). Basically, glucose can react reversibly with protein amino groups, resulting in Schiff's base formation which, in turn, can rearrange to form an Amadori product. The Amadori product can subsequently degrade into dicarbonyl compounds with MG being one of

the most important of them (Skamarauskas et al. 1996). Previous studies have demonstrated that MG is capable of inducing apoptosis in hippocampal neurons through both mitochondrial and Fas-receptor pathways (Di Loreto et al. 2008). Furthermore, advanced glycation and oxidative stress are closely linked (Wang et al. 2007), and both phenomena are referred to as "glycoxidation". All steps of glycoxidation generate reactive oxygen species, some of them intersecting with lipidic peroxidation pathways (Reddy and Beyaz 2006). It is known that oxidative stress, AGEs products and apoptosis are involved in the impairment of cognitive processes (Markesbery 1997; Nagy and Esiri 1997), which allows to hypothesize that MG cytotoxicity may be responsible for the related impairment of cognitive functions (Desai et al. 2010). Indeed, higher levels of serum MG have been linked to a faster rate of cognitive decline and may be indicative of the initiation of brain cell injury before clinically evident cognitive compromise (Beeri et al. 2010).

Pyridoxamine (PM) is one of three natural occurring forms of vitamin B6, the other two being pyridoxal and pyridoxine; and is a critical transient intermediate in catalysis of transamination reactions by vitamin B6-dependent enzymes (Edeas et al. 2010). PM treatment has been shown to reveal a significant protective effect in rat models of diabetes (Alderson et al. 2003; Metz et al. 2003), accompanied by a substantial decrease in plasma levels of the reactive carbonyl compounds glyoxal and MG (Nagaraj et al. 2002).

In this work, we aimed to evaluate the effects of MG and PM in brain mitochondrial function and oxidative status. For that purpose, several parameters were appraised: respiratory chain parameters [states 3 and 4 of respiration, respiratory control ratio (RCR), and ADP/O index], phosphorylation system [transmembrane potential ($\Delta\Psi_m$), ADP-induced depolarization, repolarization lag phase, and ATP levels], malondialdehyde (MDA) levels, H_2O_2 production rate, mitochondrial aconitase activity, non-enzymatic [glutathione-to-glutathione disulfide (GSH/GSSG) ratio, α-tocopherol levels] and enzymatic [glutathione peroxidase (GPx), glutathione disulfide reductase (GR) and manganese superoxide dismutase (MnSOD) activities] antioxidant defenses.

Materials and methods

Chemicals Methylglyoxal (2-oxopropanal) and pyridoxamine were obtained from Sigma (St. Louis, MO, USA). All the chemicals were of the highest grade of purity commercially available.

Animals treatment Male Wistar rats (12-weeks-old) were obtained from the local breeding colony in Coimbra (Portugal) and were maintained under controlled light (12 h day/night cycle) and humidity with free access to water and powdered rodent chow. Rats were orally treated with MG during 10 weeks. In the first 6 weeks the treatment consisted in 50 mg/Kg body weight/day that was changed to 60 mg/Kg body weight/day in the last 4 weeks. After this period of time, the rats were randomly divided in two groups and one group was orally treated with 1 g/l of PM during 4 weeks. The same treatment was given to one group of Wistar control rats to evaluate the effects of PM treatment per se. Animal handling and sacrifice followed the procedures approved by the Federation of European Laboratory Animal Science Associations (FELASA).

Determination of blood glucose levels Blood glucose concentration was determined from the tail vein using a commercial glucometer (Glucometer-Elite, Bayer, Portugal).

Preparation of brain mitochondrial fraction Brain mitochondria were isolated from rats according to Moreira et al. (Moreira et al. 2001, 2002), as previously described (Cardoso et al. 2013). The final mitochondrial pellet was resuspended in the washing medium and mitochondrial protein was determined by the biuret method calibrated with BSA (Gornall et al. 1949).

Mitochondrial respiration measurements Oxygen consumption of the brain mitochondria was registered polarographically with a Clark oxygen electrode (Estabrook 1967) connected to a suitable recorder in a thermostated water-jacketed closed chamber with magnetic stirring. The reactions were carried out as described elsewhere (Cardoso et al. 2013) with 1 mg of protein for Control, MG and MG + PM and 0.8 mg of protein for PM. States 3 and 4 (consumption of oxygen after ADP phosphorylation) of respiration, respiratory control ratio (RCR = state 3/state 4), and ADP/O index (a marker of the mitochondrial ability to couple oxygen consumption to ADP phosphorylation during state 3 of respiration) were determined according to Chance and Williams (Chance and Williams 1956).

