Amino Acids (2015) 47:1931–1939
DOI 10.1007/s00726-015-1973-6

ORIGINAL ARTICLE

Differential in vitro effects of homoarginine on oxidative stress in plasma, erythrocytes, kidney and liver of rats in the absence and in the presence α-tocopherol, ascorbic acid or L-NAME

Simone Sasso[1] · Leticia Dalmedico[2] · Débora Delwing-Dal Magro[3] · Eduardo Manoel Pereira[2] · Angela T. S. Wyse[4] · Daniela Delwing- de Lima[5]

Received: 12 January 2015 / Accepted: 26 March 2015 / Published online: 18 April 2015

Abstract In the present study, we evaluated the in vitro effects of homoarginine (hArg) at 1, 10 and 20 μM on thiobarbituric acid-reactive substances (TBA-RS), total sulfhydryl content and on the activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in plasma, erythrocytes, kidney and liver of rats (60 days old). We also investigated the influence of the antioxidants (each at 1 mM) α-tocopherol and ascorbic acid, as well as of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) at 1 mM, on the effects elicited by hArg on the parameters tested. In plasma, hArg at concentrations of 10 and 20 μM decreased moderately the total sulfhydryl content. At 20 μM, hArg enhanced moderately TBA-RS in the plasma. In plasma, the effects of hArg (20 μM) on TBA-RS and total thiol content were abolished by α-tocopherol, ascorbic acid and L-NAME. At all concentrations tested, hArg did not exert any effect on CAT, SOD or GSH-Px activity in the erythrocytes. In the kidney, hArg exerted effects only at 20 μM and in a different manner: TBA-RS levels increased and total thiol content and CAT activity decreased, while SOD and GSH-Px activity increased. In the renal medulla, α-tocopherol and ascorbic acid but not L-NAME abolished the effects of hArg (20 μM) on TBA-RS, while all agents inhibited the hArg-induced increase in SOD activity. In the renal cortex, α-tocopherol, ascorbic acid and L-NAME abolished the effects of hArg (20 μM) on the total sulfhydryl content and GSH-Px activity, but L-NAME did not reverse the inhibitory effects of hArg on CAT activity. In the liver, no effects of hArg were observed of all biomarkers measured. At the pathologically high concentration of 20 μM, as it may occur in plasma in hyperargininemia, hArg may enhance lipid peroxidation and thiol oxidation and inhibit CAT activity, but may increase SOD and GSH-Px activity predominantly in the kidney.

Keywords Homoarginine · Oxidative stress · Antioxidants · Blood · Kidney · Liver

Abbreviations

Arg	Arginine
CAT	Catalase
CSF	Cerebrospinal fluid
DTNB	5,5′-Dithiobis(2-nitrobenzoic acid)
GSH-Px	Glutathione peroxidase
IEM	Inborn error of metabolism
L-NAME	N^G-nitro-L-arginine methyl ester
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide

✉ Daniela Delwing- de Lima
daniela.delwing@univille.br

[1] Mestrado em Saúde e Meio Ambiente, Universidade da Região de Joinville-Univille, Rua Paulo Malschitzki, 10-Zona Industrial Norte, Joinville, SC CEP 89201-972, Brazil

[2] Departamento de Farmácia, Universidade da Região de Joinville-Univille, Rua Paulo Malschitzki, 10-Zona Industrial Norte, Joinville, SC CEP 89201-972, Brazil

[3] Departamento de Ciências Naturais, Centro de Ciências Exatas e Naturais, Universidade Regional de Blumenau, Rua Antônio da Veiga, 140, Blumenau, SC CEP 89012-900, Brazil

[4] Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600-Anexo, Porto Alegre, RS, Brazil

[5] Departamento de Medicina, Universidade da Região de Joinville-Univille, Rua Paulo Malschitzki, 10-Zona Industrial Norte, Joinville, SC CEP 89201-972, Brazil

ROS	Reactive oxygen species
SOD	Superoxide dismutase
SPSS	Statistical Package for the Social Sciences
TBA-RS	Thiobarbituric acid-reactive substances

Introduction

Hyperargininemia is a rare inborn error of metabolism (IEM) that affects the activity of the enzyme arginase, which is the final enzyme of the urea cycle and catalyzes the conversion of arginine (Arg) to urea and ornithine (Ash 2004). It is an autosomal recessive disorder and is characterized by early childhood progressive neurologic impairment with spastic paraplegia, epilepsy, developmental delay or regression (Cederbaum and Crombez 2004; Crombez and Cederbaum 2005). Other uncommon presentations, such as persistent neonatal jaundice with biopsy-proven hepatic cirrhosis, have been reported (Braga et al. 1997). In addition, the slight increase in transaminase values and the decrease in prothrombin activity observed in some patients could indicate mild hepatic dysfunction related to hyperargininemia, as suggested by some authors (Crombez and Cederbaum 2005; Scaglia and Lee 2006). Since hyperargininemia is a very rare disease and only a limited number of patients have been described, the clinical course of hyperargininemia remains poorly characterized (Carvalho et al. 2012).

Arg and its metabolites, including those containing the guanidine group, may act as neurotoxins. Guanidine compounds can cause demyelination with consequent upper motor neuron signals and can affect GABAergic neurotransmission and decrease the fluidity of the plasma membrane by inhibiting Na^+K^+-adenosine triphosphatase, resulting in epileptogenic properties. In addition, Arg acts as a substrate for nitric oxide (NO) synthase (Crombez and Cederbaum 2005; Scaglia and Lee 2006). In fact, elevated Arg and other guanidine compounds may also result in neurotoxicity via augmented NO synthesis, thereby leading to oxidative damage to the brain (Luiking et al. 2010). The concentration of several guanidine compounds has been previously shown to be increased in plasma and cerebrospinal fluid (CSF), as well as in brain tissue of hyperargininemic patients (Mizutani et al. 1987; Deignan et al. 2010) and in a mouse model (Deignan et al. 2008).

