Toxicology and Applied Pharmacology 262 (2012) 22–31

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Activation of K^+ channels and Na^+/K^+ ATPase prevents aortic endothelial dysfunction in 7-day lead-treated rats

Jonaina Fiorim [a,*], Rogério Faustino Ribeiro Júnior [a], Bruna Fernades Azevedo [a], Maylla Ronacher Simões [a], Alessandra Simão Padilha [a], Ivanita Stefanon [a], Maria Jesus Alonso [c], Mercedes Salaices [b], Dalton Valentim Vassallo [a]

[a] Department of Physiological Sciences, Federal University of Espirito Santo, Vitoria, ES, Brazil
[b] Departamento de Farmacología, Universidad Autónoma de Madrid, Instituto de Investigación Hospital Universitario La Paz (IdiPaz), Spain
[c] Departamento de Ciencias de la Salud III, Universidad Rey Juan Carlos, Alcorcón, Spain

ARTICLE INFO

Article history:
Received 6 February 2012
Revised 10 April 2012
Accepted 12 April 2012
Available online 23 April 2012

Keywords:
Lead
Nitric oxide
Na^+/K^+-ATPase activity
Potassium channels

ABSTRACT

Seven day exposure to a low concentration of lead acetate increases nitric oxide bioavailability suggesting a putative role of K^+ channels affecting vascular reactivity. This could be an adaptive mechanism at the initial stages of toxicity from lead exposure due to oxidative stress. We evaluated whether lead alters the participation of K^+ channels and Na^+/K^+-ATPase (NKA) on vascular function. Wistar rats were treated with lead (1st dose 4 μg/100 g, subsequent doses 0.05 μg/100 g, im, 7 days) or vehicle. Lead treatment reduced the contractile response of aortic rings to phenylephrine (PHE) without changing the vasodilator response to acetylcholine (ACh) or sodium nitroprusside (SNP). Furthermore, this treatment increased basal O_2^- production, and apocynin (0.3 μM), superoxide dismutase (150 U/mL) and catalase (1000 U/mL) reduced the response to PHE only in the treated group. Lead also increased aortic functional NKA activity evaluated by K^+-induced relaxation curves. Ouabain (100 μM) plus L-NAME (100 μM), aminoguanidine (50 μM) or tetraethylammonium (TEA, 2 mM) reduced the K^+-induced relaxation only in lead-treated rats. When aortic rings were precontracted with KCl (60 mM/L) or preincubated with TEA (2 mM), 4-aminopyridine (4-AP, 5 mM), iberiotoxin (IbTX, 30 nM), apamin (0.5 μM) or charybdotoxin (0.1 μM), the ACh-induced relaxation was more reduced in the lead-treated rats. Additionally, 4-AP and IbTX reduced the relaxation elicited by SNP more in the lead-treated rats. Results suggest that lead treatment promoted NKA and K^+ channels activation and these effects might contribute to the preservation of aortic endothelial function against oxidative stress.

Introduction

Lead has historically been used in a wide variety of human activities, which has significantly increased its emission into the atmosphere (Patrick, 2006). Therefore, all humans have an associated lead burden due to exposure to exogenous sources (Levin and Goldberg, 2000). The adverse effects of lead on the heart and vessels have been previously demonstrated (Fiorim et al., 2011; Silveira et al., 2010; Vassallo et al., 2008). Numerous studies have revealed that chronic or acute lead exposure increases oxidative stress (Silveira et al., 2010; Vaziri et al., 1999a), lipid peroxidation (Ding et al., 1998; Vaziri et al., 1999b), and affects antioxidant reserves (Farmand et al., 2005; Vaziri et al., 2003).

Vascular endothelium is highly sensitive to oxidative stress, and this stress is the main cause of the endothelial dysfunction observed in cardiovascular diseases such as atherosclerosis, hypertension and stroke (Chatterje and Catravas, 2008; Forstermann and Munzel, 2006). It is well established that lead exposure induces endothelial dysfunction, and therefore, it could be considered an important cardiovascular risk factor and a serious problem for public health (Patrick, 2006; Poreba et al., 2011; Silveira et al., 2010; Vaziri et al., 1999a).

Recently, we demonstrated that a 7-day treatment with a low concentration of lead acetate increases NO bioavailability and Na^+/K^+-ATPase activity in the rat aorta (Fiorim et al., 2011). NO, a short lived gas, is an important protective molecule in the vasculature, especially in conductance arteries. NO is synthesized in the endothelium and diffuses from it to the vascular lumen being a potent inhibitor of platelet aggregation, adhesion and proliferation of vascular smooth muscle cells, and it prevents the development of atherosclerosis (Chatterje and Catravas, 2008; Forstermann and Munzel, 2006; Poreba et al., 2011; Triggle et al., 2003). Thus, in response to various neurohumoral

* Corresponding author at: Programa de Pós-Graduação em Ciências Fisiológicas, CCS/UFES, Av. Marechal Campos, 1468, Maruípe, 29040-091,Vitoria, ES, Brazil. Fax: +55 27 2122 7330.
E-mail addresses: nanafiorim@hotmail.com (J. Fiorim), faustino43@oi.com.br (RF. Ribeiro Júnior), brunafernandes.azevedo@gmail.com (BF. Azevedo), yllars@hotmail.com (MR. Simões), ale_spadilha@yahoo.com.br (AS. Padilha), ivanita@pq.cnpq.br (I. Stefanon), mariajesus.alonso@urjc.es (MJ. Alonso), mercedes.salaices@uam.es (M. Salaices), daltonv2@terra.com.br (DV. Vassallo).

0041-008X © 2012 Elsevier Inc. Open access under the Elsevier OA license.
doi:10.1016/j.taap.2012.04.015

stimuli, endothelial cells release NO, which produces vasodilation of the vascular smooth muscle cells. In addition, NO could also stimulate Na^+/K^+-ATPase activity (Gupta et al., 1994) and open K^+ channels (Bolotina et al., 1994; Félétou and Vanhoutte, 2006), which contribute to maintain adequate vascular function.

The Na^+/K^+-ATPase is responsible for maintaining the cellular membrane potential and contributes to the regulation of vascular tone and blood pressure. Thus, alterations in the Na^+/K^+-ATPase could be related to cardiovascular disease (Marín and Redondo, 1999). In a previous report, it has been shown that chronic lead exposure causes cardiovascular disease by inhibiting Na^+/K^+-ATPase (Weiler et al., 1990). However, our recent studies have shown that acute (Simões et al., 2011) or 7-day lead exposure increases Na^+/K^+-ATPase activity and the expression of the alpha-1 subunits of Na^+/K^+-ATPase (Fiorim et al., 2011).

K^+ channel activation has been identified as an important component in vascular tone regulation (Ko et al., 2008; Nelson and Quayle, 1995). Activation of K^+ channels in vascular smooth muscle leads to hyperpolarization, decreases the activity of voltage-gated L-type Ca^{2+} channels, reduces $[Ca^{2+}]i$ and induces vasodilation (Ledoux et al., 2006). Many subtypes of K^+ channels have been identified in endothelial and smooth muscle cells (Félétou, 2009; Félétou and Vanhoutte, 2009; Standen and Quayle, 1998). In vascular smooth muscle cells, K_v channels are activated by membrane depolarization in the physiological range (Nelson and Quayle, 1995), while the large conductance K_{Ca} channels (BK_{Ca}) are activated mainly by increases in $[Ca^{2+}]i$ (Eichhorn and Dobrev, 2007; Ledoux et al., 2006). The involvement of K^+ channels in cardiovascular disorders depends on the vascular tissue or species studied (Ko et al., 2008). Thus, BK_{Ca} channels play a key role in regulating vascular tone in resistance arteries (Briones et al., 2009; Eichhorn and Dobrev, 2007), while aortic tone is strongly dependent on the activity of K_v channels (Tammaro et al., 2004).

