Oxidative stress-induced by 30 days of mercury exposure triggers acceleration of hypertension development in prehypertensive young SHR rats

Graziele Zandominegue Ronchetti
Universidade Federal do Espírito Santo
Maylla Ronacher Simões
Universidade Federal do Espírito Santo
Ingridy Reinholz Grafites Schereider (ingridy_grafit@hotmail.com)
Universidade Federal do Espírito Santo
Marcos André Soares Leal
Universidade Federal do Espírito Santo
Giulia Alessandra Wiggers Peçanha
Universidade Federal do Pampa
Alessandra Simão Padilha
Universidade Federal do Espírito Santo
Dalton Valentim Vassallo
Universidade Federal do Espírito Santo

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Abstract

Mercury is considered a risk factor for hypertension development and other cardiovascular diseases. Objectives: We investigated whether the effects of mercury exposure on hemodynamic parameters of young Wistar and prehypertensive SHR animals might influence the time course of hypertension development. Main methods: Young (4 weeks) male Wistar and SHR rats were randomly assigned to 4 groups: untreated Wistar rats (Wistar Ct), Wistar rats exposed to mercury chloride (Wistar Hg); untreated SHR rats (SHR Ct) and SHR rats exposed to mercury chloride (SHR Hg) for 30 days. Non-invasive and invasive arterial pressure were measured to investigate pressure reactivity; nitrite/nitrate levels, ACE activity, and lipid peroxidation were measured in plasma. Results: Systolic blood pressure (SBP) of Wistar groups did not change but increased in SHR from the second week to the last one. Hg exposure accelerated the increase of SBP in SHR rats. L-NAME administration increased SBP and diastolic blood pressure (DBP) in all groups, but this increase was smaller in SHR animals exposed to Hg. A decrease of plasma nitrite and nitrate in SHR Hg group suggested that mercury reduced NO bioavailability. Tempol reduced blood pressure, suggesting that the superoxide anion played a role in the marked increase of this parameter. These findings provide evidence that the effects of exposure to Hg might trigger mechanisms to accelerate the hypertension development including NO bioavailability reduction. Therefore, mercury might enhance the risk to cardiovascular disorders not only for adults but also for pre-disposed young ones enhancing hypertension development.

Introduction

Among toxic agents mercury (Hg) is considered a high-risk environmental pollutant for public health.[1,2] According to the US Environmental Protection Agency and the Centers for Disease Control, Hg is classified as one of the top three pollutants[3,4] and represents a considerable risk factor for the development of cardiovascular diseases.[5-14] Currently, human beings are exposed to Hg, mainly by consumption of contaminated fish, the administration of thimerosal in vaccines and the inhalation of Hg vapor during professional exposure.[15-19]

Mercury has become a public health problem, due to its high capacity for bioaccumulation and the variety of its harmful effects on biological systems, including increased blood pressure.[20] Therefore, such characteristics seem to occur only after longer periods of exposure. Carmignani & Boscolo (1984)[21] reported that rats receving 50 µg/mL of mercuric chloride ($HgCl_2$) in drinking water from weaning to 350 days presented arterial hypertension. Those reports showed hypertension development under conditions obtained with exposure to high levels of mercury and longer duration. In addition, we already reported that chronic exposure to smaller mercury concentrations increases vascular reactivity without inducing hypertension at short periods of Hg exposure.[22-24] Mercury chloride administration for 30 days to normotensive rats at levels similar to those found in exposed individuals produce oxidative stress, COX, ACE and NADPH oxidase activation at vascular level, and consequently, vasoconstriction.[22, 24, 25] However, hypertension does not develop. It appears only after longer periods of exposure.[23]

Previously, we demonstrated that mercury exposure for 30 days accelerates the development of hypertension in young SHR animals.[26, 27] However, the systemic mechanisms by which this metal induces the early development of hypertension in these animals has not yet been described. As mentioned above, mercury exposure induces an increase ACE activation at vascular level that enhances oxidative stress reducing NO bioavbailability.[22, 25, 26, 27] On the other hand, the systemic renin-angiotensin system (RAS) plays a role for hypertension development, especially in SHR. Therefore, we hypothetized that low-mercury exposure could accelerate the increment of blood pressure in SHR animals by a systemic mechanism that involves increase of renin-angiotensin system activity and reduction of NO bioavailability.

Materials And Methods

2.1 Animals and Mercury exposure protocol

Young Wistar rats (*Rattus norvegicus*) and SHR (*Spontaneously Hypertensive Rats*) 30 days old and weighing 50 to 80 g, were randomly divided into four groups and treated for 30 days: a) untreated Wistar rats (Wistar Ct) - saline solution 0.9% i.m.; b) Wistar rats exposed to mercury chloride (Wistar Hg) - $HgCl_2$ first dose 4.6 µg/kg and subsequent doses of 0.07 µg/kg/day, i.m. (Wiggers et al., 2008); c) untreated SHR rats (SHR Ct) - saline solution 0.9% i.m.; d) SHR rats exposed to mercury chloride (SHR Hg) - at the same dose as the Wistar Hg group. Rats were housed under conditions of constant temperature (22°C), humidity and light cycle (12:12h light-dark) with free access to water and feed rat chow *ad libitum*. The experiments were carried out in compliance with the Guidelines for Biomedical Research, as stated by the Brazilian Societies of Experimental Biology. The experimental protocols were used according to the ethical principles of animal experimentation recommended by the Research Ethics Committee in Experimental Animal Research of the Federal University of Espírito Santo (CEUA/UFES – approval No. 22/2018).

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2.2 Hemodynamic measurements

2.2.1 Non-invasive Systolic Arterial Blood pressure measurement

Systolic arterial blood pressure (SBP) was assessed weekly by tail plethysmography (IITC Life Science Woodland Hills, CA, USA) according to the manufacturer's recommendations. Before initiating the measurements, animals were subjected to an acclimatization period of three days. This adaptation was performed by placing the animal in the apparatus and exposing it to the measurement protocol to reduce stress. SBP was evaluated weekly and is reported as the arithmetic mean of three recorded measurements.

2.2.2 Invasive blood pressure measurements

After 30 days of mercury treatment, animals were anesthetized with urethane (1.2 mg/kg, intraperitoneal (*i.p.*), the carotid artery and jugular vein were cannulated with a polyethylene catheter (PE-50/Clay-Adams) filled with heparin (50 U/mL) in saline to measure blood pressure and for drug infusion, respectively. The arterial cannula was connected to pressure transducer (TSD 104A - Biopac Systems, Inc; CA, USA) connected to a preamplifier and an acquisition system (MP 30 Biopac Systems, Inc; CA, USA) for recording pressure measurements. After 20 min of stabilization SBP and diastolic blood pressure (DBP) were recorded. Then, the effects of the following drugs on those parameters were analyzed by intravascular injecting of: N^G-nitro-L-arginine methyl ester (L-NAME, 3 mg/kg), a non-selective nitric oxide synthase (NOS) inhibitor; losartan (10 mg/kg), an AT1 receptor antagonist; and hidroxy tempol (Tempol 12 mg/kg) a mimetic of the enzyme superoxide dismutase (SOD).