Oxidative stress is commonly observed in some inborn errors of intermediary metabolism (Colome et al. 2000; Wajner et al. 2004) and results from an imbalance between the production of reactive oxygen species (ROS) and the cell's ability to mitigate damage through antioxidant pathways or mechanisms that repair or eliminate damaged molecules (Du et al. 2013). Accumulation of intracellular ROS in normal cells contributes to the oxidation of various components, including nucleic acids, proteins and lipids (Gueraud et al. 2010). Antioxidant mechanisms that can be either enzymatic (including catalases, dismutases and peroxidases) or non-enzymatic (such as vitamin A, C or E) are critical to protect cells against ROS-induced damage, both at steady state and upon acute oxidative stress (Oakley et al. 2009).

According to Wyse et al. (2001), guanidine compounds increase lipid peroxidation and decrease enzymatic and non-enzymatic antioxidant defenses in the rat brain. As such, we investigated the in vitro effects of different concentrations of hArg including those found in plasma and CSF of hyperargininemic patients, on lipid peroxidation including the thiobarbituric acid-reactive substances (TBA-RS), protein damage, namely their total sulfhydryl content, and the activity of the main antioxidant enzymes catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in blood, kidney and liver of rats. According to Deignan et al. (2010), the levels of hArg found in serum/plasma of hyperargininemic patients ranged between 2 and 15 μM, i.e., they were increased 2–15 times compared to healthy control (about 1 μM), while those found in CSF were increased fivefold compared to healthy subjects (about 0.4 μM). In the present study, we also investigated the influence of two antioxidants, α-tocopherol (trolox) and ascorbic acid, and of N^G-nitro-L-arginine methyl ester (L-NAME) on the effects elicited by hArg.

Materials and methods

Animals and reagents

Sixty-day-old male Wistar rats (180–200 g), obtained from Tecpar Company (Curitiba, Brazil), were used in the experiments. The animals from our own breeding stock were maintained on a 12 h light/12 h dark cycle at a constant temperature (22 ± 1 °C), with free access to water and commercial protein chow. The "Principles of Laboratory Animal Care" (NIH publication 85–23, revised 1985) were followed in all the experiments and the experimental protocol was approved by the Ethics Committee for Animal Research of the University of Region Joinville, Joinville, Brazil, under the protocol number 019/2013 PRPPG/CEP. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

In vitro studies

For in vitro experiments, erythrocytes, plasma, liver and kidney supernatants were pre-incubated for 1 h at 37 °C in the presence of hArg at final concentrations of 1.0, 10.0 and 20.0 μM. Control experiments were performed without

hArg addition. After incubation, aliquots were taken to measure TBA-RS, total sulfhydryl content and the activity of antioxidant enzymes.

Effects of trolox (α-tocopherol), ascorbic acid and L-NAME

The assays were divided into eight groups: group 1 (control-saline), group 2 (hArg), group 3 (control − 1 mM trolox), group 4 (hArg + 1.0 mM trolox), group 5 (control − 1 mM ascorbic acid), group 6 (hArg + 1 mM ascorbic acid), group 7 (control − 1 mM L-NAME), group 8 (hArg + 1 mM L-NAME). The concentrations of trolox, ascorbic acid and L-NAME utilized in the present study were chosen according to previous studies (Wyse et al. 2002; Silva et al. 2004; Qi et al. 1995), respectively.

Erythrocyte and plasma preparation

Erythrocytes and plasma were isolated from whole blood samples of rats. Whole blood was collected and transferred to heparinized tubes for erythrocyte separation. Blood samples were centrifuged at 1000×*g*, and plasma was removed by aspiration and frozen at −80 °C until further use. Erythrocytes were washed three times with cold saline solution (153 mM NaCl). Lysates were prepared by the addition of 1 mL of distilled water to 100 μL of washed erythrocytes and frozen at −80 °C. For antioxidant enzyme activities determination, erythrocytes were frozen and thawed three times and finally centrifuged at 13,500×*g* for 10 min. The supernatant was diluted to contain approximately 0.5 mg/mL of protein.

Tissue preparation

After decapitation, the liver and kidney were removed, decapsulated and kept in ice-cold buffered sodium phosphate (20 mM, pH 7.4, 140 mM KCl). The renal cortex was carefully separated from the renal medulla. The liver, renal cortex and renal medulla were homogenized in ten volumes (1:10, w/v) of the buffer. Homogenates were prepared using a Potter–Elvehejem homogenizer (Remi motors, Mumbai, India) by passing five pulses and centrifuged at 800×*g* for 10 min at 4 °C to discard nuclei and cell debris. The pellet was discarded and the supernatant was saved in aliquots and stored at −20 °C for assaying the activity of antioxidant enzymes, total sulfhydryl content and estimation of lipid peroxidation (Ferreira et al. 2012).

Thiobarbituric acid-reactive substances (TBA-RS)

TBA-RS were determined according to the method described by Esterbauer and Cheeseman (1990). TBA-RS methodology measures malondialdehyde (MDA), a product of lipid peroxidation caused mainly by hydroxyl free radicals, and many lipid peroxidation substances. For the measurements, plasma and tissues were mixed with 10 % trichloroacetic acid and 0.67 % thiobarbituric acid and heated in a boiling water bath for 25 min. TBA-RS were determined by measuring the absorbance at 535 nm. A calibration curve was obtained using 1,1,3,3-tetramethoxypropane as the MDA precursor and each curve point was subjected to the same treatment as that of the supernatants. TBA-RS content was calculated as nanomoles of MDA formed per milligram of protein.

Total sulfhydryl content

The total thiol group concentration was determined by the method of Ellman (Ellman 1959). Briefly, 50 μl of homogenate was added to 1 ml of phosphate-buffered saline (PBS), pH 7.4, containing 1 mM EDTA. The reaction was started by the addition of 30 μL of 10 mM 5,5´-dithiobis(2-nitrobenzoic acid) (DTNB) and incubated for 30 min at room temperature in a dark room. Total sulfhydryl content was determined by measuring the absorbance at 412 nm. Analyses of blank (DTNB absorbance) was also performed. Results are reported as nmol 3-thio-2-nitrobenzoic acid (TNB)/mg protein.