Mitochondrial membrane potential measurements The transmembrane potential ($\Delta\Psi_m$) was monitored by evaluating the transmembrane distribution of the lipophilic cation TPP^+ (tetraphenylphosphonium) with a TPP^+-selective electrode prepared according to Kamo et al. (Kamo et al. 1979) using an Ag/AgCl-saturated electrode (Tacussel, model MI 402) as reference, as described elsewhere (Cardoso et al. 2013). Brain mitochondria (1 mg/ml for Control, MG and MG + PM; and 0.8 mg/ml for PM) were energized with 5 mM succinate (substrate of complex II) in the presence of 2 μM rotenone (mitochondrial complex I inhibitor) in order to activate the mitochondrial electron transport chain. After a steady-state distribution of TPP^+ had been reached (ca. 1 min of recording), $\Delta\Psi_m$ fluctuations were recorded.

Determination of adenine nucleotide levels Adenine nucleotide levels were determined as previously described (Cardoso et al. 2013). Adenine nucleotides were identified by their chromatographic behaviour (retention time, absorption spectra and correlation with standards).

Measurement of aconitase activity Aconitase activity was determined according to Krebs and Holzach (Krebs and Holzach 1952), as described elsewhere (Cardoso et al. 2013). One unit was defined as the amount of enzyme necessary to produce 1 μM cis-aconitate per minute.

Measurement of hydrogen peroxide (H_2O_2) levels H_2O_2 levels were measured fluorimetrically using a modification of the method described by Barja (Barja 1999), as described elsewhere (Cardoso et al. 2013). The H_2O_2 levels were calculated using a standard curve of H_2O_2 and expressed as pmol/mg protein/15 min.

Measurement of malondialdehyde (MDA) levels MDA levels were determined by HPLC (Wong et al. 1987), as previously described (Cardoso et al. 2013). The MDA content of the samples was calculated from a standard curve prepared using the thiobarbituric acid-MDA complex and was expressed as mmol/mg protein.

Measurement of glutathione (GSH) and glutathione disulfide (GSSG) levels GSH and GSSG levels were determined with fluorescence detection after reaction of the supernatant containing H_3PO_4/NaH_2PO_4-EDTA or H_3PO_4/NaOH, respectively, of the deproteinized homogenates solution with ophthalaldehyde (OPT), pH 8.0, according to Hissin and Hilf (Hissin and Hilf 1976), as described elsewhere (Cardoso et al. 2013). The GSH and GSSG levels were determined from comparisons with a linear GSH or GSSG standard curve, respectively.

Measurement of α-tocopherol (vitamin E) content Extraction and separation of reduced α-tocopherol (vitamin E) from brain mitochondria were performed by following a previously described method by Vatassery and Younoszai (Vatassery and Younoszai 1978). The content of mitochondrial vitamin E was calculated as mmol/mg protein.

Measurement of glutathione disulfide reductase (GR) activity GR activity was determined as previously described (Cardoso et al. 2013) using the molar extinction coefficient 6,220 M^{-1} cm^{-1} and expressed as nmol/min/mg protein (Carlberg and Mannervik 1985).

Measurement of glutathione peroxidase (GPx) activity GPx activity was determined spectrophotometrically at 340 nm by following the method of Flohé and Gunzler (Flohe and Gunzler 1984), as previously described (Cardoso et al. 2013). GPx activity was determined using the molar extinction coefficient 6,220 M^{-1} cm^{-1} and expressed as nmol/min/mg protein.

Measurement of manganese superoxide dismutase (MnSOD) activity MnSOD activity was determined spectrophotometrically, at 550 nm (Flohe and Otting 1984), as described elsewhere (Cardoso et al. 2013). The activity of MnSOD was calculated using a standard curve, prepared with different concentrations of SOD.

Statistical analysis Data were analyzed and results are presented as mean ± SEM of the indicated number of experiments. Statistical significance was defined using one-way ANOVA test for multiple comparisons, followed by the posthoc Tukey-Kramer test. A p-value<0.05 was considered significant.

Results

Characterization of the experimental groups

MG and/or PM treatments did not promote significant alterations in body and brain weight, and fasting glycemia (Table 1). However, at the same time, we observed that PM treatment alone slightly promoted an increase in body weight and fasting glycemia levels (Table 1).