2.3 Determination of Nitrite and Nitrate

The NO production was evaluated by the amount of nitrite and nitrate accumulation in the plasma using the Griess colorimetric assay as previously described.[28] Briefly, 100 µL of plasma or standards serial dilutions of $NaNO_2$ in non-conditioned media were mixed with 100 µL of Griess reagent. For nitrite measurement 50 µL of 1% sulfanilamide and 50 µL of 0.1% naphtylethylene-diamine-dihydrochloride in 2.5 M H_3PO_4 were added and for nitrate measurement, 8 mg/mL of vanadium was added to the sample. Absorbance was measured at 540 nm.

2.4 Angiotensin Converting Enzyme-1 (ACE-1) activity

The fluorescence resonance energy transfer (FRET) was used to determine the ACE activity with the peptide Abz-FRK(Dnp)P-OH used as a substrate. To obtain tissue samples homogenization in 0.1 M Tris-HCl buffer, pH 7.0 containing 50 mM NaCl and centrifugation for 10 min at 1000 g was performed. The assays using 0.1 M Tris-HCl buffer, pH 7.0 containing 50 mM NaCl and 10 µM $ZnCl_2$, were performed at 37°C. The ACE enzymatic activity of heart homogenates was then obtained by measuring the hydrolysis rate of the substrate Abz- FRK (Dnp) P-OH (10 µM), after incubation for 90 min at 37°C. This assay method was adapted to a 96-well plate reader and the fluorescence measured at 320 nm excitation and 420 nm emission using a Varioskan LUX Multimode Microplate Reader. Protein quantification was performed using the Bradford method[29] for normalization and ACE activity was expressed as nmol Abz-FRK (Dnp) P-OH x min^{-1} x µg protein.

2.5 Determination of thiobarbituric acid-reactive substances (TBARS)

The level of plasma lipid peroxidation was determined using the thiobarbituric acid-reactive substances (TBARS) spectrophotometric assay based on the reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA) according Ohkawa et al (1979).[30] Briefly, 50 µL of plasma was diluted with 125 µL of 17.5% trichloroacetic acid and 125 µL of 0.6% TBA. After being vortexed, the tubes were boiled for 30 min and cooled in ice before being diluted with 125 µL of 70% trichloroacetic acid. The samples were then centrifuged, and the upper phase was read at 532 nm using a spectrophotometer and quantified as nmol of MDA/mg protein.

2.6 Chemicals

The following drugs were used: heparin (Roche Q.F.S.A., Brazil); urethane, mercury chloride, L-NAME (N^G-nitro-L-arginine methyl ester), losartan, tempol, naphtylethylene-diamine-dihydrochloride, sulfanilamide, Tris-HCl, zinc chloride and $NaNO_2$ (Sigma-Aldrich, St. Louis, MO); TCA, TBA and sodium nitrite (Merck KGaA, Darmstadt, Germany); Abz-FRK(Dnp)P-OH trifluoro acetate salt (Santa Cruz Biotecnology).

2.7 Data analysis and statistics

Results were analyzed using the unpaired Student t-test and one or two-way analysis of variance (ANOVA). When a significant one-way ANOVA was obtained the Tukey post hoc test was used to compare means. For two-way ANOVA, the Sidak post hoc test was used. Differences were considered significant at $P<0.05$. Results are expressed as the mean ± standard error of the mean (SEM) for the number of rats indicated in each experiment. The data were analyzed and the figures plotted using GraphPad Prism ~~T M~~ (Version 6.0, GraphPad Software, San Diego, CA, USA).

Results

3.1 Effect of mercury chloride on hemodynamic parameters

SHR treated with mercury showed a significant increase of the SBP obtained by tail plethysmography from the second week of exposure compared to the untreated SHR. Note that SHR animals show an increase in SBP still within the expected limits for this model, while SHR Hg animals already reach hypertension levels in the 3rd and 4th weeks. No significant changes in BP were observed in both Wistar groups (Figure 1).

After 30 days of Hg treatment rats were anesthetized, and SBP and DBP were assessed (invasive recording). As expected SHR had SBP and DBP higher than Wistar rats. However, the mercury treatment did not change SBP and DBP in the Wistar, but did it in SHR group (Table 1).

3.2 Effect of mercury chloride on nitric oxide modulation

To clarify the mechanisms involved in the acceleration of hypertension development in SHRs the blood pressure modulation by NO was evaluated. To assess whether exposure to mercury alters NO modulation, the effect of an inhibitor of NO synthase was investigated. L-NAME increased both SBP and DBP of control and Hg exposure Wistar rats, suggesting a role of NO performing a negative modulation of blood pressure (Figure 2A and 2C). L-NAME also increased SBP and DBP of control SHRs, (Figure 2B and 2D). However, in SHR exposed to mercury, L-NAME administration did not change the SBP (Figure 2B) and just a small increase of the DBP was observed (Figure 2D). This finding suggests that the exposure to mercury reduced NO bioavailability and its negative modulation. Corroborating the hemodynamic results, no difference regarding total nitrite/nitrate between exposed and unexposed Wistar groups was observed (Figure 3). These results reinforce findings that show that Hg did not modify the NO modulation of blood pressure in normotensive rats. But an increased nitrite/nitrate concentration was observed in young untreated SHR when compared to the Wistar control group, suggesting that young SHR do have an increased production of NO. Moreover, the SHR group exposed to Hg showed a decrease in nitrate/nitrite production compared to the SHR Control group, suggesting that mercury exposure decreased NO bioavailability, as previously suggested, by the lack of change of SBP in the protocol using L-NAME.

3.3 Involvement of the superoxide anion in the modulation of oxidative stress on blood pressure

In order to explain this reduced bioavailability of NO in the mercury treated SHR group we investigated the involvement of oxidative stress. Tempol, a mimetic of SOD was used to evaluate the role of superoxide anion. The SBP in the Ct and Hg Wistar groups was not altered (Figure 4A). Moreover, in SHR animals the SBP decreased but only in the Hg treated group (Figure 4B). This finding suggested that in treated SHR the Hg exposure could be increasing the superoxide anion production. Thereby, we investigate the possibility of oxidative stress in the studied animals measuring the plasma concentrations of malondialdehyde (MDA). We did not observe differences in the amount of plasma MDA in both groups or treatment conditions. However, it was seen in treated and untreated SHRs that TBARS levels were higher than in the Wistar group. This finding suggested that the increased oxidative stress was taking place but the Hg exposure was not enough to enhance the lipid peroxidation in young prehypertensive rats (Figure 5).