Catalase activity assay (CAT)

CAT activity was assayed using a UV–visible Shimadzu spectrophotometer. The method used is based on the disappearance of H_2O_2 at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1 % Triton X-100, 10 mM potassium phosphate buffer, pH 7.0, and 0.1–0.3 mg protein/mL (Aebi 1984). One CAT unit is defined as 1 μmol of H_2O_2 consumed per minute and the specific activity is calculated as CAT units/mg protein.

Glutathione peroxidase (GSH-Px) activity assay

GSH-Px activity was measured using *tert*-butyl-hydroperoxide as substrate (Wendel 1981). NADPH disappearance was monitored at 340 nm using a UV–visible Shimadzu spectrophotometer. The medium contained 2 mM GSH, 0.15 U/mL GSH reductase, 0.4 mM azide, 0.5 mM *tert*-butyl-hydroperoxide and 0.1 mM NADPH. One GSH-Px unit is defined as 1 μmol of NADPH consumed per minute and the specific activity is presented as GSH-Px units/mg protein.

Superoxide dismutase (SOD) activity assay

The method used to assay SOD activity is based on the capacity of pyrogallol to autoxidize, a process highly

dependent on superoxide ($O_2^{-\cdot}$) which is a substrate for SOD (Marklund 1985). Briefly, to 15 µl of each sample, 215 µl of a mixture containing 50 mM Tris buffer, pH 8.2, 1 mM EDTA and 30 mM CAT were added. Then, 20 µl of pyrogallol was added and the absorbance was immediately recorded each 30 s for 3 min at 420 nm using a UV–visible Shimadzu spectrophotometer. The inhibition of autoxidation of pyrogallol occurs in the presence of SOD, of which activity can be then indirectly assayed spectrophotometrically. A calibration curve was performed with purified SOD as reference, to calculate the activity of SOD present in the samples. One SOD unit is defined as the amount of SOD necessary to inhibit 50 % of pyrogallol autoxidation and the specific activity is reported as SOD units/mg protein.

Statistical analysis

Data were analyzed by ANOVA, followed by the Duncan multiple range test, when the *F* test was significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software in a PC compatible computer. Values of $P < 0.05$ were considered to be significant. If not otherwise stated, results are expressed as mean ± SD for seven independent experiments (animals) performed in duplicate. $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$, compared to the control group (Duncan's multiple range tests).

Results

In vitro effects of hArg on TBA-RS, total sulfhydryl content and on the activity of antioxidant enzymes in the blood of rats

We initially verified the in vitro effects of hArg at three different concentrations (1, 10 and 20 µM) on TBA-RS and on total sulfhydryl content in the plasma and on the activities of the antioxidant enzymes CAT, SOD and GSH-Px in the erythrocytes of rats. Figure 1 shows that hArg at a concentration of 20 µM enhanced TBA-RS (40.5 %, $P < 0.01$) (Fig. 1a). In rat plasma, hArg decreased total sulfhydryl content by 39.1 % at 10 µM and by 61.8 % at 20 µM ($P < 0.001$) (Fig. 1b). In contrast, hArg did not alter CAT (Fig. 1c, $P > 0.05$), SOD (Fig. 1d, $P > 0.05$) or GSH-Px (Fig. 1e, $P > 0.05$) activity in the erythrocytes of 60-day-old rats, as compared to the control group.