The regulatory function of the endothelium is altered by cardiovascular risk factors or disorders, such as heavy metal exposure (Silveira et al., 2010; Triggle et al., 2003; Wiggers et al., 2008). We aimed to evaluate whether K^+ channels and Na^+/K^+-ATPase activation promoted by 7-day treatment with lead could be a compensatory mechanism against increased free radical production in the initial stages of lead exposure. Thus, we analyzed the effects of this treatment on the following: 1) reactive oxygen species (ROS) production and its participation in vascular responses to vasoconstrictors; 2) involvement of the NO pathway in Na^+/K^+-ATPase functional activity; and 3) participation of K^+ channels in the relaxation induced by acetylcholine and sodium nitroprusside. Our findings provide evidence that the activation of K^+ channels and Na^+/K^+-ATPase prevents the aortic endothelial dysfunction induced by increased free radicals in lead-treated rats.

Materials and methods

Animals and treatment. Male Wistar rats (250–300 g) were used for these studies. The care and use of laboratory animals were in accordance with the NIH guidelines, and all experiments were conducted in compliance with the guidelines for biomedical research as stated by the Brazilian Societies of Experimental Biology and were approved by the Institutional Ethics Committee of the Federal University of Espirito Santo (CEUA-UFES 052/2011). All rats had free access to water and were fed with rat chow *ad libitum*. The rats were divided into two groups: control (vehicle-saline, intramuscular) or treated with lead acetate for 7 days (1st dose: 4 μg/100 g, subsequent doses: 0.05 μg/100 g, intramuscular, to cover daily loss). No differences in body weight between the two groups were observed before (untreated: 260 ± 0.89 g, $n = 38$; lead-treated: 258 ± 0.99 g, $n = 40$; $P > 0.05$) or after treatment (untreated: 308 ± 2.45 g, $n = 38$; lead-treated: 312 ± 2.63 g, $n = 40$; $P > 0.05$). At the end of the treatment, the rats were anesthetized with pentobarbital (35 mg/kg, intraperitoneal) and killed by exsanguination. The thoracic aortas were carefully dissected out, and the fat and connective tissue were removed. For the vascular reactivity experiments, the aortas were divided into cylindrical segments 4 mm in length.

Vascular reactivity measurements. The aortic segments were mounted between two parallel wires in organ baths containing Krebs–Henseleit solution (KHS, in mM: 124 NaCl, 4.6 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 0.01 EDTA, 23 $NaHCO_3$) at 37 °C and gassed with 95% O_2–5% CO_2 (pH 7.4). The arterial segments were stretched to an optimal resting tension of 1 g. Isometric tension was recorded using a force transducer (TSD125C, CA, USA) connected to an acquisition system (MP100A, BIOPAC System, Inc., Santa Barbara, USA). After a 45 min equilibration period, all aortic rings were initially exposed twice to 75 mM KCl. The first exposure checks their functional integrity, and the second exposure assesses the maximal tension. Next, endothelial integrity was tested with acetylcholine (ACh, 10 μM) in segments previously contracted with phenylephrine (1 μM). A relaxation equal to or greater than 90% was considered demonstrative of the functional integrity of the endothelium. After a 45-min washout period, concentration–response curves to phenylephrine were determined. Single curves were performed in each segment. The effects of apocynin (0.3 μM, an inhibitor of NADPH oxidase), superoxide dismutase (SOD) (150 U/mL) and catalase (1000 U/mL) were investigated by adding them to the bath 30 min before performing the phenylephrine concentration–response curves.

In another set of experiments, after the 45-min equilibration period, aortic rings from untreated and lead-treated rats were pre-contracted with phenylephrine (1 μM) or 60 mmol/L KCl, and the concentration–response curves to ACh (0.1 nM–300 μM) were determined. The role of NO in the relaxation induced by ACh was analyzed by incubating the vessels with N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM, nonspecific NOS inhibitor) for 30 min before phenylephrine or KCl administration. The contribution of K^+ channels to ACh-induced relaxation was assessed in aortas previously incubated for 30 min with the K^+ channel blockers tetraethylammonium (TEA, 2 mM, nonselective blocker of K^+ channels), 4-aminopyridine (4-AP, 5 mM, K_v blocker), iberiotoxin (IbTX, 30 nM, selective BK_{Ca} blocker), apamin (0.5 μM, selective blocker of small-conductance Ca^{2+}-activated K^+ channels — SK_{Ca}) and charybdotoxin (ChTX, 0.1 μM, blocker of K_{Ca} and K_v).

In some experiments, the concentration–response curves to sodium nitroprusside (SNP, 0.01 nM–0.3 μM) were performed in segments contracted with phenylephrine (1 μM). The role of the K_v and BK_{Ca} channels in the SNP-induced relaxation was analyzed by incubating the vessels with 4-AP and IbTX, respectively, for 30 min before phenylephrine administration. The influence of the endothelium on the response to SNP in untreated and lead-treated rats was investigated after its mechanical removal, which was performed by rubbing the lumen with a needle. The absence of endothelium was confirmed by the inability of 10 μM acetylcholine (ACh) to produce relaxation.

The functional activity of the $Na^{\pm}/K^+$-ATPase was measured in segments from untreated and lead-treated rats using K^+-induced relaxation, as described by Weeb and Bohr (1978) and modified by Rossoni et al. (2002). After a 30-min equilibration period in normal Krebs, the preparations were incubated for 30 min in K^+-free Krebs. The vessels were subsequently pre-contracted with phenylephrine, and once a plateau was attained, the KCl concentration was increased stepwise (1, 2, 5 and 10 mM) with each step lasting for 2.5 min. After a washout period, the preparations were incubated with 100 μM ouabain (OUA) for 30 min to inhibit sodium pump activity, and the K^+-induced relaxation curve was repeated. To study the involvement of NO, inducible NO synthase (iNOS) and K^+ channels in OUA-sensitive Na^+K^+-ATPase functional activity, the rings were incubated with L-NAME (100 μM), aminoguanidine (50 μM) and TEA (2 mM), respectively. Moreover, the influence of the endothelium was investigated, repeating the same protocols after its mechanical removal.

In situ detection of vascular O_2^- production. The oxidative fluorescent dye dihydroethidium (DHE) was used to evaluate O_2^- production in situ, as previously described by Wiggers et al. (2008). Hydroethidine freely permeates cells and is oxidized in the presence of O_2^- to ethidium bromide, which is trapped by intercalation with DNA. Ethidium bromide is excited at 546 nm and has an emission spectrum of 610 nm. Frozen tissue segments were cut into 10-μm-thick sections and placed on a glass slide. Serial sections were equilibrated under identical conditions for 30 min at 37 °C in Krebs–HEPES buffer (in mM: 130 NaCl, 5.6 KCl, 2 $CaCl_2$, 0.24 $MgCl_2$, 8.3 HEPES, and 11 glucose, pH = 7.4). Fresh buffer containing DHE (2 μM) was applied topically to each tissue section, covered with a cover slip, incubated for 30 min in a light-protected humidified chamber at 37 °C, and then viewed with a inverted fluorescence microscope (NIKON Eclipse Ti-S, x40 objective) using the same imaging settings in the untreated and lead-treated rats. Fluorescence was detected with a 568-nm long-pass filter. For quantification, eight frozen tissue segments per animal were sampled for each experimental condition and averaged. The mean fluorescence densities in the target region were calculated.

Statistical analyses. All values are expressed as the mean ± standard error of the mean (SEM). Contractile responses to phenylephrine were expressed as a percentage of the maximal response induced by 75 mM KCl. Vasodilator responses to ACh or SNP were expressed as the percentage of relaxation of the previous contraction.