3.4 Modulation of AT1 receptors in the blood pressure response

It is known that the systemic renin-angiotensin system (RAS) stimulates oxidative stress development and plays a role for hypertension development. Knowing that RAS might be activated by Hg exposure we investigated its role in the acceleration of hypertension development induced by this metal. Losartan administration decreased SBP in all groups (Figure 6A and B), confirming a modulation of SRA

in the blood pressure. However, comparing the mean of the values of SBP reduction produced by losartan, the effect of this blocker was smaller after Hg exposed (Wistar Ct: -31 ± 5 mmHg *vs* Wistar Hg: -17.0 ± 5 mmHg, **P*<0.05 and SHR Ct: -46.1 ± 8 *vs* SHR Hg**:** -23.6 ± 6 mmHg, **P*<0.05). Regarding the DBP, all groups exposed or not to mercury showed a reduction after treatment with Losartan, but without differences among them (results not shown). In addition, it is possible to observed in the Figure 7 that mercury exposure reduced the ACE activity, being this effect greater in the SHR Hg exposed group.

Discussion

In this study we suggested that mercury exposure accelerated the hypertension development in young SHR animals. This behavior seen in the mercury treated SHR appears to be caused by a reduced bioavailability of NO, which plays a crucial role in the blood pressure regulation decreasing its negative modulation by oxidative stress. These findings were reinforced by the plasma nitrite/nitrate reduction and the reduction of SBP produced by tempol in SHR after mercury exposure. Losartan reduced the SBP of all groups. In addition, after mercury exposure the mercury treated SHR group showed reduced ACE activity (Figure 7) and in a lesser extent the increment of SBP. These findings should reduce the accelerated hypertension development of mercury treated SHR group instead of increasing it. Therefore, the increase in SBP of SHR exposed group should be occuring by another mechanism involving oxidative stress and reduction of nitric oxide bioavailability.

Previous findings have shown that mercury exposure in normotensive rats, both acute and for 30 days, affects endothelial function without changing arterial blood pressure even though reducing NO bioavailability [24, 25, 31] increasing the production of free radicals.[11, 22, 32, 33] However, in normotensive animals previous report showed that chronic Hg exposure for long periods (60 days) increases blood pressure.[23, 34] Based on these findings we argued whether the association of mercury with predisposing hypertensive effects could accelerate the occurrence of hazardous consequences as increase of arterial blood pressure, oxidative stress and reduced NO modulation.

Acute exposure to small doses of mercury chloride causes elevation of SBP[35,36] but exposure for 30 days does not change SBP in Wistar animals.[22, 23, 25, 34, 36] In addition, even using high doses of chronically administered mercury chloride there is no interference with the systolic or diastolic blood pressure of adult Wistar rats.[23, 34] These findings suggest that acute mercury administration in adult Wistar rats increases blood pressure while under chronic treatment at levels similar to those found in exposed individuals adaptive mechanisms restrain such blood pressure increment.

In fact, recently we showed that mercury exposure could accelerated the hypertension development of young SHR.[26, 27] However, this effect was accompanied by a vasoprotective mechanism, which includes an increase of NO, against the early establishment of hypertension in resistance arteries of young SHR. Therefore, we evaluated the effect of mercury exposure in *in vivo* young SHR to elucidate how this metal changes blood pressure at the same time that vasoprotective mechanisms were occurring, as observed previously.[27]

Thus, we evaluated mercury effects by recording invasive and noninvasive arterial blood pressure. Our results showed an accelerated increase of the SBP (noninvasive recordings) in young SHR exposed to mercury chloride from the second week of treatment. This was not observed in the Wistar group. Invasive recordings after mercury exposure for 30 days also showed that SBP and DBP did not differ between control groups but SBP of SHR treated increased compared to SHR controls. These findings suggested that the mercury exposure of prehypertensive SHRs might influence the time course of hypertension development.

Previous reports showed that mercury exposure causes endothelial dysfunction, reduces NO production and increases oxidative stress in Wistar rats.[22, 24, 25, 37] Similar findings of endothelial dysfunction have been reported in other models as the SHR strain.[38-40] Therefore, our first attempt was to investigate the role of NO. Considering that NO acts relaxing vascular smooth muscle, we studied its bioavailability in animals exposed to Hg. It is known that eNOS expression is increased in young SHR (approximately 12 weeks)[41, 42] and decreased in adult and elderly SHR rats (approximately 36 and 72 weeks, respectively).[43] Our findings using L-NAME administration suggested that mercury chloride interfered with the bioavailability of NO at an earlier stage of animal life.

We also investigated plasma nitrate and nitrite as an indicator of NO production.[44] The nitrite/nitrate concentration was higher, as expected,[45] in the control group of young SHR rats compared with the Wistar control group. However, there was no difference between the treated and untreated Wistar groups (Figure 4). Moreover, only in the SHR group exposed to Hg, a reduction in nitrate/nitrite production was observed suggesting that mercury reduced NO production or increased its degradation (catabolism). Another possible mechanism already reported is the reduced NO production by endothelial damage produced by mercury using a similar treatment.[46] This finding reinforced our results, a condition that might contribute to accelerate the increase of the SBP.

It is well established that Hg activates NADPHoxidase and COX consequently increasing free radicals production and producing vasoconstriction.[13, 25, 26, 27] NO can react with the superoxide anion forming peroxynitrite, another vasoconstrictor.[45] So, we evaluated the involvement of superoxide anion and oxidative stress in animals exposed to Hg. Our findings showed that both Wistar groups, Hg-treated and untreated, had a decrease in systolic blood pressure when treated with Tempol (Figure 5-A), but without difference between the groups. In SHRs, the decrease of SBP was shown only in the group exposed to $HgCl_2$. These results suggest that the superoxide anion was contributing to increase the SBP in young SHR animals exposed to mercury for 30 days.