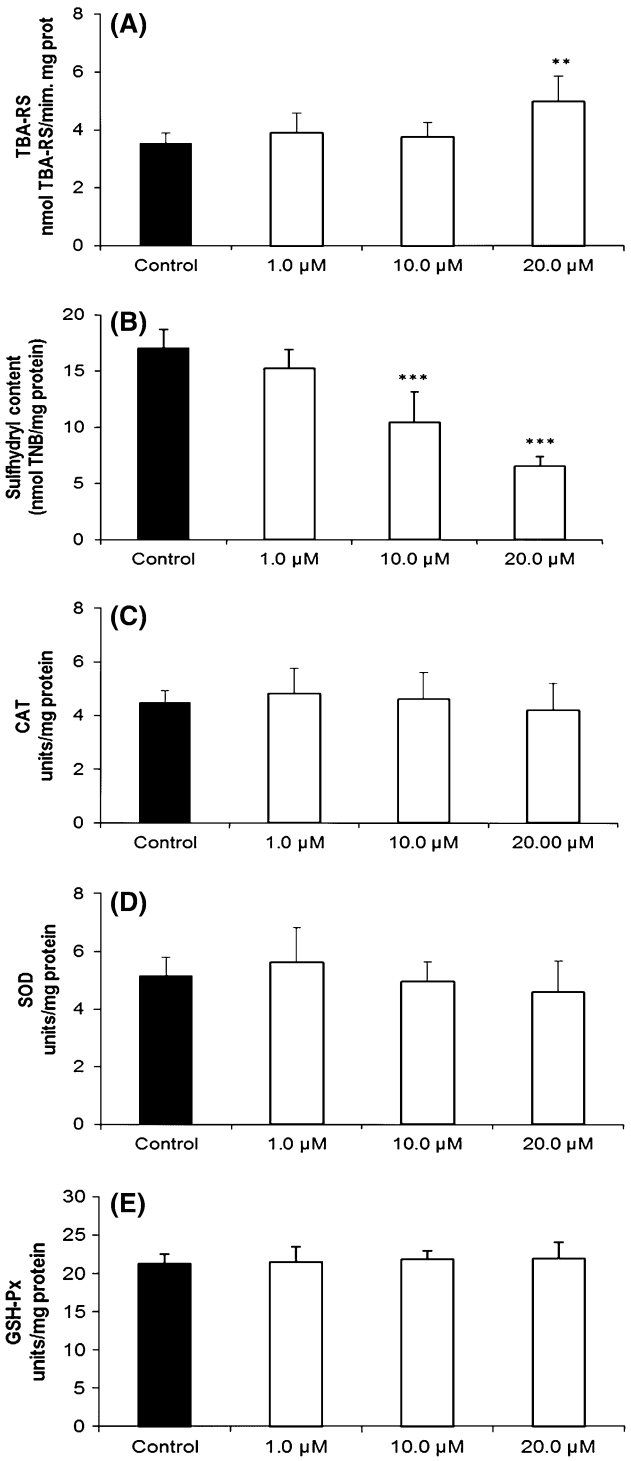

Fig. 1 In vitro effect of homoarginine (1.0–20.0 µM) on thiobarbituric acid-reactive substances (TBA-RS) (**a**) and total sulfhydryl content (**b**) in plasma and on the activities of CAT (**c**), SOD (**d**) and GSH-Px (**e**) in the erythrocytes of rats

In vitro effects of hArg on TBA-RS, total sulfhydryl content and on the activity of antioxidant enzymes in the kidney of rats

We then investigated the in vitro effects of different concentrations of hArg on TBA-RS, total sulfhydryl content and on the activity of the antioxidant enzymes CAT, SOD and GSH-Px in the kidney (cortex and medulla) of rats (Fig. 2). In the renal cortex, hArg at 20 µM significantly decreased

total sulfhydryl content by 20.2 % (Fig. 2b, $P < 0.05$), but did not alter TBA-RS (Fig. 2a, $P > 0.05$). With regard to the antioxidant enzymes, hArg at 20 µM decreased CAT activity by 31.7 % (Fig. 2c, $P < 0.05$), but increased SOD activity by 42 % (Fig. 2d, $P < 0.01$) and GSH-Px activity by 18.4 % (Fig. 2e, $P < 0.001$) in the renal cortex of rats. In the renal medulla of the rats, hArg at 20 µM increased TBA-RS by 36.5 % (Fig. 2a, $P < 0.01$) and SOD activity by 26.8 % (Fig. 2d, $P < 0.01$), but did not alter total sulfhydryl content (Fig. 2b, $P > 0.05$) and the activity of CAT (Fig. 2c, $P > 0.05$) and GSH-Px (Fig. 2e, $P > 0.05$), as compared to the control group.

In vitro effects of hArg on TBA-RS, total sulfhydryl content and on the activity of antioxidant enzymes in the liver of rats

Subsequently, the in vitro effects of hArg on the same parameters analyzed were also verified in the liver of 60-day-old rats. Figure 3 shows that hArg, when added to the incubation medium, did not alter any of the parameters analyzed in the liver of rats, at any of the concentrations tested.

Influence of trolox, ascorbic acid and L-NAME on the effects elicited by hArg in the blood and kidney of rats

Finally, we evaluated whether the alterations in TBA-RS, total sulfhydryl content and in the activity of antioxidant enzymes caused by hArg administration in the blood and kidney were mediated by NO and/or $ONOO^-$ and the production of other free radicals. With this idea in mind, we examined the possible action of the antioxidants trolox and ascorbic acid, as well as of L-NAME, a synthetic NO synthase inhibitor, on the effects produced by hArg on these parameters. Post hoc analyses showed that trolox, ascorbic acid and L-NAME did not alter these parameters per se or when tested in combination with hArg, since the results were not different from the control group. Unlike antioxidants and L-NAME differed significantly from the hArg group in several parameters. As can be seen in Fig. 4, trolox, ascorbic acid and L-NAME were able to prevent the increase in TBA-RS in the plasma (Fig. 4a, $P < 0.001$). Furthermore, trolox and ascorbic acid, but not L-NAME, were able to prevent the increase in TBA-RS (Fig. 4b, $P < 0.001$) in the renal medulla of rats. With regard to total sulfhydryl content, trolox and L-NAME, but not ascorbic acid, were able to prevent the reduction caused by hArg in the plasma of rats (Fig. 4c, $P < 0.001$); moreover, trolox, ascorbic acid and L-NAME were able to prevent the reduction caused by hArg in total sulfhydryl content in the renal cortex of rats (Fig. 4d, $P < 0.001$). With regard to the antioxidant enzymes, Fig. 5 shows that trolox and ascorbic acid, but not L-NAME, prevented the reduction in CAT activity in the renal cortex (Fig. 5a, $P < 0.001$). Trolox, ascorbic acid and L-NAME also prevented the increase in SOD activity (Fig. 5b, $P < 0.001$) in the renal cortex and in the renal medulla (Fig. 5c, $P < 0.001$), and the increase in GSH-Px activity in the renal cortex (Fig. 5d, $P < 0.001$) of the rats.

Discussion

The aim of the present study was to investigate the in vitro effects of hArg on the status of oxidative stress in plasma, erythrocytes, kidney and liver of rats. To our knowledge, this issue has not been investigated thus far. The added hArg concentrations tested were 1.0, 10 and 20 µM. This range covers physiological and pathological concentrations including those occurring in hyperargininemia (Deignan et al. 2010). We measured different biomarkers of oxidative stress, i.e., TBA-RS, total thiol content and the activity of the antioxidant enzymes CAT, SOD and GSH-Px. As guanidine compounds exert oxidative stress (Wyse et al. 2001; Sasso et al. 2014), we also investigated the effects of the antioxidants α-tocopherol (vitamin E) and ascorbic acid (vitamin C), representing a lipophilic and a hydrophilic vitamin, respectively, as well as of L-NAME, a synthetic inhibitor of NO synthase activity. These substances were used at the suprapharmacologically high concentration of 1 mM each. Our study revealed different effects of hArg on parameters of oxidative stress in the biological samples investigated, which do not allow drawing a conclusion.