Effects of chronic MG administration and/or PM treatment in the respiratory chain and oxidative phosphorylation system of brain mitochondria

The mitochondrial respiratory chain complexes pump protons out of the matrix across the inner membrane. The coupling of electron transfer with the pumping of protons through the complexes establishes the proton motive, which is a combination of both an electrical ($\Delta\Psi_m$) and chemical (ΔpH) gradients. $\Delta\Psi_m$ is essential for oxidative phosphorylation to occur, which results in the conversion of ADP to ATP via ATP synthase. MG administration caused a significant decrease in RCR and PM exacerbated this effect (Fig. 1). Interestingly, PM alone promoted a significant increase in ADP/O index and a slight stimulation in state 4 of respiration (Fig. 1). On the other hand, even though the administration of MG and PM alone did not promote any effect in ATP levels, in animals pre-treated with MG, PM evoked a significant decrease in $\Delta\Psi_m$ (Table 2).

Table 1 Characterization of the experimental animal models

	Control	PM	MG	MG + PM
Body weight (g)	429.6±17.2	484.3±21.7	431.2±17.8	432.6±11.9
Brain weight (g)	2.0±0.03	2.05±0.02	2.00±0.03	1.99±0.03
Fasting glycemia (mg/dl)	63.3±1.0	67.1±2.4	62.9±1.5	64.3±2.0

Data are the mean ± SEM of 7–12 animals from each condition studied

Effects of chronic MG administration and/or PM treatment in the oxidative status of brain mitochondria

Evidence suggests that the cytotoxicity of MG often occurs through the induction of oxidative stress-mediated apoptosis and may contribute to neurodegeneration (Kikuchi et al. 1999; Shinpo et al. 2000; Amicarelli et al. 2003). In this line, we evaluated the oxidative status of rat brain mitochondria in our experimental groups. Mitochondrial aconitase activity is a sensitive redox sensor of reactive oxygen and nitrogen species in cells. Interestingly, a significant decrease in this enzyme activity was found in all the experimental groups and especially in the PM-treated animals when compared with mitochondria from control animals (Fig. 2). However, the administration of PM to MG-treated rats did not counteract MG-mediated effects (Fig. 2). On the other hand, PM significantly decreased MDA levels, an index of oxidative damage, in mitochondria from both control and MG (Fig. 3). In contrast, H_2O_2 production rate was significantly increased in MG-treated rats and PM administration was not able to reverse this effect (Fig. 4).

Effects of chronic MG administration and/or PM treatment in the antioxidant defense system of brain mitochondria

To evaluate the impact of MG administration in the brain mitochondrial ROS-defense network, we analyzed non-enzymatic antioxidants namely glutathione and α-tocopherol, and also antioxidant enzymes such as GR, GPx and MnSOD. Fig. 5a shows that GSH/GSSG ratio was not significantly affected by MG and/or PM treatment (Fig. 5a), while α-tocopherol levels were significantly increased in the control group treated with PM (Fig. 6b). Regarding the antioxidant enzymes, we observed that GR activity was significantly decreased in MG-treated rats compared to control mitochondria (Fig. 6a) and PM alone induced a significant increase in GPx and MnSOD activities (Fig. 6b and c).