For each concentration–response curve, the maximal effect (R_{max}) and the concentration of agonist that produced 50% of the maximal response (log EC_{50}) were calculated using non-linear regression analysis (GraphPad Prism, GraphPad Software, Inc., San Diego, CA). The sensitivities of the agonists were expressed as pD_2 (− log EC_{50}). To compare the effects of L-NAME, TEA, 4-AP, IbTX, ChTX and apamin on the relaxation responses to ACh, some results were expressed as the differences in the area under the concentration–response curves (dAUC) for the control and experimental groups. These values indicate whether the magnitude of the effect of L-NAME, TEA, 4-AP, IbTX, ChTX and apamin is different in the untreated or lead-treated rats.

The results were expressed as the mean ± SEM of the number of rats indicated (n). The differences were analyzed using Student's *t*-test or two-way ANOVA followed by a Bonferroni test. $P<0.05$ was considered to be significant.

Drugs and reagents. Lead acetate, l-phenylephrine hydrochloride, ACh chloride, SNP, sodium pentobarbital, apocynin, SOD, catalase, OUA, L-NAME, TEA, 4-AP, IbTX, CbTX and apamin were purchased from Sigma-Aldrich (St. Louis, USA). The salts and reagents used were of analytical grade from Sigma-Aldrich and Merck (Darmstadt, Germany).

Results

Effects of lead treatment on vascular reactivity

Lead exposure did not affect the response to KCl (untreated E^+: 3.46 ± 0.04 g, n = 38; lead-treated E^+: 3.43 ± 0.11 g, n = 40; untreated E^-: 3.49 ± 0.03 g, n = 20; lead-treated E^-: 3.43 ± 0.09 g, n = 20; $P>0.05$). Pre-contraction to phenylephrine used before performing ACh and SNP relaxation curves was similar in the groups (untreated E^+: 2.46 ± 0.05 g, n = 10; lead-treated E^+: 2.63 ± 0.03 g, n = 10; untreated E^-: 2.55 ± 0.11 g, n = 10; lead-treated E^-: 2.57 ± 0.04 g, n = 10 $P>0.05$). However, this metal reduced vascular reactivity to phenylephrine in the aortic rings (Table 1). However, lead did not change the relaxation induced by acetylcholine (Table 2) or the relaxation produced by SNP (Table 3).

Table 1
Effects of apocynin, SOD and catalase on the vascular response to phenylephrine (R_{max} and pD_2) in aortas from untreated and lead-treated rats.

	Untreated		Lead treated	
	R_{max}	pD_2	R_{max}	pD_2
Control	81.80 ± 4.74	6.55 ± 0.10	66.22 ± 4.66$^{\#}$	6.29 ± 0.06
Apocynin	82.15 ± 9.69	6.25 ± 0.15	47.23 ± 5.22*	6.15 ± 0.10
SOD	79.70 ± 3.71	6.32 ± 0.08	51.19 ± 5.32*	6.07 ± 0.06*
Control	94.12 ± 4.07	6.11 ± 0.10	78.18 ± 0.71$^{\&}$	5.99 ± 0.12
Catalase	92.39 ± 3.73	5.93 ± 0.15	66.61 ± 1.53^{+}	5.86 ± 0.10

The results are expressed as mean ± SEM of the number of animals shown in Fig. 1. R_{max}, maximal effect (expressed as a percentage of the maximal response induced by 75 mM KCl); pD_2, − log one-half R_{max}. $P<0.05$ *vs.* untreated control rats ($^{\#\&}$) and lead-treated control rats ($^{*+}$). Note that apocynin and SOD incubation have one control and catalase has another one.

Effect of lead treatment on oxidative stress

The basal O_2^- production in the aortas from the lead-treated rats was greater than that from the controls (Fig. 1A). To investigate whether the vascular oxidative stress induced by lead treatment was involved in the observed alterations of vascular reactivity to phenylephrine, we used apocynin (0.3 nM), which is a NADPH oxidase inhibitor; SOD, (150 U/mL), which is a superoxide anion scavenger; and catalase (1000 U/mL), which is a hydrogen peroxide scavenger. These drugs reduced the vasoconstrictor response induced by phenylephrine in the aortas from lead-treated rats but did not in the aortas from untreated rats (Figs. 1B–D and Table 1).

Effects of lead treatment on Na^+/K^+-ATPase activity

We previously reported that lead treatment for 7 days increased the activity of the sodium pump and protein expression of the Na^+/K^+-ATPase alpha-1 subunit in aortic rings from treated rats (Fiorim et al., 2011). After endothelium removal, the KCl-induced relaxation was reduced in the aortic rings from both groups (Fig. 2A), but this reduction was greater in the aortas from lead-treated rats. To investigate the involvement of NO in Na^+/K^+-ATPase activity, we used L-NAME (100 μM), a nonselective NOS inhibitor, and aminoguanidine (50 μM), a selective iNOS inhibitor. After incubating the rings with L-NAME, the KCl-induced relaxation was reduced in the aortic rings from both groups (Fig. 2B), but this reduction was greater in the aortas from the treated group compared to the untreated rats. Incubation with aminoguanidine did not modify the relaxation induced by potassium in aortas from untreated rats but reduced the relaxation induced by potassium in lead-treated rats (Fig. 2C). Similarly, after coincubation of the

Table 2
Effects of N^G-nitro-L-arginine methyl ester (L-NAME), tetraethylammonium (TEA), 4-aminopyridine (4-AP), iberiotoxin (IbTX), apamin and charybdotoxin (ChTX) on the vascular responses to acetylcholine (R_{max} and pD_2) in phenylephrine contracted aortas from untreated and lead-treated rats.

	Untreated		Lead treated	
	R_{max}	pD_2	R_{max}	pD_2
Control	99.91 ± 0.09	7.12 ± 0.13	99.80 ± 0.10	7.11 ± 0.09
L-NAME	6.91 ± 3.51$^{\#}$	7.14 ± 0.58	6.91 ± 2.86*	7.11 ± 0.09
TEA	71.90 ± 2.47$^{\#}$	6.62 ± 0,12$^{\#}$	59.69 ± 3.16*	5.72 ± 0.31*
4-AP	88.84 ± 3.03	6.45 ± 0.22$^{\#}$	54.03 ± 2.46*	6.72 ± 0.52
IbTX	94.14 ± 2.48$^{\#}$	6.40 ± 0.18$^{\#}$	81.43 ± 4.36*	6.44 ± 0.18*
Apamin	91.65 ± 2.22$^{\#}$	6.87 ± 0.09	91.47 ± 2.23*	6.45 ± 0.07*
ChTX	92.01 ± 1.88$^{\#}$	6.93 ± 0.08	90.41 ± 3.19*	6.42 ± 0.13*

The results are expressed as mean ± SEM of the number of animals shown in Figs. 2, 3, 4 and 5. R_{max}, maximal effect; pD_2, − log one-half R_{max}. $P<0.05$ *vs.* untreated control rats ($^{\#}$) and lead-treated control rats (*).

Table 3
Effects of 4-aminopyridine (4-AP) and iberiotoxin (IbTX) on the vascular response to sodium nitroprusside (R_{max} and pD_2) in phenylephrine contracted aortas from untreated and lead-treated rats.

	Untreated		Lead treated	
	R_{max}	pD_2	R_{max}	pD_2
Control	99.90 ± 0.10	7.49 ± 0.07	99.96 ± 0.10	7.37 ± 0.06
IbTX	99.47 ± 0.83	6.35 ± 0.10#	91.50 ± 2.20*	6.57 ± 0.10*
4-AP	93.26 ± 2.04#	6.77 ± 0.17#	89.01 ± 2.39*	6.55 ± 0.16*

The results are expressed as mean ± SEM of the number of animals shown in Fig. 6. R_{max}, maximal effect; pD_2, −log one-half R_{max}. P<0.05 *vs.* untreated control rats (#) and lead-treated control rats (*).

rings with OUA (100 μM) plus L-NAME or aminoguanidine, the KCl-induced relaxation was reduced in aortic rings from treated rats but not in aortas from untreated rats (Figs. 2B and C). After endothelium removal, incubation with OUA, further reduced the KCl-induced relaxation in aortic rings from both groups (Fig. 2A), but this reduction was greater in aortas from lead-treated rats. These results reinforce the previous findings regarding the increase of NKA activity after lead treatment.