It seems that appreciable effects of hArg on oxidative stress require the quite high concentration of 20 µM. At this concentration, hArg increases plasma TBA-RS and decreases total thiol content in plasma, suggesting that hArg acts oxidatively in plasma with respect to lipid peroxidation and oxidation of thiols, respectively. In contrast, at 20 µM hArg did not change the activity of the most relevant antioxidative enzymes in red blood cells.

In rat liver, we did not observe any effects of hArg. This finding could be due to rapid metabolism of hArg in the liver. In the kidney, the effects of hArg were more evident, although also not uniform with respect to the individual parameters of oxidative stress. Interestingly and surprisingly, hArg exerted different effects in the kidney with respect to CAT, SOD and GSH-Px. Thus, in the renal cortex but not in renal medulla, hArg (at 20 µM) decreased CAT activity, but increased SOD and GSH-Px activity. This diametrically opposite observation is difficult to explain. A direct, oxidative stress-unrelated effect of hArg on CAT (i.e., inhibition), SOD and/or GSH-Px (i.e., activation) cannot be excluded and remains to be investigated in further studies.

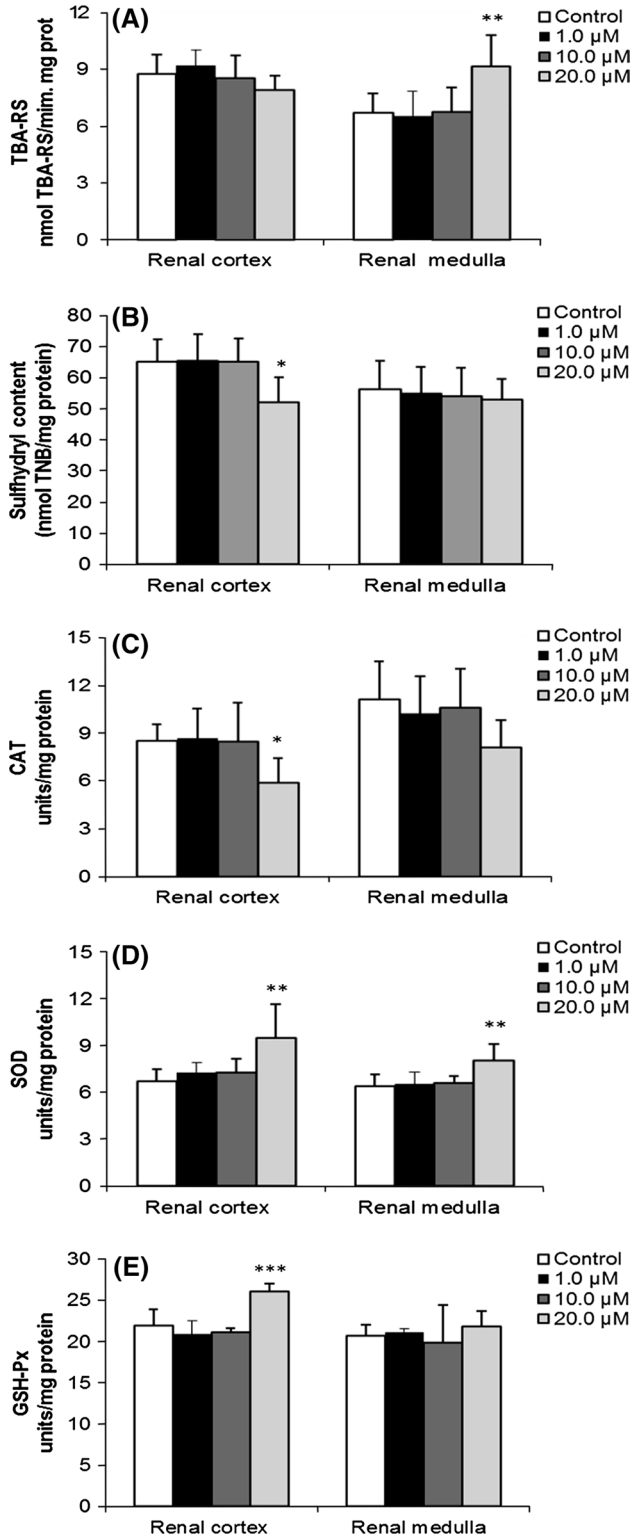

Fig. 2 In vitro effect of homoarginine (1.0–20.0 µM) on thiobarbituric acid-reactive substances (TBA-RS) (**a**), total sulfhydryl content (**b**) and on the activities of CAT (**c**), SOD (**d**) and GSH-Px (**e**) in the kidney (renal cortex and medulla) of rats