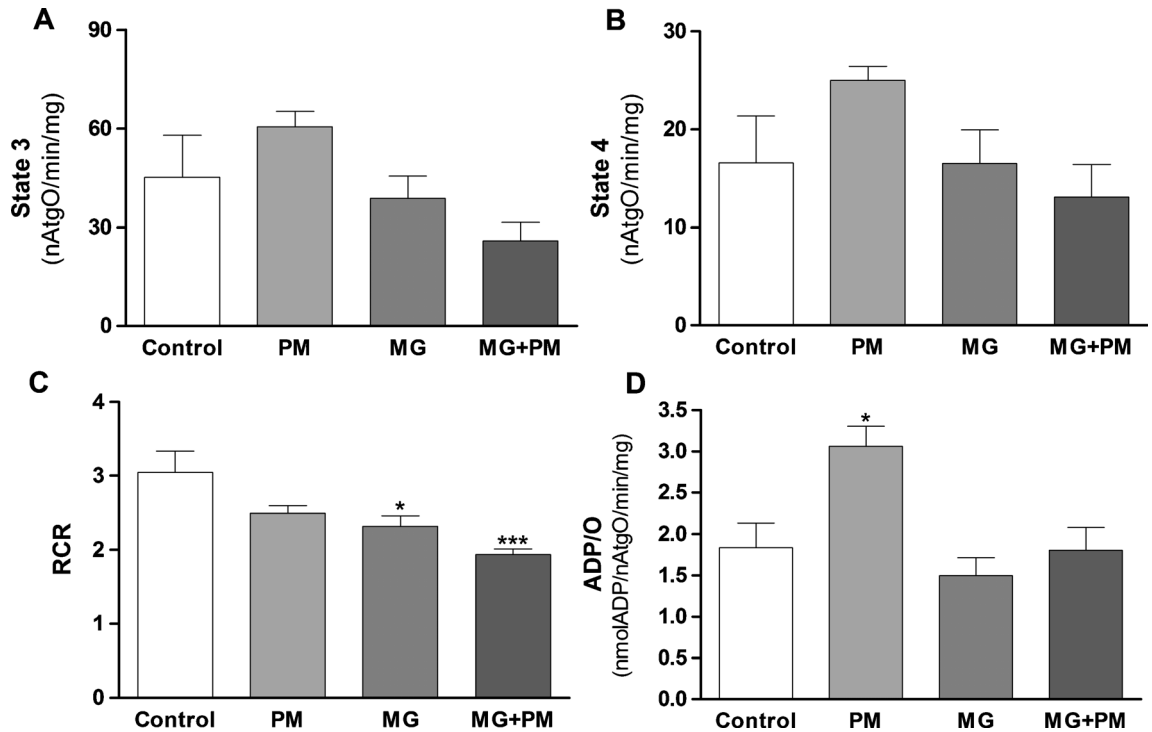

Fig. 1 Effects of MG and/or PM treatment in brain mitochondrial respiratory chain parameters: states 3 **a** and 4 **b** of respiration, RCR **c** and ADP/O ratio **d**. Data are the mean ± SEM of 5–6 animals from each condition studied. $^{***}p<0.001$; $^{*}p<0.05$ when compared with brain control mitochondria

Table 2 Effects of MG and/or PM treatment in brain mitochondrial oxidative phosphorylation system ($\Delta\Psi_m$, ADP-induced depolarization, repolarization lag phase and ATP levels)

	Control	PM	MG	MG + PM
$\Delta\Psi_m$ (−mV)	185.6±5.5	188.2±3.8	184.7±4.6	171.1±4.6*
ADP-induced depolarization (−mV)	16.5±2.67	20.0±2.15	10.8±1.39	9.2±1.46
Repolarization lag phase (min)	1.7±0.3	0.7±0.1	2.1±0.4	2.6±0.4
ATP levels (nmol/mgprotein)	176.3±30.5	100.0±13.1	189.3±19.1	148.4±16.4

The oxidative phosphorylation parameters were evaluated in freshly isolated brain mitochondrial fractions (1 mg for Control, MG and MG + PM; and 0.8 mg for PM) in 1 ml of the reaction medium supplemented with 3 μM of TPP^+ and energized with 5 mM succinate in the presence of 2 μM rotenone. Data are the mean ± SEM of 5–6 animals from each condition studied. * $p<0.05$ when compared with control group

Discussion

Chemical modifications of circulating, cellular and matrix proteins by glucose lead to the formation of AGEs, which are thought to be a major factor in aging process and in the pathogenesis of diabetes, atherosclerosis, and neurodegenerative diseases (Beisswenger et al. 2003). As previously reported (Sena et al. 2012), our experimental protocol was based on the fact that chronic administration of MG in a progressive dose (starting at 50 mg/kg and ending at 60 mg/kg), is able to mimic the allegedly continuous production of MG in the body and, in this way, the excessive peaks in plasma associated with repeated intraperitoneal or subcutaneous injections are avoided. Our data show that high levels of MG impair brain mitochondria by altering the function and oxidative status of these organelles. In addition, PM treatment did not reverse MG-induced mitochondrial alterations.