To evaluate the extent of oxidative stress, we investigated lipid peroxidation. It has also been reported that male SHR adult animals have an increase in oxidative stress.[47] However, we did not observe an increase in the lipid peroxidation in the animals exposed to $HgCl_2$ of both SHR groups. In young SHR animals, a previous study showed that they have an activated antioxidant capacity by superoxide dismutase and catalase,[48] which explains our findings. Regarding adult Wistar rats, it has been reported

in a 30-day mercury exposure that, at the end of the treatment, superoxide dismutase and catalase were higher when compared to the untreated group.[49] These findings suggest that the chronic mercury administration to young SHR might activate mechanisms that reduce NO bioavailability and explains the accelerated increase of arterial blood pressure.[22, 23, 35, 37]

Another mechanism that generates oxidative stress and contributes to increase arterial blood pressure is the renin angiotensin system. Its product, angiotensin II, interacts with AT 1 receptors and increases blood pressure. Therefore, we investigated the modulation of these receptors in animals exposed to Hg. When the animals were treated with Losartan, a decrease of SBP was observed in all groups. However, it was noticed that the decrease of SBP was smaller in the animals exposed to $HgCl_2$.

However, we should emphasize that when comparing untreated rats from both groups ACE activity was increased in the SHRs. This result suggested that ACE activity was interfering only in the young SHRs. Based on these findings it was possible to postulate that in young SHRs the renin angiotensin system is involved in the increase of arterial pressure. In the SHR model in animals with previously established hypertension, we demonstrate that ACE activity levels increase with exposure to mercury.[50] However, before establishing hypertension in SHRs the metal exposure caused the opposite effect, a reduction in this enzyme activity. Mercury exposure accelerated the increase of SBP in SHR although the percentage of the losartan effect was reduced and ACE activity was also reduced in this exposed group. Therefore, the enhanced increase in SBP of SHR group exposed to Hg, should be occuring by a mechanism involving oxidative stress and reduction of nitric oxide. So, is there then a link between oxidative stress and the renin-angiotensin system in such conditions? Recently, Saleem et al., (2016)[51] suggest a mechanism that might explain such possibility. Superoxide, but not H2O2, acting via Sp3 transcription factor up-regulates angiotensin II AT1 receptor expression and function in human kidney-2 cells. Rizzetti et al., (2018)[24] also reported that Hg exposure might play a role upregulating AT-1 receptors by increasing oxidative stress and reducing NO bioavailability.

Conclusion

Our results suggest that chronic mercury exposure accelerates SBP development in young SHR. These findings provide evidence that the effects of chronic exposure to Hg might trigger mechanisms that accelerate hypertension development, dependent on oxidative stress induced by superoxide anion. Consequently, reduction of NO bioavailability and the renin-angitensin system activation, accelerated the development of hypertension in young SHRs. Thereby, mercury must be considered a risk factor for cardiovascular diseases development not only for adults, but also for pre-disposed young individuals accelerating the progress of arterial blood pressure increment.

Declarations

Compliance with Ethical Standard

Ethical Approval: All experimental procedures were performed in accordance with the guidelines for the care and handling of laboratory animals as recommended by the Brazilian Societies of Experimental Biology, and the study protocols were previously approved by the Ethics Committee in Animal Research of the Federal University of Espirito Santo (22/2018 CEUA-UFES).

Conflict of interest: The authors declare no conflict of interest with the contents of this article.

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5463.12148

Tables

Table 1 – Effects of chronic mercury exposure on invasive blood pressure measurements

	Wistar Ct	Wistar Hg	SHR Ct	SHR Hg
SBP	120 ± 2 (n=10)	114 ± 3 (n=9)	131 ± 3 (n=17)$^{\#}$	141 ± 2 (n=18)*
DBP	84 ± 5 (n=6)	78 ± 6 (n=6)	90 ± 5 (n=7)	103 ± 6 (n=7)

The values are expressed as the mean ± SEM. The sample number is indicated in parentheses. *P<0.05 *vs*. SH