The K^+ channel blocker TEA (2 mM) did not modify the relaxation induced by potassium in aortas from untreated rats but reduced that relaxation in lead-treated rats. However, after coincubation with OUA (100 μM), the KCl-induced relaxation was not different compared to ouabain alone in either the treated or untreated rats (Fig. 2D).

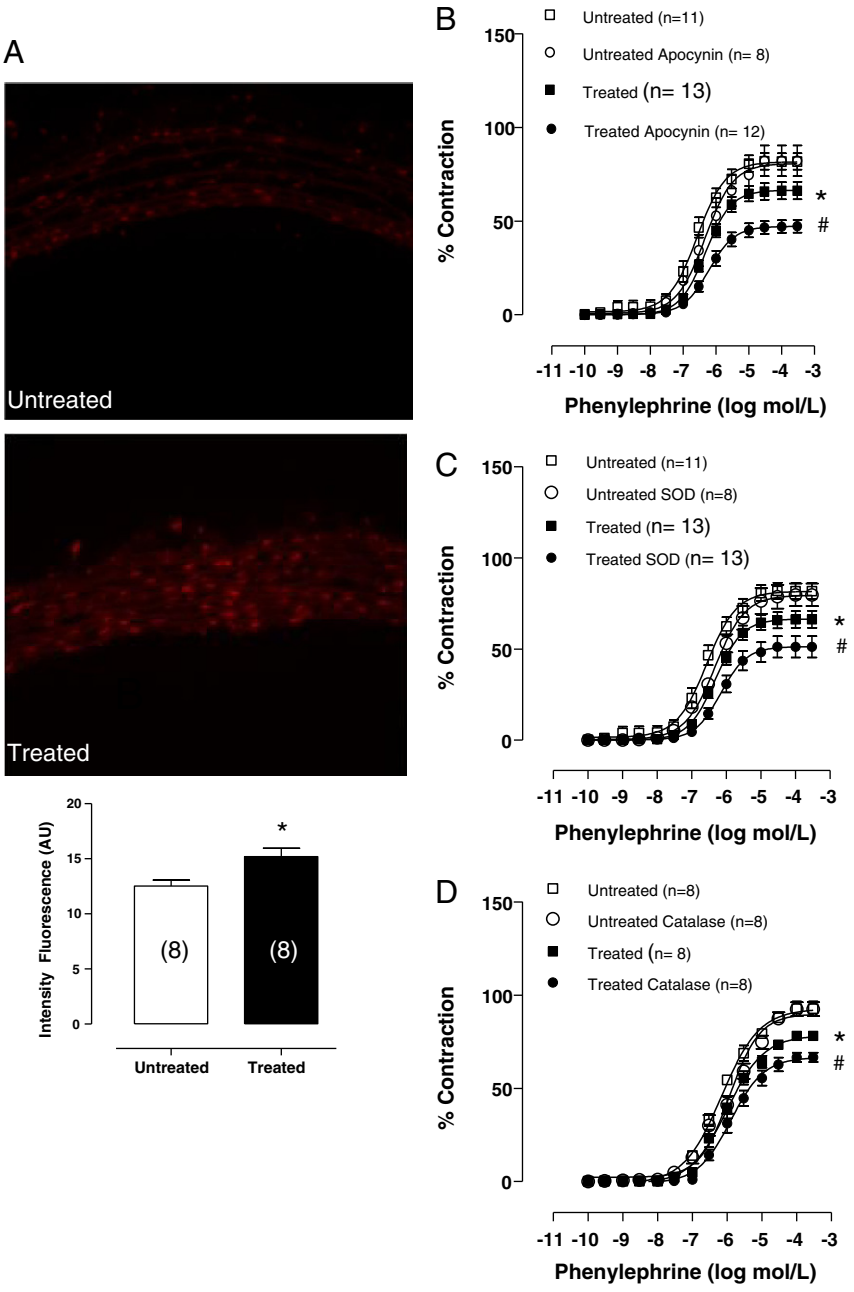

Fig. 1. Vascular superoxide anion production in segments of aorta from untreated and lead-treated rats. Representative fluorescent photomicrographs of inverted microscopic arterial sections labeled with the oxidative dye hydroethidine and vascular superoxide anion quantification (A). The effect of apocynin (0.3 μM) (B), superoxide dismutase (SOD) (150 U/mL) (C) and catalase (1000 U/mL) (D) on the concentration–response curves to phenylephrine in aortic rings from untreated and lead-treated rats. The results are expressed as mean ± SEM. *P<0.05 (untreated *vs.* treated); #P<0.05 (treated apocynin or SOD or catalase *vs.* treated), Student's *t*-test. The number of animals is indicated in parentheses.

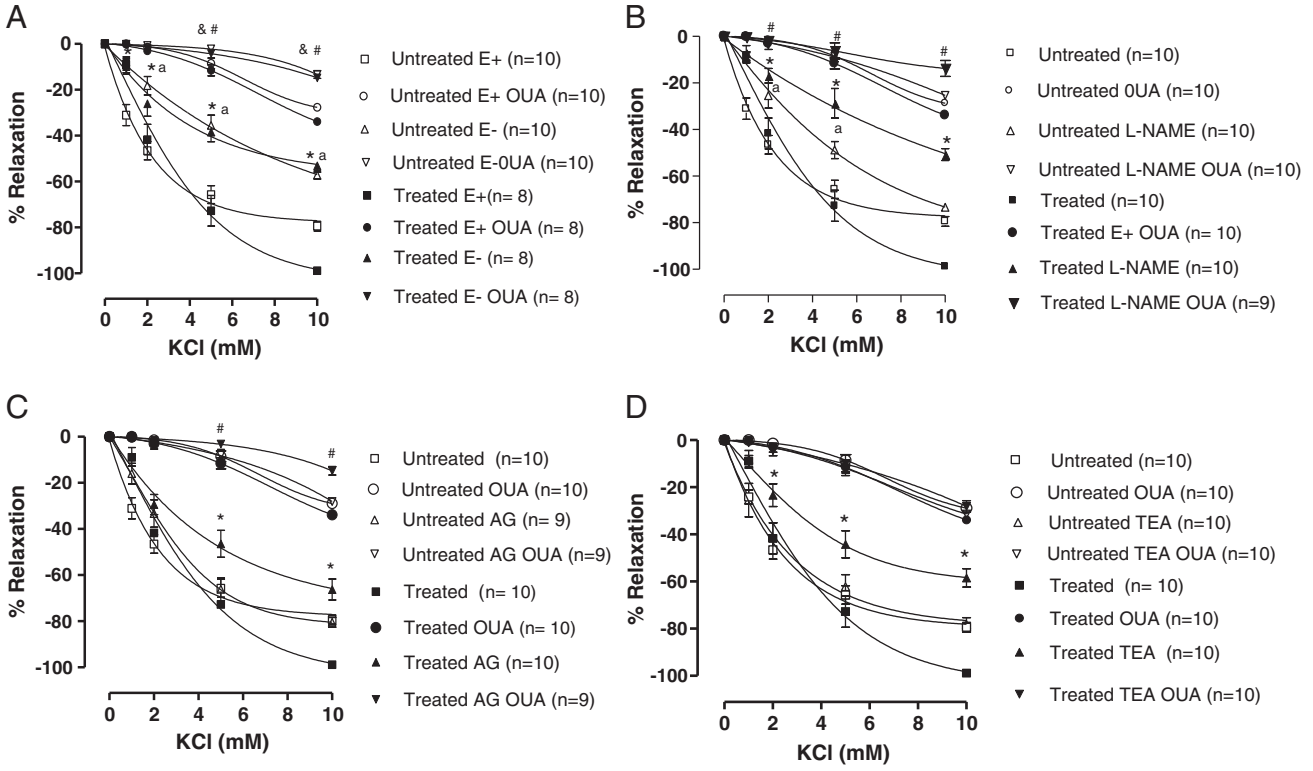

Fig. 2. The effects of endothelium removal (A), N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) (B), aminoguanidine (AG, 50 μM) (C) and TEA (2 mM) (D) on potassium-induced relaxation in the aortic rings from untreated and lead-treated rats previously incubated in a K^+-free medium and contracted with phenylephrine before and after incubation with 100 μM ouabain (OUA). The results are expressed as mean ± SEM. ^{a}P<0.05 (untreated E^- or L-NAME *vs.* untreated E^+); *P<0.05 (treated E^- or L-NAME or AG or TEA *vs.* treated E^+); $^{\&}$P<0.05 (untreated OUA E^- *vs.* untreated OUA E^+) $^{\#}$P<0.05 (treated OUA E^- or L-NAME or AG *vs.* treated OUA E^+), all by two-way ANOVA followed by a Bonferroni test. The number of animals is indicated in parentheses.