Mitochondria are central to eukaryotic cells. These organelles consume ~95 % of the O_2 inspired to generate most of the cell's ATP, control cell death, contain much of central metabolism, and modulate calcium and redox signaling (Breuer et al. 2012). The harmful effects of MG to mitochondria have been previously described with renal mitochondria presenting a concentration-dependent decrease in RCR after in vitro incubation with MG (Rosca et al. 2002). Similarly, the in vitro incubation of MG in SH-SY5Y neuroblastoma cells promoted a decrease in $\Delta\Psi_m$ and intracellular ATP levels (de Arriba et al. 2007). In the present study, the chronic MG administration promoted a significant decrease in RCR of brain mitochondria and PM treatment did not reverse this effect (Fig. 1). In fact, PM promoted a significant decrease in $\Delta\Psi_m$ in mitochondria isolated from MG-treated rats (Table 2) suggesting that under certain conditions PM administration could have deleterious effects. In fact, for a normal mitochondrial function, $\Delta\Psi_m$ must be maintained within a specific range, since deviations in $\Delta\Psi_m$ can induce mitochondrial dysfunction and activate cell death pathways (Kim-Han and Dugan 2005).

Closely linked to mitochondrial energy production, oxidative stress caused by an excessive mitochondrial reactive oxygen species (ROS) production is often considered to play a key role in diabetic complications, aging and neurodegeneration (Karbowski and Neutzner 2012). ROS are particularly active in the brain and, depending on their levels, can be involved in numerous cellular functions, including cell death or survival. Evidence has demonstrated that MG concentrations exert detrimental effects on cultured cortical (Kikuchi et al. 1999) and hippocampal (Di Loreto et al. 2008) neurons through a mechanism involving oxidative stress and increased

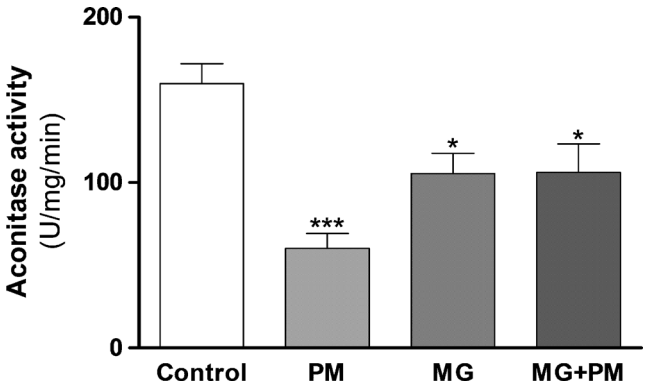

Fig. 2 Effects of MG and/or PM treatment on mitochondrial aconitase activity. Aconitase activity was measured as described in the material and methods section. Data are the mean ± SEM of 5–6 animals from each condition studied. *** $p<0.001$; * $p<0.05$ when compared with control mitochondria

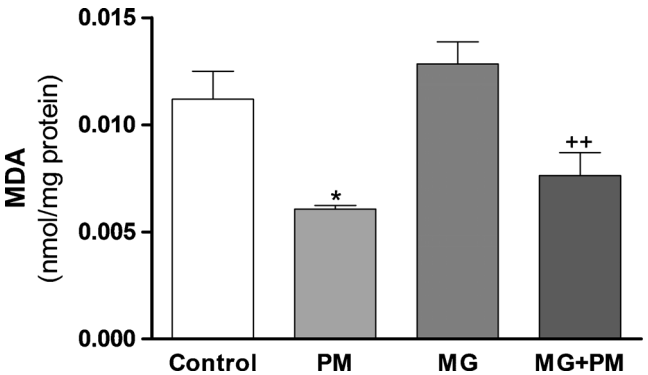

Fig. 3 Effects of MG and/or PM treatment on lipid peroxidation measured by MDA levels. Data are the mean ± SEM of 5–6 animals from each condition studied. * $p<0.05$ when compared with control mitochondria; ++ $p<0.01$ when compared with MG-treated mitochondria

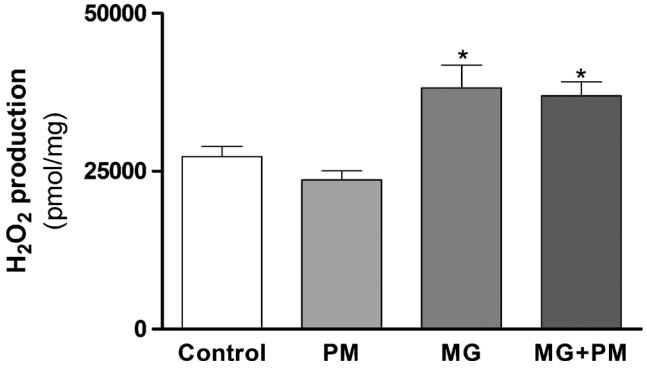

Fig. 4 Effects of MG and/or PM treatment on hydrogen peroxide (H_2O_2) production rate. Data are the mean ± SEM of 5–6 animals from each condition studied. $^{*}p<0.05$ when compared with control mitochondria