Figures

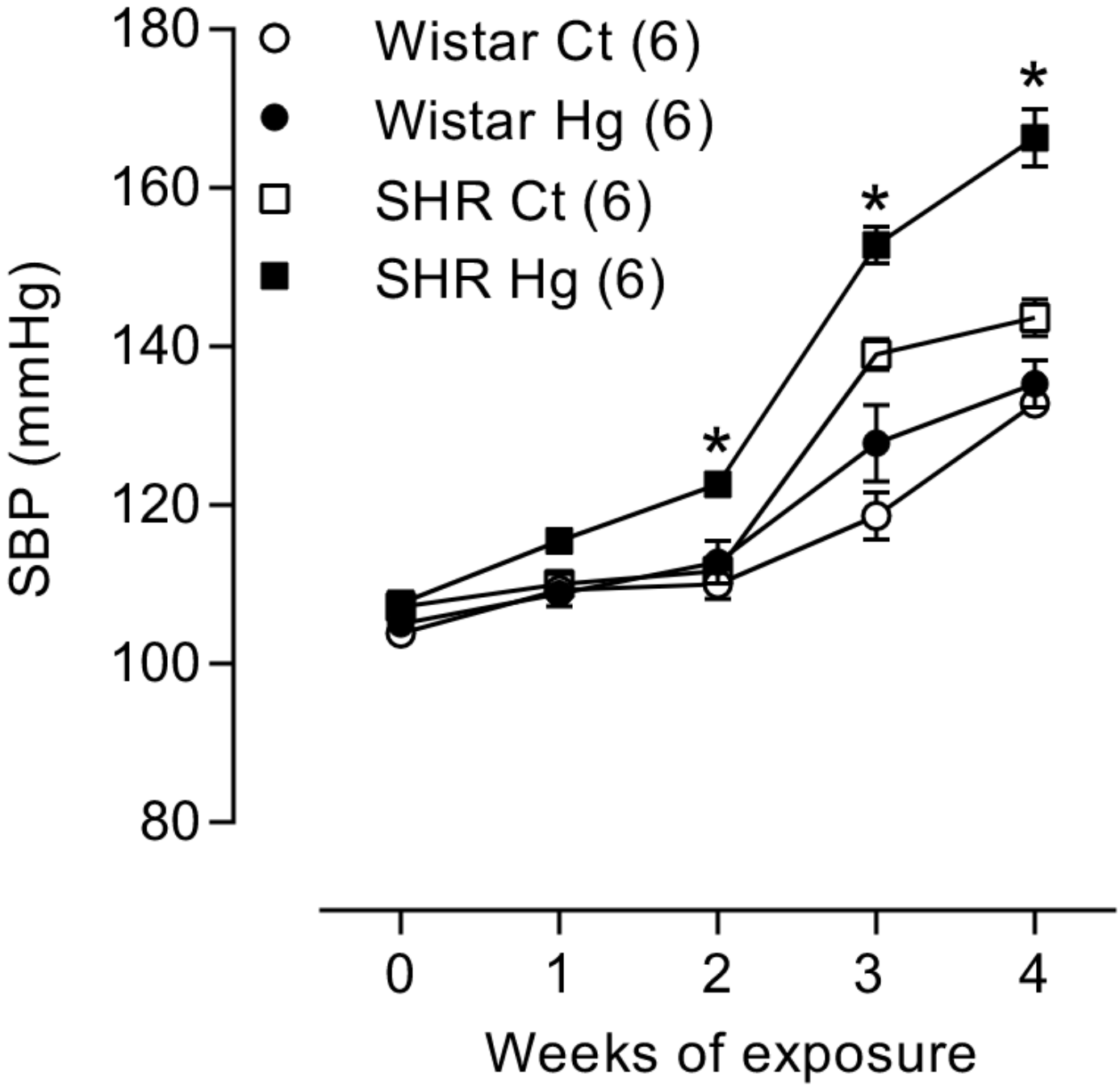

Figure 1

Effects of 4-week exposure to mercury on non-invasive measure of systolic blood pressure (SBP) in young Wistar and SHR rats (N = 6 for all groups). SBP is shown in mmHg and presented in weeks. Time 0 represents the start of the study and the fourth week of exposure corresponds to the end of treatment. The symbols represent the mean ± SEM. *P<0.05 *vs*. SHR Ct using two-way ANOVA, followed by the Sidak post-test

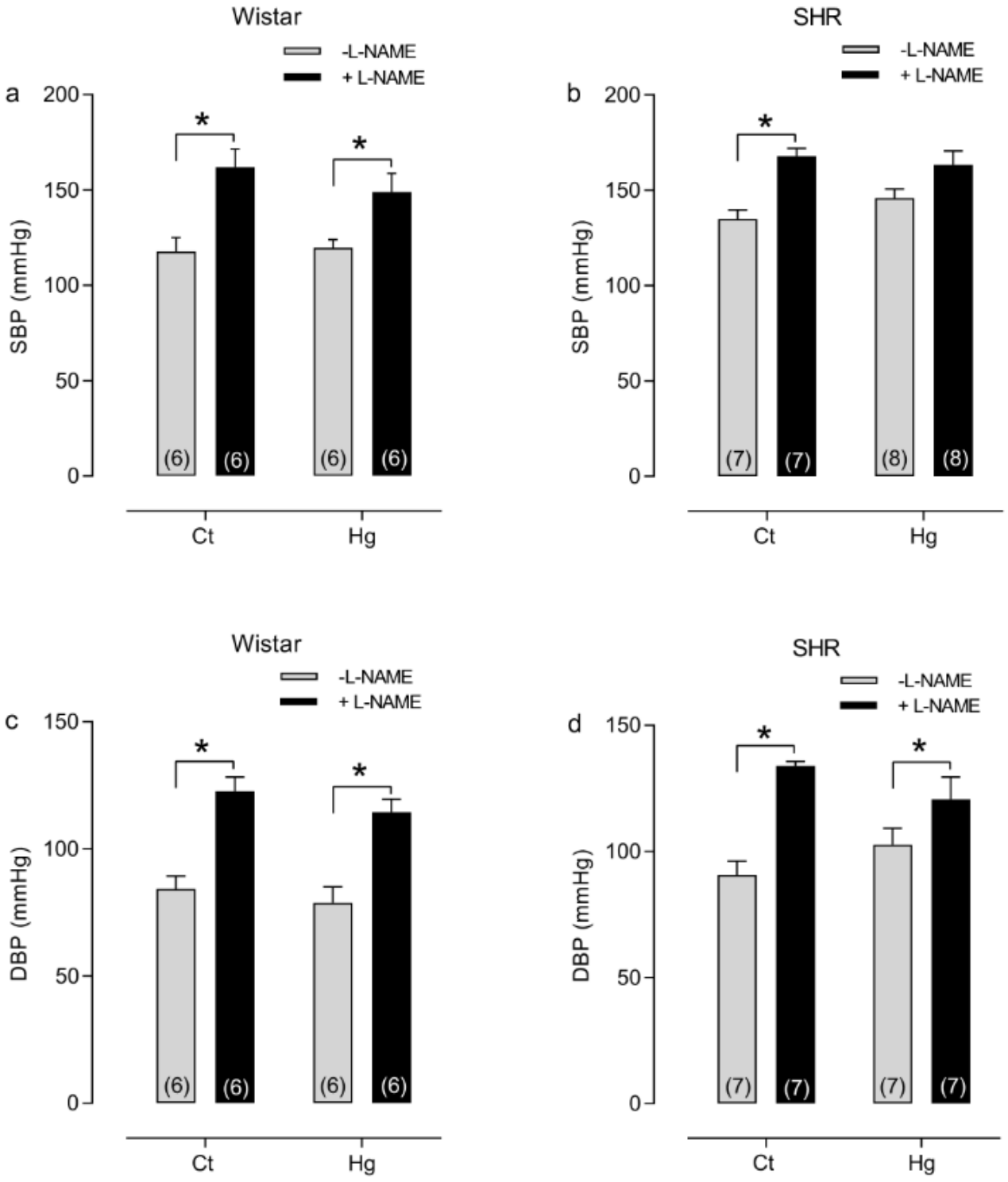

Figure 2

(A) Systolic blood pressure (SBP) of Wistar rats, (B) of SHRs, (C) diastolic blood pressure (DBP) of young Wistar, and (D) of young SHR rats in control and mercury exposure condition before (- LNAME: grey bar) and after (+ LNAME: black bar) intravascular injecting of NO synthase inhibitor, L-NAME (3 mg/kg). The number of animals is indicated on the respective bars. Results are presented as mean ± SEM. *P<0.05 *vs.* - L-NAME and $^{\#}P$<0.05 for comparison between Wistar and SHR groups by Student's t-test