Effects of lead treatment on the functional activity of potassium channels

As mentioned, the endothelium-dependent relaxation induced by ACh in arteries pre-contracted with phenylephrine was similar in aortic rings from untreated and lead-treated animals (Table 2). In arteries pre-contracted with 60 mmol/L KCl, the relaxation induced by ACh was reduced both in untreated (R_{max} for phenylephrine pre-contraction: 99.91 ± 0.09%, n = 10; for KCl pre-contraction: 56.14 ± 2.83%, n = 10, P<0.05) and lead-treated rats (R_{max} for PHE pre-contraction: 99.99 ± 0.01%, n = 10; for KCl pre-contraction: 25.97 ± 3.29% n = 10, P<0.05). However, this reduction was much more marked in arteries obtained from lead-treated rats (Fig. 3A). The participation of NO in ACh-induced relaxation was investigated using L-NAME (100 μM), which was added before phenylephrine or high K^+. Under these conditions, the relaxation induced by ACh was negligible in arteries from both groups contracted with either phenylephrine (Fig. 3B) or KCl (Fig. 3C), indicating that NO accounted for most of the endothelium-dependent relaxation. However, the greater reduction in ACh relaxation observed in arteries from lead-treated rats pre-contracted with KCl compared to untreated rats suggests a different contribution of hyperpolarizing mechanisms in the aortas from both groups. Therefore, we tested the effects of some potassium channel blockers on the basal tone and relaxation induced by ACh.

TEA (2 mM), a nonselective K^+ channel blocker, and 4-AP (5 mM), a specific inhibitor of K_v channels, increased the basal tone in aortic segments from both groups, but these effects were greater in the lead-treated rats compared to the untreated rats (Fig. 4A), suggesting a relevant role for K_v channels in controlling arterial tone. In addition, TEA and 4-AP reduced the relaxation induced by ACh in aortic segments from both groups (Figs. 4B, D and Table 2), and this effect was greater in preparations from lead-treated rats compared to untreated rats as shown by the dAUC values (Figs. 4C and E).

To evaluate the role of K_{Ca} channels, the aortic rings were incubated with the selective BK_{Ca} blocker (IbTX), the selective SK_{Ca} blocker (apamin) and the K_{Ca} and K_v blocker (ChTX). Only iberiotoxin increased the basal tone in aortic segments from the lead-treated and untreated rats, but this effect was similar in both groups (Fig. 4A). Moreover, the three calcium-activated potassium channel inhibitors reduced the relaxation induced by ACh in aortic segments from both groups (Figs. 5A, C, E and Table 2). However, this effect was greater in preparations from lead-treated than untreated rats as shown by the dAUC values (Figs. 5B, D and F).

Because lead treatment increases the contribution of K_v and K_{ca} channels upon ACh-induced relaxation, which is mainly mediated by NO, we analyzed the participation of these K^+ channels in the relaxation induced by the NO donor, SNP. The endothelium-independent relaxation induced by SNP in arteries pre-contracted with phenylephrine was similar in aortic rings from untreated and lead-treated animals (Table 3). After IbTX and 4-AP incubation, there was a decrease in the relaxation induced by SNP in aortic segments from either group (Figs. 6A, C and Table 3), and this decrease was greater in preparations from lead-treated than untreated rats, as shown by the dAUC values (Figs. 6B and D). Endothelium removal did not affect SNP relaxation in any group, being the relaxation induced by the NO donor similar between groups (Fig. 6E).

Discussion

The results presented here show that a 7-day treatment with a low concentration of lead acetate increased free radicals production, despite the reduction in vascular reactivity to phenylephrine, but did not change the relaxation induced by ACh and SNP. On the other hand, our findings also suggest that activation of the K^+ channel as well as the increased Na^+/K^+ ATPase activity masked a putative endothelial

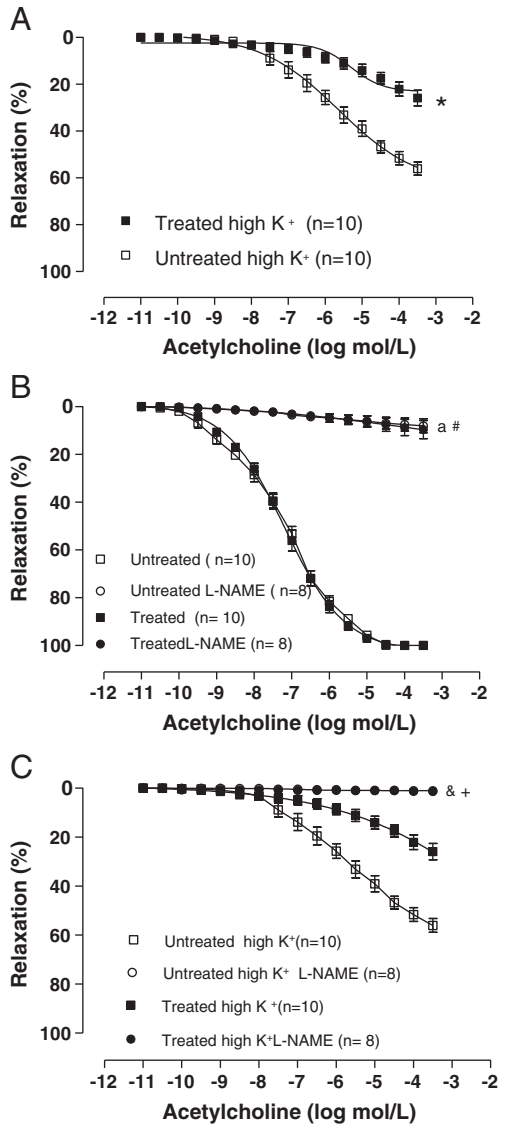

Fig. 3. Concentration–response curves to acetylcholine (ACh) in aortic rings from untreated and lead-treated rats precontracted with 60 mmol/L KCl (A and C) or phenylephrine (B) in the absence or presence (B and C) of L-NAME (100 μM). The results are expressed as mean ± SEM. *P<0.05 (untreated high K^+ *vs.* treated high K^+); ^{a}P<0.05 (untreated L-NAME *vs.* untreated); $^{\#}$P<0.05 (treated L-NAME *vs.* treated); $^{\&}$P<0.05 (untreated high K^+ L-NAME *vs.* untreated high K^+); $^{+}$P<0.05 (treated high K^+ L-NAME *vs.* treated high K^+), Student's *t*-test. The number of animals is indicated in parentheses.

dysfunction in lead-treated rats induced by the increased oxidative stress.