ROS production, namely H_2O_2 (Kikuchi et al. 1999). Similarly, in the present study, the administration of MG led to a significant increase in mitochondrial H_2O_2 production (Fig. 4). However, this effect was not reversed by the treatment with PM (Fig. 4). Likewise, the evaluation of aconitase activity shows a significant decrease both in MG-and MG plus PM-treated groups (Fig. 2). The loss of the mitochondrial aconitase activity is an important marker of oxidative stress. The prolonged exposure of mitochondria to oxidants results in disassembly of the [4Fe-4S] $^{2+}$ cluster and concomitant release of Fe^{2+}, carbonylation, and inactivation of the enzyme, potentially establishing the link between oxidative stress and mitochondrial aconitase inactivation (Yan et al. 1997; Addabbo et al. 2009). Superoxide anions ($O_2^{\bullet -}$) and H_2O_2 are toxic byproducts of respiration and glucose metabolism that can cause a wide range of oxidative damage within the cell. Surprisingly, PM administration to control rats also caused

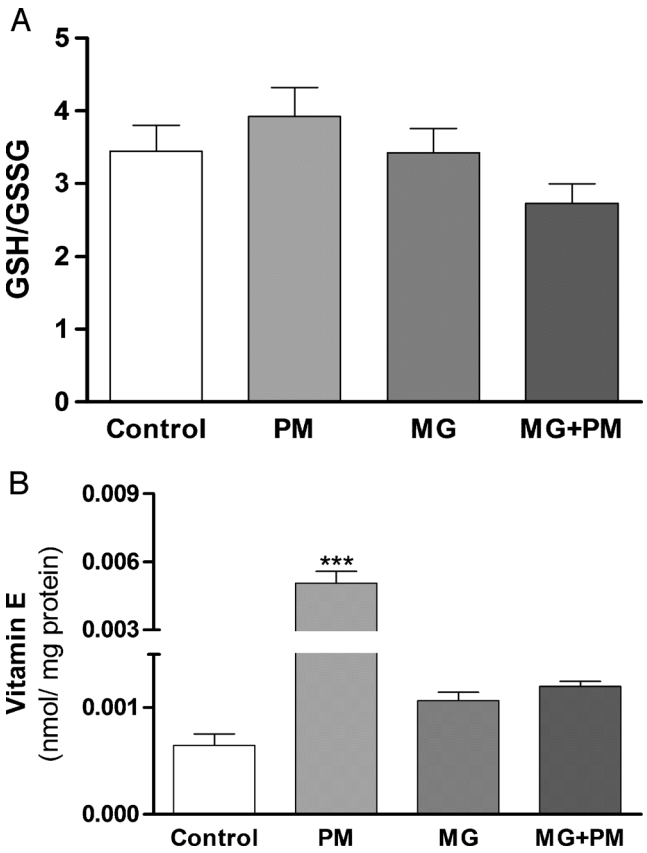

Fig. 5 Effects of MG and/or PM treatment on non-enzymatic antioxidant defenses: GSH/GSSG (**a**) and α-tocopherol (vitamin E) (**b**) levels. Data are the mean ± SEM of 5–6 animals from each condition studied. $^{***}p<0.001$ when compared with control mitochondria