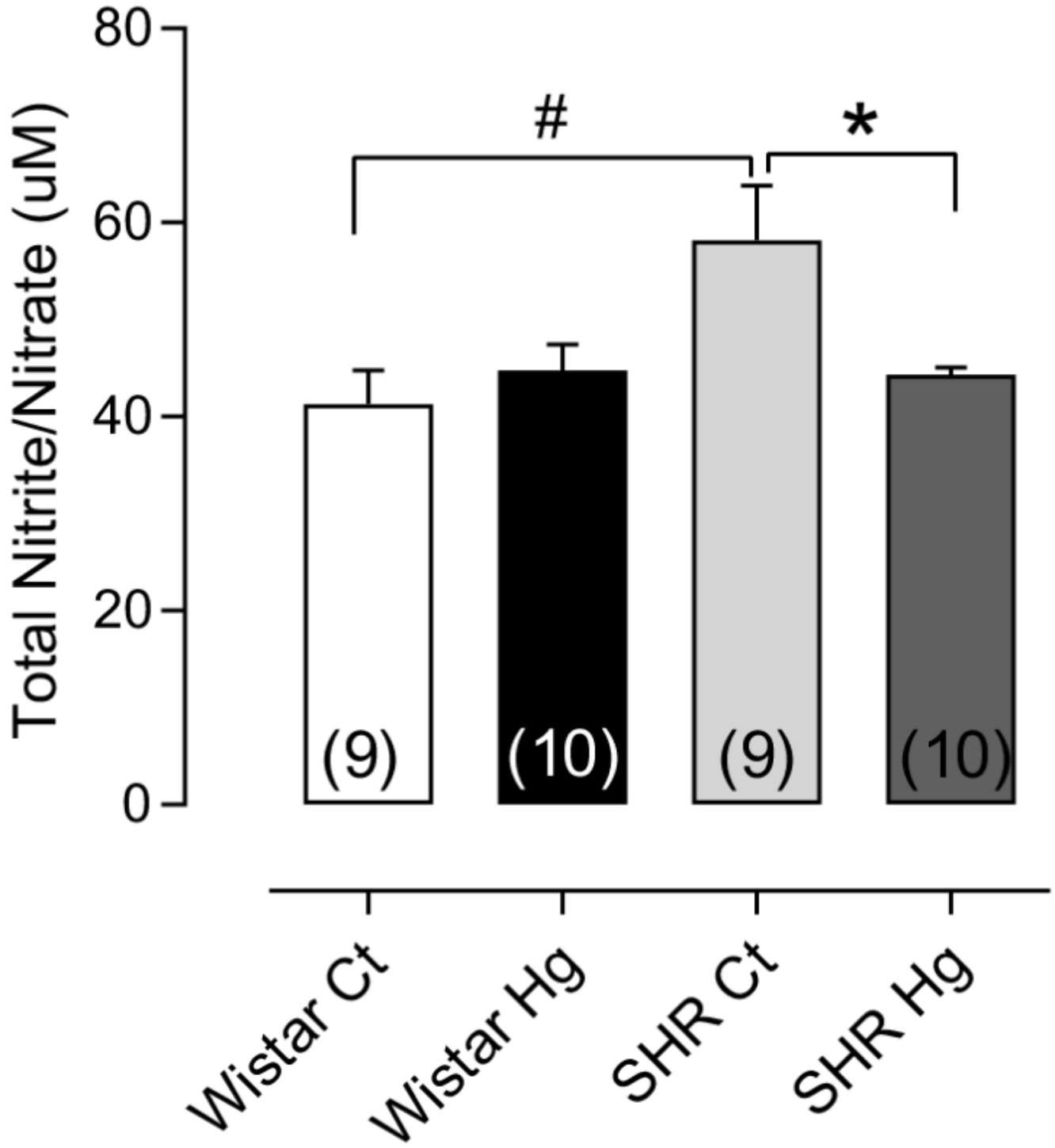

Figure 3

Plasma nitrite and nitrate production. Results are expressed as the mean ± SEM. The number of animals is indicated on the respective bars. One-way ANOVA, followed by Tukey's post-test. *P<0.05 vs. SHR Ct and $^{\#}P$<0.05 for comparison between Wistar and SHR groups

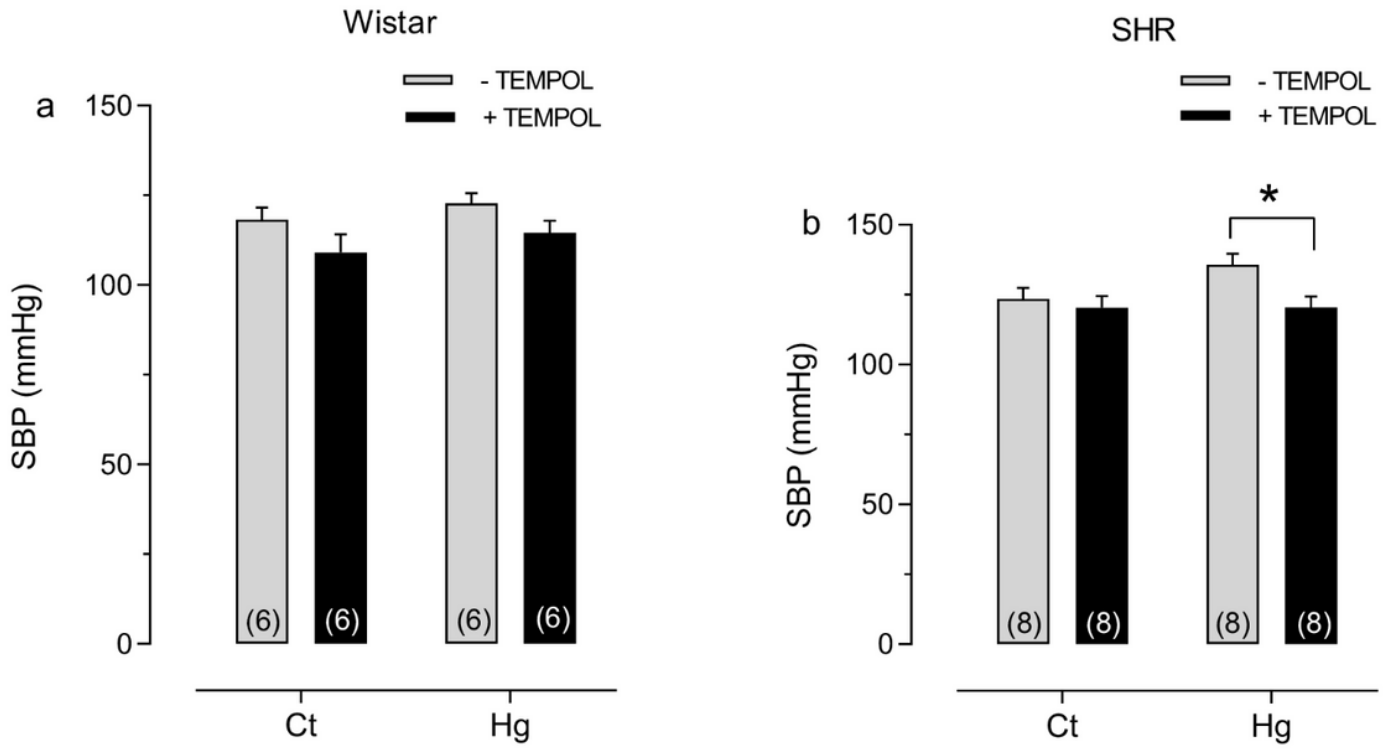

Figure 4

(A) Systolic blood pressure (SBP) of young Wistar rats, (B) of young SHR rats in the control group and in the mercury exposure condition before (- TEMPOL: grey bar) and after (+ TEMPOL: black bar) intravascular injecting of SOD mimetic, TEMPOL (12 mg/kg). The number of animals is indicated on the respective bars. Results are presented as mean ± SEM. *P<0.05 vs. -TEMPOL in SHR by Student's t-test