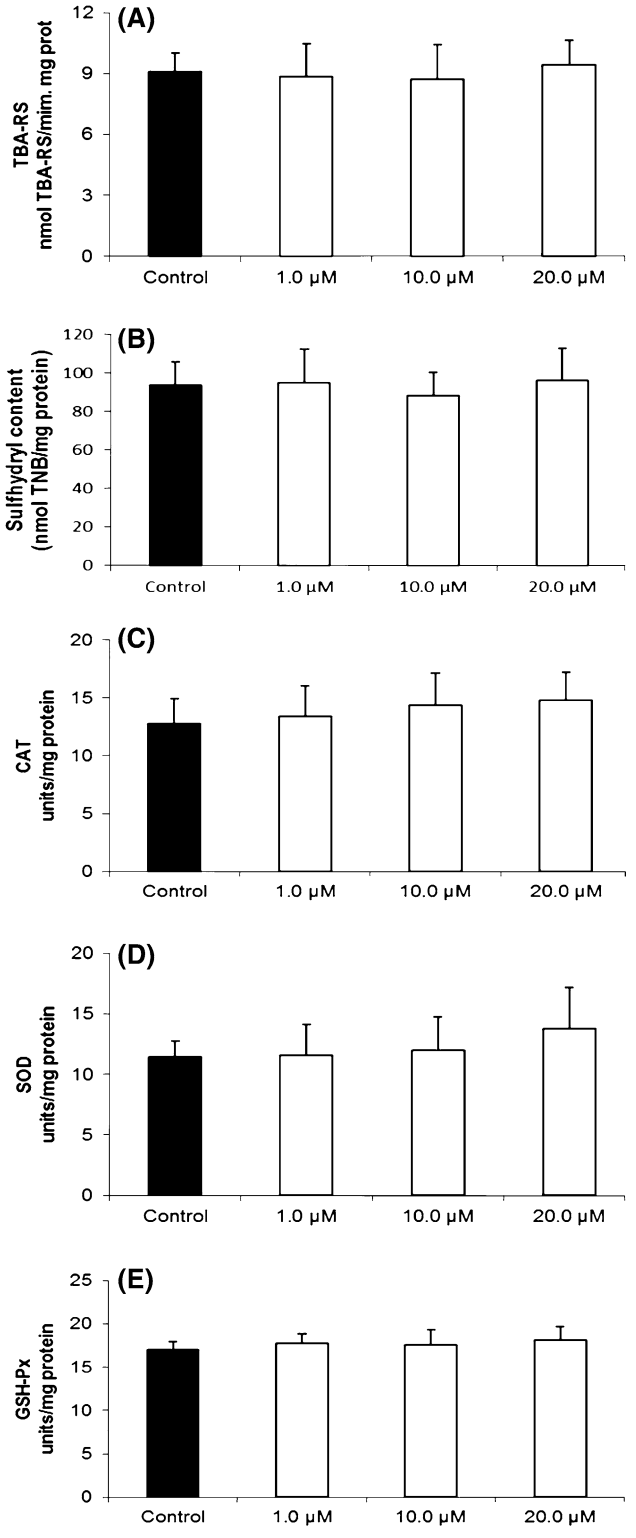

Fig. 3 In vitro effect of homoarginine (1.0–20.0 µM) on thiobarbituric acid-reactive substances (TBA-RS) (**a**), total sulfhydryl content (**b**) and on the activities of CAT (**c**), SOD (**d**) and GSH-Px (**e**) in the liver of rats

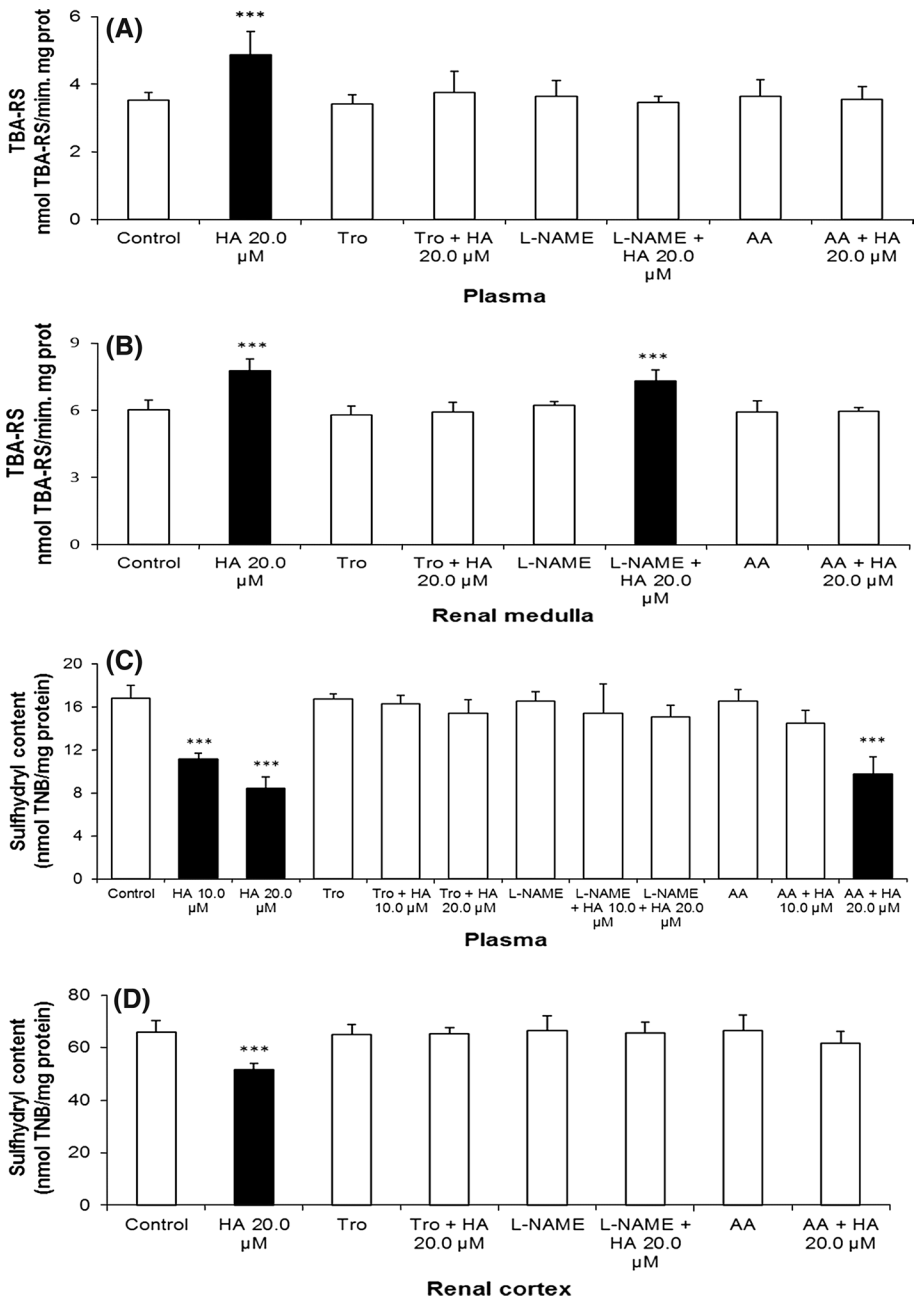

Fig. 4 In vitro effects of trolox, ascorbic acid and L-NAME on thiobarbituric acid-reactive substances (TBA-RS) in the plasma (**a**) and in the renal medulla (**b**), and on total sulfhydryl content in the plasma (**c**) and in the renal cortex (**d**) of 60-day-old rats in the presence or absence of homoarginine