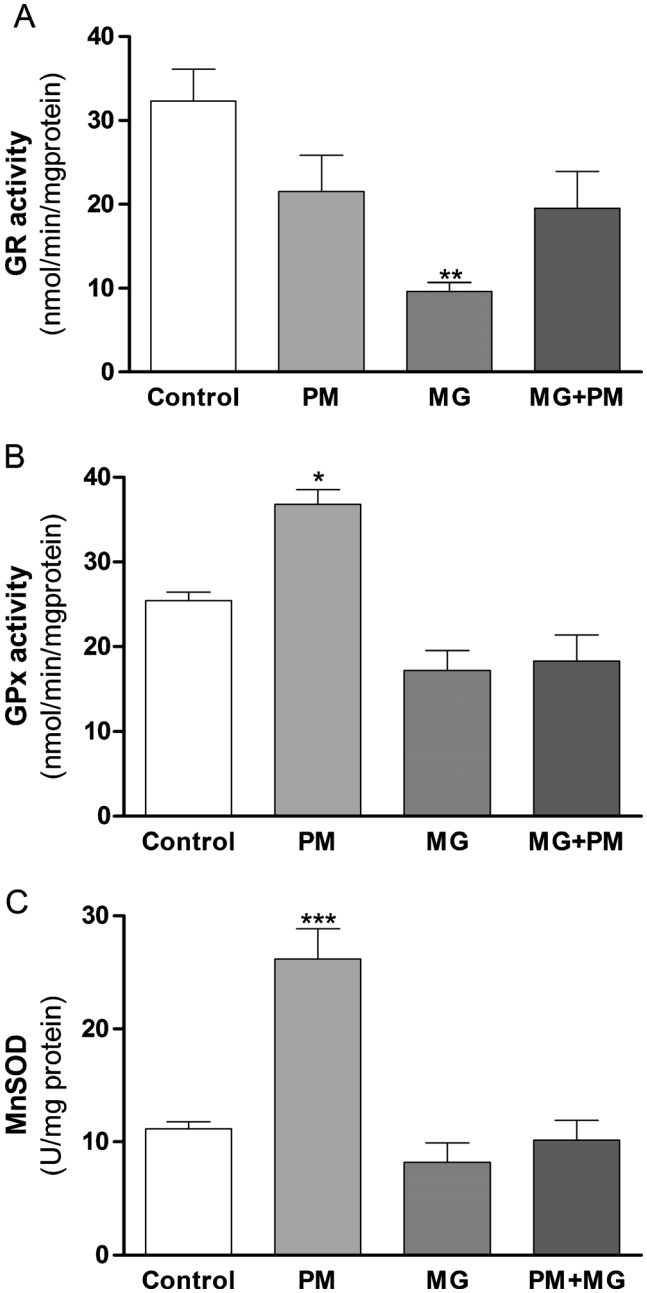

Fig. 6 Effects of MG and/or PM treatment on enzymatic antioxidant defenses: glutathione disulfide reductase (GR) **a** glutathione peroxidase (GPx) **b** and manganese superoxide dismutase (MnSOD) **c** activities. Data are the mean ± SEM of 5–6 animals from each condition studied. $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$ when compared with control mitochondria

the loss of aconitase activity (Fig. 2). According to the literature, the mechanism of action of PM includes: inhibition of AGE formation by blocking oxidative degradation of the Amadori intermediate of the Maillard reaction; scavenging of toxic carbonyl products of glucose and lipid degradation; and trapping of ROS (Onorato et al. 2000; Voziyan and Hudson 2005). A previous study documented that an excess of vitamin B6 intake with increasing treatment periods can have deleterious effects to cerebral cortex leading to an increased number of damaged mitochondria, lipofuscin granules and vacuoles together with decreased synaptic density (Demir et al. 2005). Nevertheless, the effects of vitamin B6 are controversial with studies: 1) showing cortical dendritic loss and hippocampal axonal swelling in rats under a diet deficient in vitamin B6 (Root and Longenecker 1983); 2) no benefits of vitamin B6 in improving mood or cognitive functions in older people (Malouf and Grimley 2003); and 3) dose-related adverse effects promoted by the vitamin B6 derivate pyridoxine in work recognition and visual retention tests in humans (Molimard et al. 1980).

Brain contains large quantities of iron and copper, which in the presence of H_2O_2 may catalyse the formation of the highly reactive hydroxyl radical and induce lipid peroxidation and other cell damages (Valko et al. 2007). MDA, as one of the most important intermediates of lipid peroxidation, is a reactive unsaturated dicarbonyl that can readily bind to and crosslink biomacromolecules such as structural and functional proteins and nucleic acids (Esterbauer et al. 1991). Data have demonstrated that PM inhibits the formation of advanced lipoxidation end products (ALEs) by directly detoxifying MDA at physiological conditions (Kang et al. 2006). In fact, a similar profile was found in the control rats treated with PM (Fig. 3). In turn, the administration of MG caused a slight increase in MDA levels (Fig. 3), which was reversed by PM treatment (Fig. 3) demonstrating the ability of PM to inhibit ALEs formation.