Lead has been identified as a hazard and risk factor for developing cardiovascular diseases (Navas-Acien et al., 2007). The Agency for Toxic Substances and Disease Registry (ATSDR) considers the reference blood lead concentration level to be 60 μg/dL (ATSDR, 2005; Kosnett et al., 2007; Patrick, 2006). Several studies have supported the association between high blood lead levels and hypertension (Glenn et al., 2006; Harlan, 1988; Navas-Acien et al., 2007). In a recent study, using controlled lead administration, we found a blood lead concentration of 9.98 μg/dL after a 7-day treatment with a low dose of lead acetate (Fiorim et al., 2011). Although this value was below the blood lead reference, it was sufficient to increase SBP and to decrease the contractile responses induced by phenylephrine in the rat aorta. In accordance, a blood lead concentration of 37 μg/dL (below the blood lead reference) that was reached after acute administration also induced an increase in SBP (Simões et al., 2011). Thus, these results provide guidance for revising the lead concentrations considered to be safe.

Several studies have shown that lead exposure in animals or humans induces the generation of ROS with subsequent oxidative damage to several organs and systems and also alters antioxidant defense systems (Ding et al., 1998; Farmand et al., 2005; Ni et al., 2004; Vaziri et al., 1999b). Similarly, we observed increased superoxide anion production in the aorta from lead-treated rats. In addition, the inhibition of NADPH oxidase as well as SOD and catalase reduced the vasoconstrictor response induced by phenylephrine only in the aortas from lead-treated rats, suggesting that both superoxide anion production and hydrogen peroxide are involved in the vascular alterations promoted by lead. In agreement, Silveira et al. (2010) demonstrated the involvement of free radicals after acute administration of lead acetate in the tail vascular bed reactivity. Ni et al. (2004) showed that lead exposure increased superoxide and hydrogen peroxide production in coronary endothelial cells.

Despite the involvement of ROS in this experimental model, which could increase vasoconstriction, we previously observed a decrease in vascular reactivity to phenylephrine in the aortas from lead-treated rats and an increase in the modulator effects by NO (Fiorim et al., 2011). Other investigators have also demonstrated that incubation with 1 ppm lead acetate for 24 h increases NO production in endothelial cells from human coronary arteries (Vaziri and Ding, 2001). Furthermore, several reports have suggested that lead exposure increases the expression of iNOS in aorta (Vaziri et al., 1999a, 1999b, 2001; Fiorim et al., 2011), heart (Vaziri et al., 2001) and kidney (Gonick et al., 1997; Vaziri et al., 2001). NO produces vasodilation of the vascular smooth muscle cells in all types of blood vessels, especially in conductance arteries. Moreover, NO could also stimulate Na^+/K^+-ATPase activity (Gupta et al., 1994) and open K^+ channels (Bolotina et al., 1994; Félétou and Vanhoutte, 2006, 2009), which contribute to reduced vascular tone.

The activation of Na^+/K^+-ATPase activity is an important mechanism contributing to the maintenance of vascular tone and membrane potential in vascular smooth muscle cells (Blaustein, 1993; Marín and Redondo, 1999). We previously reported that a 7-day treatment with a low concentration of lead acetate increased the protein expression of the Na^+/K^+-ATPase alpha-1 subunit and Na^+/K^+-ATPase activity in the rat aorta (Fiorim et al., 2011). K^+-induced relaxation was used as an index of Na^+/K^+-ATPase functional activity (Weeb and Bohr, 1978). Endothelium removal and the non specific NOS inhibitor L-NAME reduced such relaxation more in aortic rings from lead-treated compared to the untreated rats, and the iNOS inhibitor aminoguanidine only had effect in rings from treated rats. These findings suggest that the increased of Na^+/K^+-ATPase functional activity induced by lead could be mediated by the NO pathway.

In addition to guanylate cyclase activation, NO is also a hyperpolarizing factor that increases K^+ channel permeability (Bolotina et al., 1994; Félétou and Vanhoutte, 2006). Our results showed that the non specific K^+ channels blocker TEA did not modify K^+-induced relaxation in the aortas from untreated rats but reduced it in treated rats. After co-incubation of the rings with TEA plus OUA, K^+-induced relaxation was not different between the groups, suggesting a similar action between K^+ channels and $Na^{+/}K^+$ATPase activity in the lead-treated rats.

Lead treatment did not modify ACh-induced relaxation in phenylephrine pre-contracted aortas, as previously reported (Fiorim et al., 2011). The importance of endothelial NO in controlling vascular tone in conductance arteries is well established (Urakami-Harasawa et al., 1997; Félétou and Vanhoutte, 2006). In agreement, we found that ACh-induced relaxation in the aorta was entirely dependent on NO release because it was abolished by L-NAME. As mentioned, NO can also hyperpolarize vascular smooth muscle cells by activating different K^+ channels, depending on the vascular bed or species studied (Bolotina et al., 1994; Félétou and Vanhoutte, 2006, 2009; Félétou et al., 2010). ACh induced-relaxation was partially blocked by high K^+ concentration suggesting that it is mediated, in part, by K^+ channels