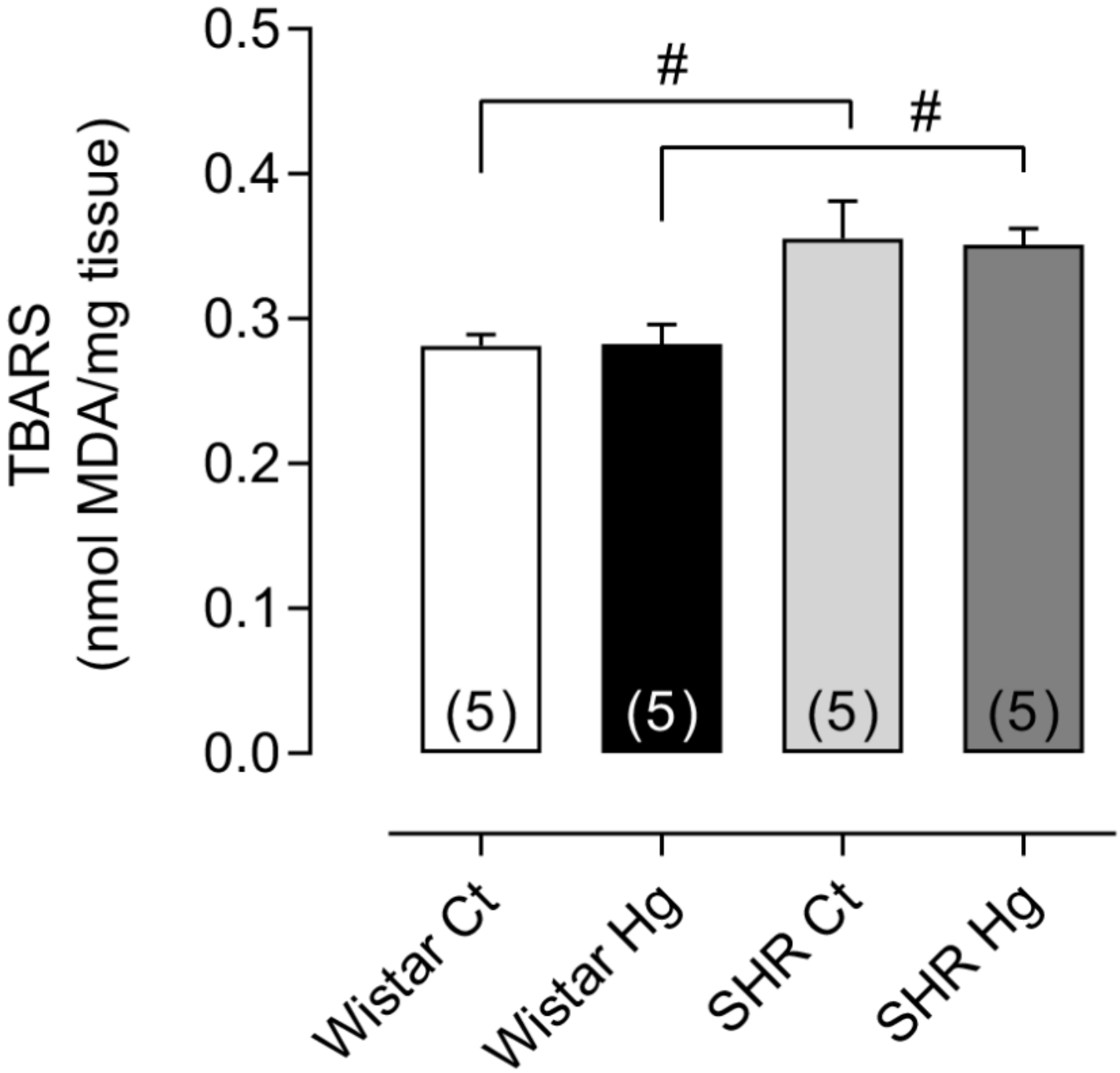

Figure 5

Plasma level of lipid peroxidation determined by thiobarbituric acid reactive substances (TBARS). Results are expressed as the mean ± SEM. The number of animals is indicated on the respective bars. $^{\#}P<0.05$ vs Wistar Ct and Wistar Hg by one-way ANOVA, followed by Tukey's post-test