The effects of α-tocopherol, ascorbic acid and L-NAME were also uniform in response to hArg in the biological samples investigated. That L-NAME was able to prevent hArg-induced increase in TBA-RS in rat plasma suggested that L-NAME did not act as an NO synthase inhibitor, because rat plasma did not contain any NO synthase proteins. In the kidney, L-NAME may have exerted its effects, at least in part, by inhibiting NO synthase activity. Yes, this issue has not been addressed in the present study as we did not measure NO synthase activity and this should be investigated in further studies. Such studies also have to consider the possible direct effects of agents such as α-tocopherol, ascorbic acid and L-NAME on the biomarkers investigated (Orr 1966; Rotzinger et al. 1995).

In conclusion, our study indicates that hArg may exert different effects on biomarkers of oxidative stress (lipid peroxidation, thiol oxidation) and main antioxidative enzymes (CAT, SOD, GSH-Px). Effects on these parameters were seen at 10 µM and especially 20 µM of hArg. These hArg concentrations are measured in hyperargininemia, but not in healthy subjects. Whether the detrimental

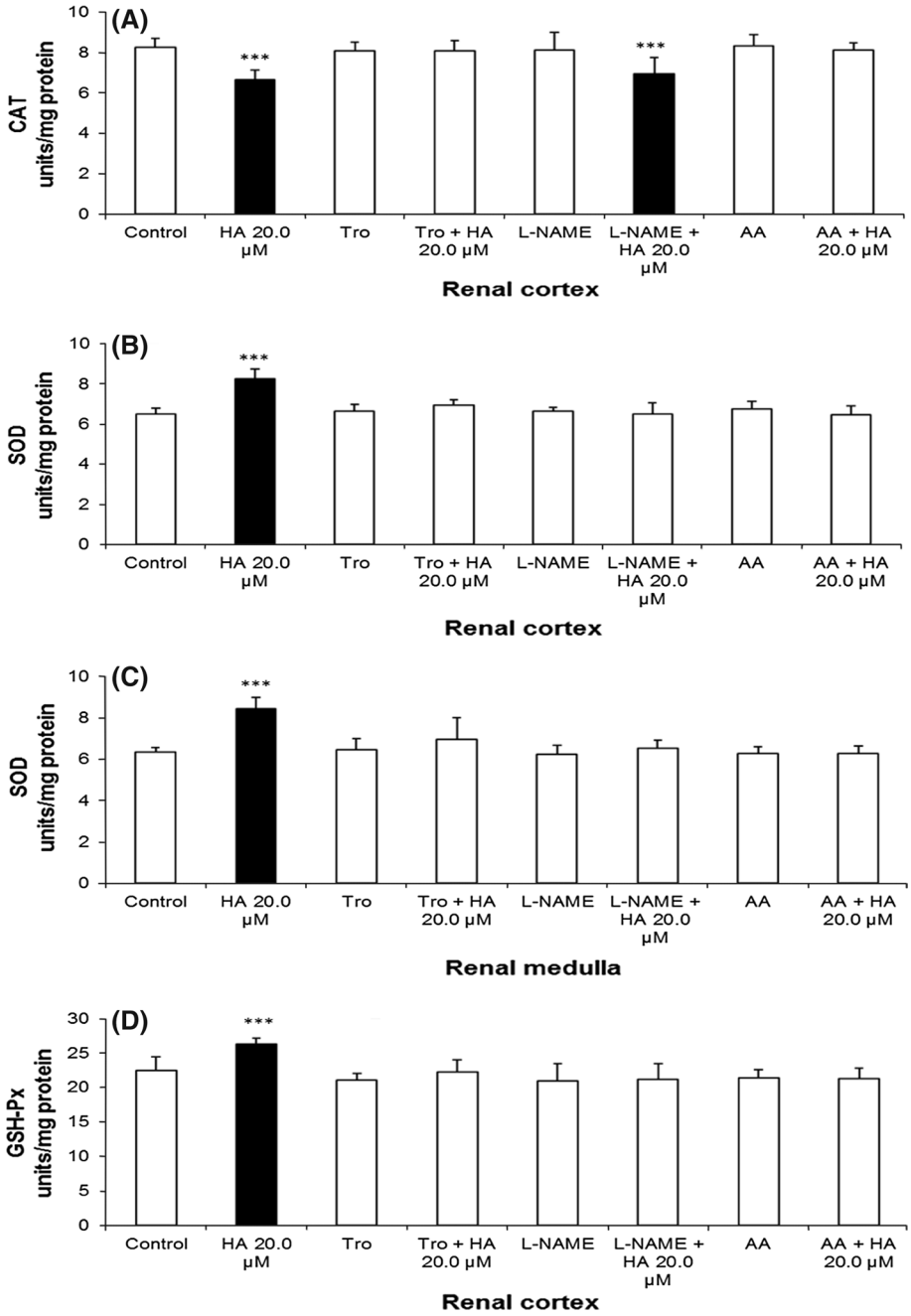

Fig. 5 In vitro effects of trolox, ascorbic acid and L-NAME on the activities of CAT (**a**) in the renal cortex, SOD in the renal cortex (**b**) and in the renal medulla (**c**); and on GSH-Px (**d**) in the renal cortex of 60-day-old rats in the presence or absence of homoarginine

effects seen in hyperargininemia are due to the elevated hArg concentrations are unknown. The roles and the functions of hArg in health and disease are currently under investigation and incompletely understood. Our study indicates that hArg at concentrations found in the blood of hyperargininemic patients may exert different effects on oxidative stress in vitro in the rat. Further studies on the pathophysiology including oxidative stress of hArg in blood and organs such as kidney and liver, the major producers of hArg, are warranted.