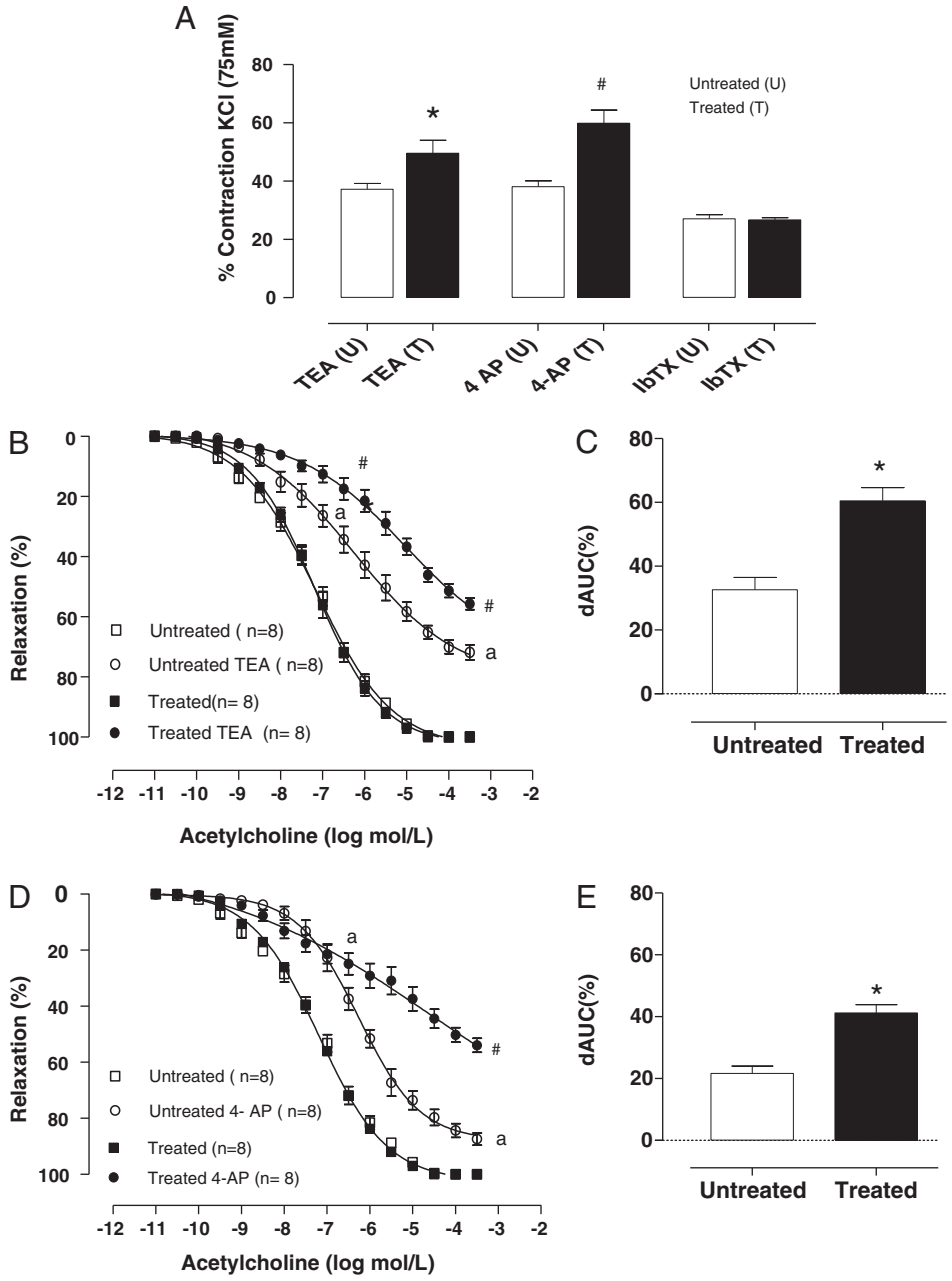

Fig. 4. The contractile effect induced by TEA (2 mM), 4-aminopyridine (4-AP, 5 mM) and iberiotoxin (IbTX, 30 nM) in aortas from untreated and lead-treated rats (A). The results are expressed as mean ± SEM *P<0.05 *vs.* untreated TEA and #P<0.05 *vs.* untreated 4-AP, Student's *t*-test. The effect of TEA (2 mM) (B) and 4-AP, (5 mM) (D) on the concentration–response curves to acetylcholine (ACh) in endothelium-intact aortic segments from untreated and lead-treated rats. The inset shows differences in dAUC in the absence and presence of TEA (C) or 4-AP (E). [a]P<0.05 (untreated TEA or 4-AP *vs.* untreated); [#]P<0.05 (treated TEA or 4-AP *vs.* treated); *P<0.05 (untreated *vs.* treated % dAUC), all by Student's *t*-test. The number of animals is indicated in parentheses.

activation. However, this reduction was much more marked in arteries obtained from lead-treated rats. The residual relaxation to ACh in high-KCl precontracted vessels was abolished by L-NAME indicating an additional effect of NO, independent of K^+ channel activation, on ACh-induced relaxation.

TEA was initially used to evaluate the overall contribution of K^+ channels to the basal tone and ACh-induced relaxation. TEA increased basal tone more in preparations from the lead-treated rats compared to the untreated rats and reduced the relaxation induced by ACh more in aortic segments from the lead-treated than untreated group; these results suggest a greater contribution of K^+ channels in both basal tone and ACh-induced relaxation after lead treatment. Accordingly, Fiorim et al. (2011) observed that TEA potentiated the phenylephrine response more strongly in aortic rings from lead-treated rats compared to untreated rats. In addition, patch clamp observations of K^+ currents in human erythrocytes showed that lead exposure activates K^+ channels (Kempe et al., 2005).

Different K^+ channels are involved in cardiovascular disorders, such as atherosclerosis, hypertension and stroke (Nelson and Quayle, 1995; Callera et al., 2004; Ledoux et al., 2006). Lead treatment increased NO bioavailability in the rat aorta (Fiorim et al., 2011) and as mentioned NO could open K^+ channels. Therefore, we investigated the participation of diverse K^+ channels in regulating basal tone and in NO-mediated ACh-induced relaxation in lead-treated rats. It has been shown that aortic tone is strongly dependent on the activity of K_v channels (Tammaro et al., 2004). In addition, Cheong et al. (2002) also has

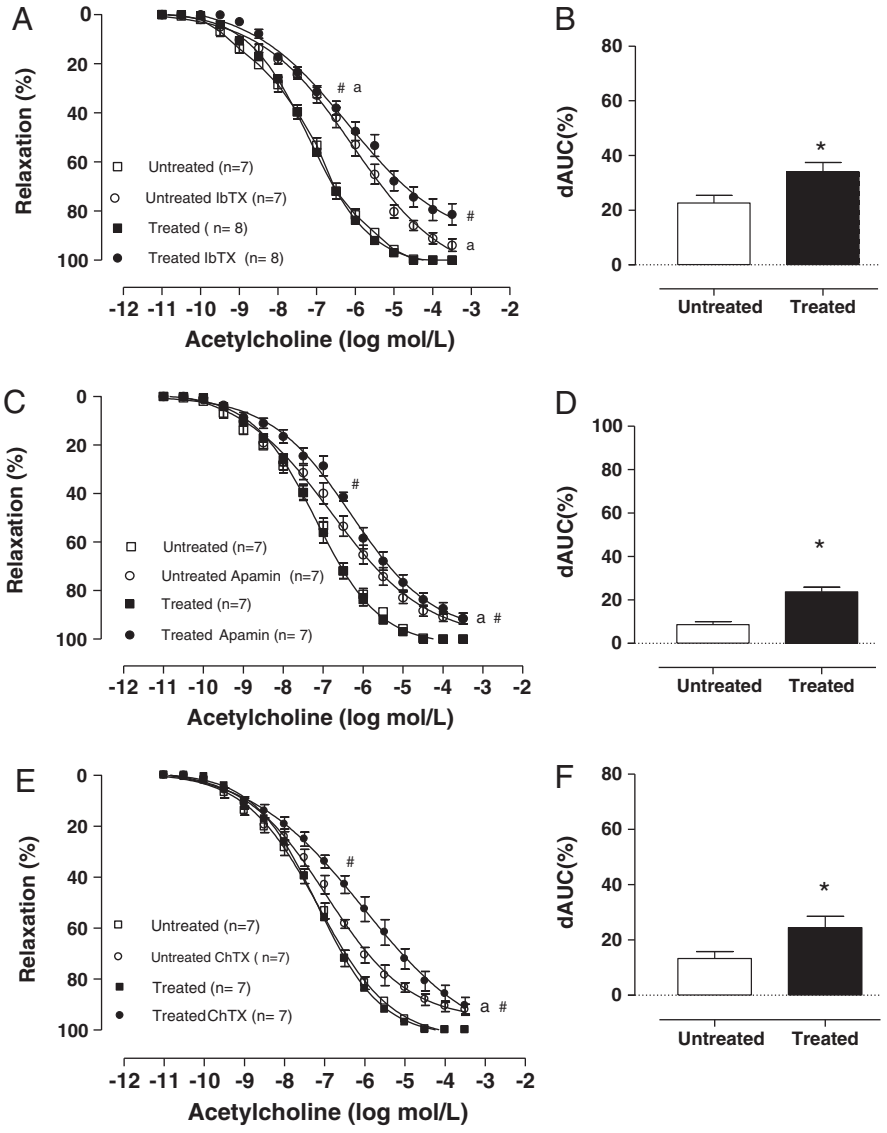

Fig. 5. The effect of iberiotoxin (IbTX, 30 nM) (A), apamin (0.5 μM) (C) and charybdotoxin (ChTX, 0.1 μM) (E) on the concentration–response curve to acetylcholine (ACh) in aortic rings from untreated and lead-treated rats. The inset shows differences of area under the concentration–response curves (dAUC) in the absence and presence of IbTX (B), apamin (D) or ChTX (F). The results are expressed as mean ± SEM. $^{a}P<0.05$ (untreated IbTX or apamin or ChTX *vs.* untreated); $^{\#}P<0.05$ (treated IbTX or apamin or ChTX *vs.* treated); $^{*}P<0.05$ (untreated *vs.* treated % dAUC), Student's *t*-test. The number of animals is indicated in parentheses.

shown the participation of K_v channel currents in small blood vessels. Our results showed that 4-aminopyridine, a selective inhibitor of K_v channel, induced a greater increase in basal tone in aortic segments from lead-treated than in untreated rats. Furthermore, this inhibitor reduced the relaxation induced by ACh to a greater extent in preparations from lead-treated compared to untreated rats. These results suggest that K_v channels contribute to the regulation vascular tone in the rat aorta and that channels contribute more to the basal tone and ACh-induced relaxation in the lead-treated rats.