Organisms have evolved a series of defense mechanisms that can involve preventive and/or repair mechanisms, physical defenses, and antioxidant defenses (Valko et al. 2007). No alterations in GSH/GSSG ratio was found in MG-treated group (Fig. 5a), even though a significant decrease was found in GR activity, the enzyme responsible for GSH regeneration (Fig. 6a). On the other hand, a slight decrease in GSH/GSSG was observed in MG plus PM-treated group compared with the MG-alone (Fig. 5a). It was previously reported that in vivo treatment of diabetic rats with PM did not counteract the decreased levels of GSH found in diabetic lysed erythrocyte preparations, but instead led to a slight decrease compared with the diabetic group (Nagaraj et al. 2002). Evidence shows that a ROS-dependent process plays a central role in the generation of intracellular AGEs, and that inhibition of oxidant pathways prevents intracellular AGE formation (Giardino et al. 1996). The mitochondrial pool of GSH is considered vital for cell survival and the principal functional mitochondrial antioxidant (Han et al. 2003), having an important role in oxygen radicals removal and in the recycling of important antioxidants, such as α-tocopherol (Victor et al. 2011). α-Tocopherol is an important physiological antioxidant that protects the cells from oxidative damage and its levels were significantly enhanced by PM in mitochondria from control rats (Fig. 5b) showing the ability of PM in inhibiting glycation reactions and the formation of AGEs (Voziyan and Hudson 2005).

H_2O_2 is produced through the activity of MnSOD that promotes the dismutation of $O_2^{\bullet-}$ into H_2O_2, which can be scavenged by either the enzyme GPx in the mitochondrial matrix that uses GSH as a reducing equivalent to reduce H_2O_2 to form GSSG and water; or by the enzyme catalase in the cytosol, which converts H_2O_2 to water and oxygen. Similarly to other studies (Di Loreto et al. 2008), GPx activity is slightly decreased in the MG-treated group (Fig. 6b) while MnSOD activity remained unchanged (Fig. 6c) (Di Loreto et al. 2004). The treatment with PM did not change these profiles in MG-treated rats but significantly increased GPx (Fig. 6b) and MnSOD activities (Fig. 6c) in control rats. When the production of ROS is prolonged, the endogenous reserves of antioxidants become insufficient, leading to cell damage. Thus, our data may be indicative of a defective scavenging capacity in response to the MG-mediated ROS production. In addition, it can suggest that PM under normal conditions exert significant effects in brain mitochondria. Data show that brain mitochondria from PM-treated animals besides presenting a more coupled status characterized by an increase in ADP/O index (Fig. 1) and low levels of lipid peroxidation (Fig. 3), also show an attempt to overcome the increased oxidative stress, as demonstrated by the loss of aconitase activity (Fig. 2), by increasing the activities of the antioxidant enzymes GPx (Fig. 6b) and MnSOD (Fig. 6c) and levels of α-tocopherol (Fig. 5b). On the other hand, in the presence of a toxic insult, like chronic MG administration, PM is not able to respond adequately in order to maintain brain mitochondria homeostasis. PM beneficial effects against MG-induced damage have been extensively described and its administration has been proven to ameliorate nephropathy and retinopathy in several animal models of diabetes (Stitt et al. 2002; Zheng et al. 2006; Chetyrkin et al. 2008). Similarly, PM-mediated reduction of MG-induced glycation promoted the restoration of heart survival pathways during ischemia (Almeida et al. 2013) and the improvement of the microvascular lesions of the adipose tissue (Rodrigues et al. 2013) caused by MG administration. Furthermore, previous studies have reported that the neurotoxicity induced by MG can be overcome by the co-treatment but not with the pretreatment with aminoguanidine (Kikuchi et al. 1999). Similarly, N-acetylcysteine proved to be ineffective when added at 1 h post-MG exposure to undifferentiated PC12 cells, which suggest an early window of redox signaling

(Okouchi et al. 2005). Thus, it cannot be ruled out the possible existence of tissue specific-effects of PM and the influence of the experimental protocol applied in the findings obtained.

Altogether our results indicate that high levels of MG impair brain mitochondrial function and weaken the mitochondrial antioxidant defense system predisposing to oxidative events. Furthermore, in our experimental conditions, PM treatment was not effective against MG-mediated effects.

Acknowledgments The authors' work is supported by the Fundação para a Ciência e a Tecnologia (FCT) (PTDC/SAU-NEU/103325/2008) co-funded by Fundo Europeu de Desenvolvimento Regional (FEDER) via Programa Operacional Factores de Competitividade (COMPETE).

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