Acknowledgments This work was supported by grants from Universidade da Região de Joinville and Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina–FAPESC.

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval The "Principles of Laboratory Animal Care" (NIH publication 85–23, revised 1985) were followed in all the experiments and the experimental protocol was approved by the Ethics Committee for Animal Research of the University of Region Joinville, Joinville, Brazil, under the protocol number 019/2013 PRPPG/CEP.

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Ash D (2004) Structure and function of arginases. J Nutr 134:2765–2767

Braga AC, Vilarinho L, Ferreira E, Rocha H (1997) Hyperargininemia presenting as persistent neonatal jaundice and hepatic cirrhosis. J Pediatr Gastroenterol Nutr 24:218–221

Carvalho DR, Brum JM, Speck-Martins CE, Ventura FD, Navarro MM, Coelho KE, Portugal D, Pratesi R (2012) Clinical features and neurologic progression of hyperargininemia. Pediatr Neurol 46:369–374

Cederbaum S, Crombez EA (2004) Arginase deficiency: Gene Reviews. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K (eds) Seattle. University of Washington, Seattle, pp 1993–2014

Colome C, Sierra C, Vilaseca MA (2000) Congenital errors of metabolism: cause of oxidative stress? Méd Clin 115:111–117

Crombez EA, Cederbaum SD (2005) Hyperargininemia due to liver arginase deficiency. Mol Genet Metab 84:243–251

Deignan JL, Marescau B, Livesay JC, Iyer RK, De Deyn PP, Cederbaum SD, Grody WW (2008) Increased plasma and tissue guanidino compounds in a mouse model of hyperargininemia. Mol Genet Metab 93:172–178

Deignan JL, De Deyn PP, Cederbaum SD, Fuchshuber A, Roth B, Gsell W, Marescau B (2010) Guanidino compound levels in blood, cerebrospinal fluid, and post-mortem brain material of patients with argininemia. Mol Genet Metab 100:31–36

Du C, Anderson A, Lortie M, Parsons R, Bodnar A (2013) Oxidative damage and cellular defense mechanisms in sea urchin models of aging. Free Radic Biol Med 63:254–263

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77

Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 186:407–421

Ferreira AGK, Cunha AA, Machado FR, Pederzolli CD, Dalazen GR, Assis AM, Lamers ML, dos Santos MF, Dutra-Filho CS, Wyse AT (2012) Experimental hyperprolinemia induces mild oxidative stress, metabolic changes, and tissue adaptation in rat liver. J Cel Biochem 113:174–183

Gueraud F, Atalay M, Bresgen N, Cipak A, Eckl PM, Huc l, Jouanin I, Siems W, Uchida K (2010) Chemistry and biochemistry of lipid peroxidation products. Free Radic Res 44:1098–1124

Luiking YC, Engelen MPKJ, Deutz NEP (2010) Regulation of nitric oxide production in health and disease. Curr Opin Clin Nutr Metab Care 13:97–104

Marklund SL (1985) Pyrogallol autoxidation. In: Greenwald RA (ed) Handbook for oxygen radical research. CRC Press, Boca Raton, pp 243–247

Mizutani N, Hayakawa C, Ohya Y, Watanabe K, Watanabe Y, Mori A (1987) Guanidino compounds in hyperargininemia. Tohoku J Exp Med 153:197–205

Oakley FD, Abbott D, Li Q, Engelhardt JF (2009) Signaling components of redox active endosomes: the redoxosomes. Antioxid Redox Signal 11:1313–1333

Orr CW (1966) The inhibition of catalase by ascorbic acid. Biochem Biophys Res Commun 23:854–860

Qi B, Yamagami T, Naruse Y, Sokejima S, Kagamimori S (1995) Effect of taurine on depletion of erythrocyte membrane Na-K ATPase activity due to ozone exposure or cholesterol enrichment. J Nutr Sci Vitaminol 41:627–634

Rotzinger S, Aragon CM, Rogan F, Amir S, Amit Z (1995) The nitric oxide synthase inhibitor NW-nitro-L-arginine methylester attenuates brain catalase activity in vitro. Life Sci 56:1321–1324

Sasso S, Dalmedico L, Delwing-Dal Magro D, Wyse ATS, Delwing-de Lima D (2014) Effect of N-acetylarginine, ametabolite accumulated in hyperargininemia, on parameters of oxidative stress in rats: protective role of vitamins and L-NAME. Cell Biochem Funct 32:511–519

Scaglia F, Lee B (2006) Clinical, biochemical, and molecular spectrum of hyperargininemia due to arginase I deficiency. Am J Med Genet C Semin Med Genet 142:113–120

Silva CG, Bueno ARF, Schuck PF, Leipnitz G, Ribeiro CA, Rosa RB, Dutra Filho CS, Wyse AT, Wannmacher CM, Wajner M (2004) Inhibition of creatine kinase activity from rat cerebral cortex by D-2-hydroxyglutaric acid in vitro. Neurochem Int 44:45–52

Wajner M, Latini A, Wyse ATS, Dutra-Filho CS (2004) The role of oxidative damage in the neuropathology of organic acidurias: insights from animal studies. J Inherit Metab Dis 27:427–448

Wendel A (1981) Glutathione peroxidase. Methods Enzymol 77:325–332

Wyse ATS, Bavaresco CS, Hagen MEK, Delwing D, Dutra-filho CS, Wajner M (2001) In vitro stimulation of oxidative stress in cerebral cortex of rats by the guanidine compounds accumulating in hyperargininemia. Brain Res 923:50–57

Wyse ATS, Zugno AI, Streck EL, Matté C, Calcagnotto T, Wannmacher CMD, Wajner M (2002) Inhibition of Na + , K + − ATPase activity in hippocampus of rats subjected to acute administration of homocysteine is prevented by vitamins E and C treatment. Neurochem Res 27:1685–1689