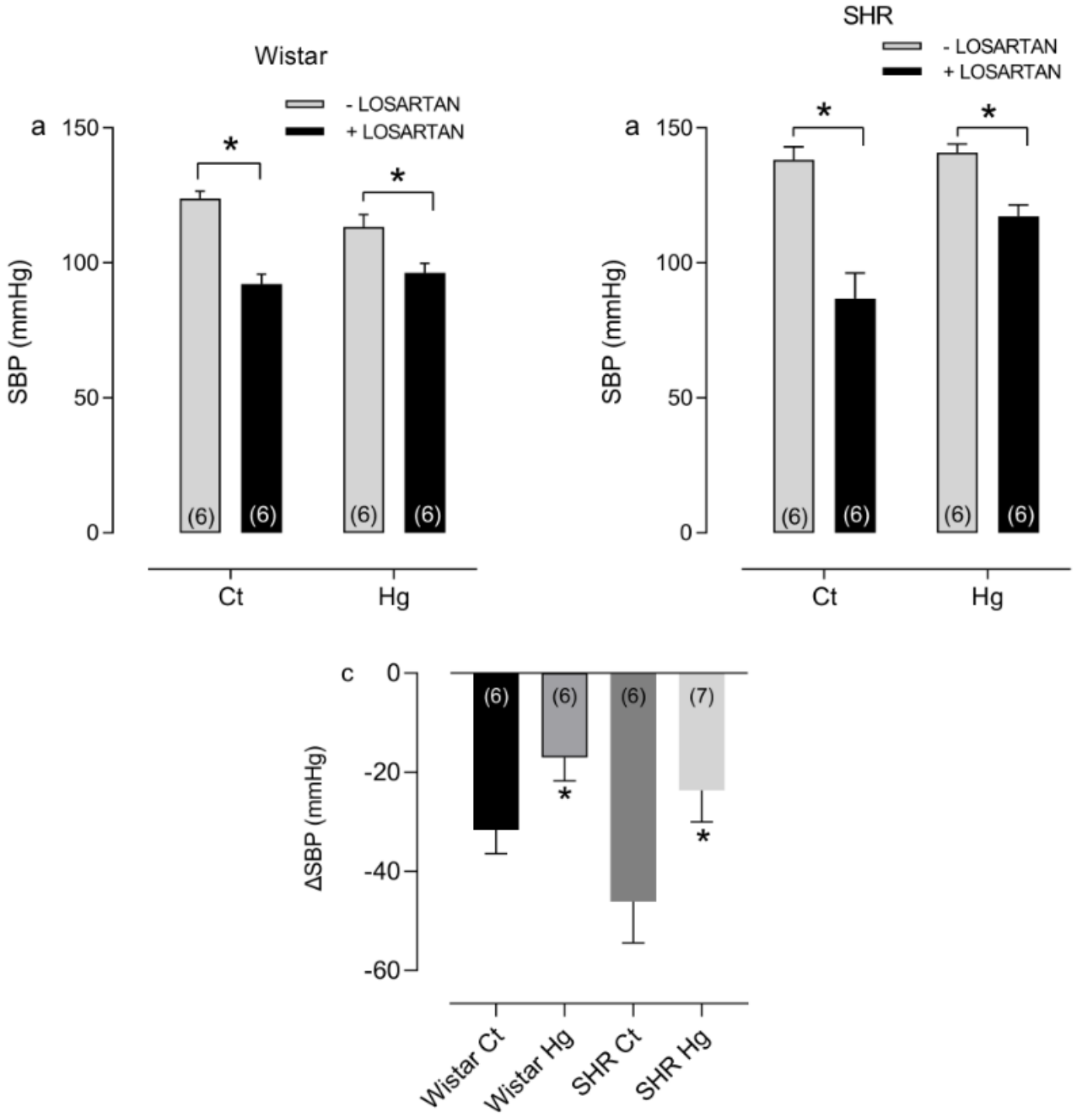

Figure 6

(A) Systolic blood pressure (SBP) of young Wistar rats, (B) of young SHR rats in the control group and in the mercury exposure condition before (- LOSARTAN: grey bar) and after (+ LOSARTAN: black bar) intravascular injecting a selective antagonist of AT1 receptors for angiotensin II, LOSARTAN (10 mg/Kg). The number of animals is indicated on the respective bars. Results are presented as mean ± SEM. *P<0.05 vs. -LOSARTAN in Wistar and SHR by Student's t-test

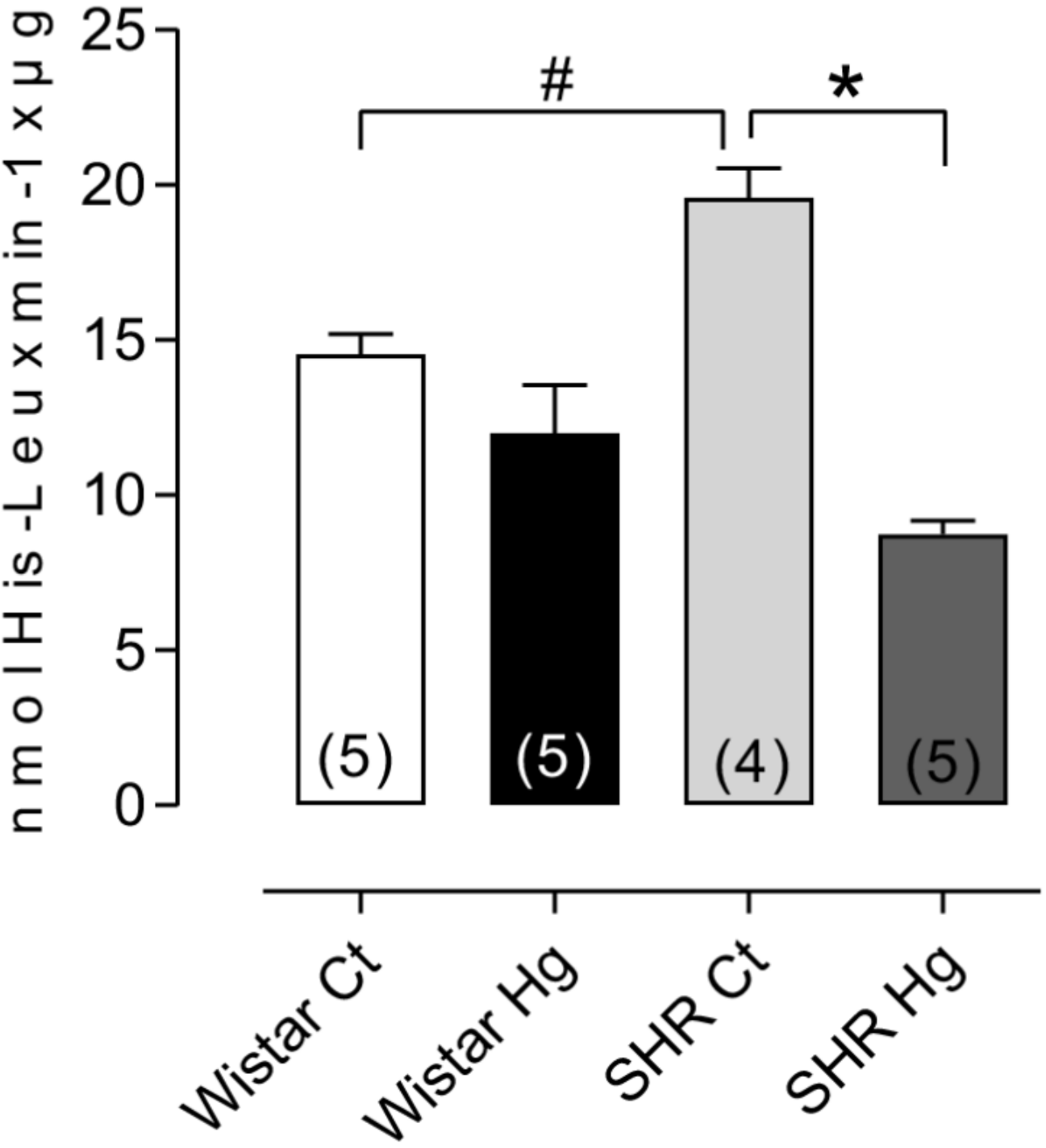

Figure 7

Plasma ACE activity. Results are expressed as the mean ± SEM. The number of animals is indicated on the respective bars. One-way ANOVA, followed by Tukey's post-test. *P<0.05 *vs* SHR Ct and $^{\#}P$<0.05 *vs* Wistar Ct or Wistar Hg