Several studies have shown that BK_{Ca} plays a key role in regulating vascular tone in different beds (Cheong et al., 2002; Eichhorn and Dobrev, 2007; Briones et al., 2009), and the activation of these channels is an important component of the EDHF response in several vascular beds (Ledoux et al., 2006). Our results show that both charybdotoxin (K_{Ca} and K_v blocker) and apamin (selective SK_{Ca} blocker) did not modify basal tone in aortic segments from both groups. However, iberiotoxin increased the basal tone in the same way in aortic segments from untreated and lead-treated rats suggesting that BK_{Ca} similarly contributes to the basal tone in the rat aorta. Similar results were reported by Briones et al. (2009) in coronary arteries from ouabain treated and untreated rats. Regarding the involvement of calcium-activated K^+ channels on ACh-induced relaxation, our results showed that ChTX, IbTX and apamin reduced the relaxation induced by ACh to a greater extent in the lead-treated than in the untreated group, suggesting that lead treatment increases the participation of K_v, BK_{Ca} and SK_{Ca} in the endothelium-dependent relaxation induced by ACh.

As mentioned before, the L-NAME effect on ACh relaxation indicates that NO is the main factor responsible for such relaxation in the aorta. Furthermore, it is known that BK_{Ca} and K_v channels are present in the vascular smooth muscle (Nelson and Quayle, 1995; Félétou and Vanhoutte, 2009). Similar to the results observed with ACh, the endothelium-independent relaxation induced by SNP was not affected by lead treatment. Importantly, after IbTX or 4-AP incubation, there was a greater decrease in the relaxation induced by SNP in aortic segments from the lead-treated rats compared to the untreated rats. These results suggest that both BK_{Ca} and K_v channels

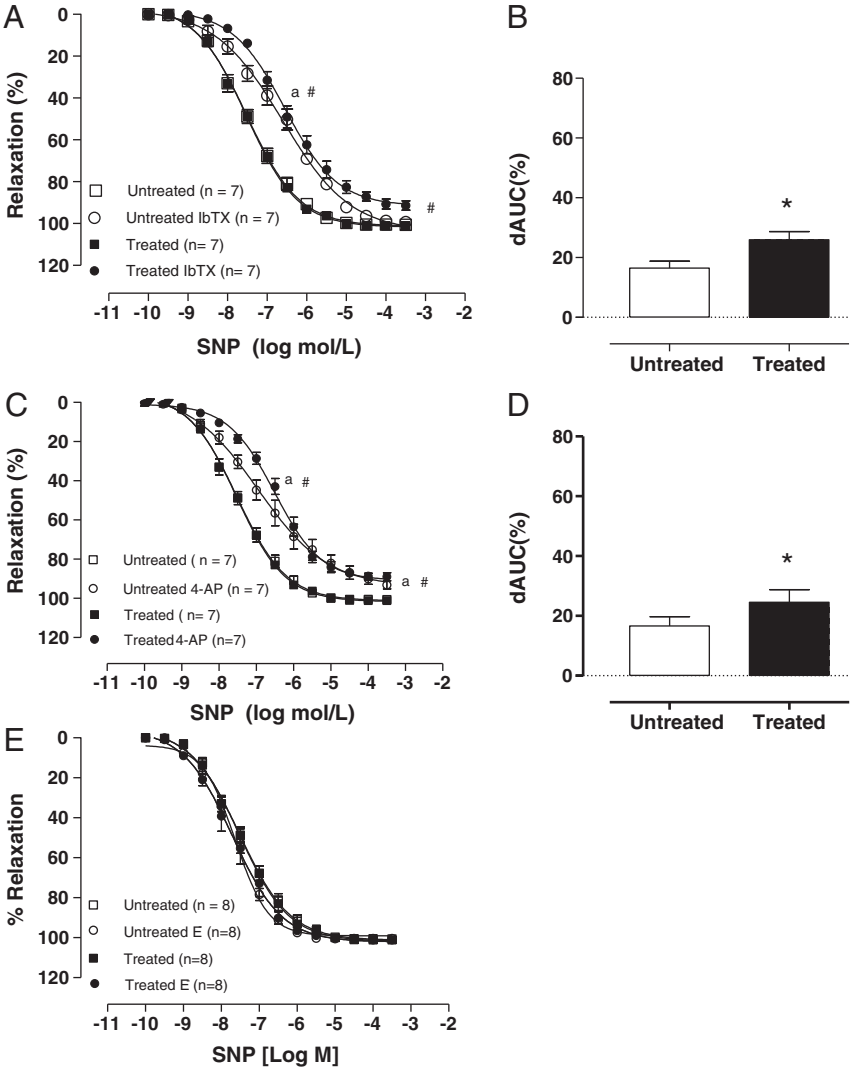

Fig. 6. The effect of iberiotoxin (IbTX, 30 nM) (A), 4-aminopyridine (4-AP, 5 mM) (C) and endothelium removal (E) on the concentration-response curves to sodium nitroprusside (SNP) in aortic rings from untreated and lead-treated rats. The inset shows differences of area under the concentration-response curves (dAUC) in the absence and presence of IbTX (B) or 4-AP (D). The results are expressed as mean ± SEM. $^{a}P< 0.05$ (untreated 4-AP vs. untreated); $^{\#}P<0.05$ (treated IbTX or 4-AP *vs.* treated); $^{*}P<0.05$ (untreated vs. treated % dAUC), Student *t*-test. The number of animals is indicated in parentheses.

are involved in NO-induced relaxation and that these channels contribute to a greater extent in lead-treated rats. However, we cannot discard alterations in NO-derived cGMP-dependent mechanisms after lead treatment and more experiments would be necessary to clarify this issue.

In summary, our results show that a 7-day treatment with a low concentration of lead acetate increases free radical production, despite the reduction in vascular reactivity to phenylephrine and did not change the relaxation induced by ACh and SNP. Our results also suggest that the activation of K^+ channels and increased Na^+/K^+ ATPase activity mask putative endothelial dysfunction in lead-treated rats. Moreover, activation of K_v and BK_{Ca} channels seems to contribute more to the control of vascular tone in the aorta from lead-treated rats. Recently, using this experimental model, we showed that lead exposure increased NO bioavailability and reduced vascular tone (Fiorim et al., 2011). Our findings suggest that the activation of K^+ channels and Na^+/K^+ ATPase could reduce vascular tone in the initial stages of lead exposure that counteracts the vasoconstrictor action of free radicals. In fact, lead exposure, at low concentrations, could be considered an important cardiovascular risk factor and a serious problem for public health.

Conflict of interest statement

None declared.

Acknowledgments

This study was supported by the "Ministerio de Ciencia e Innovación" (SAF 2009-07201), "Ministerio de Educación, Cultura y Deporte" (PHB2011-0001-PC) and "Banco Santander-Central-Hispano" in Spain and by grants from the "Coordenação de Aperfeiçoamento de Pessoal de Nível superior" (CAPES), "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), "Fundação de Amparo à Pesquisa do Espírito Santo" (FAPES) and "Fundo Estadual de Ciência e Tecnologia" (FUNCITEC — 39767531/07) in Brazil.

References

Agency for Toxic Substances and Disease Registry (ATSDR), 2005. Toxicological Profile for Lead. U.S. Department of Health and Human Services, Public Health Service, Atlanta.
Blaustein, M.P., 1993. Physiological effects of endogenous ouabain: control of intracellular Ca^{+2} stores and cell responsiveness. Am. J. Physiol. 264, 1367–1387.

Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J., Cohen, R.A., 1994. Nitric oxide directly activates calcium-dependent potassium channel in vascular smooth muscle. Nature 368 (6474), 850–853.
Briones, A.M., Padilha, A.S., Cogolludo, A.L., Alonso, M.J., Vassallo, D.V., Pérez-Vizcaino, F., Salaices, M., 2009. Activation of BK_{Ca} channels by nitric oxide prevents coronary artery endothelial dysfunction in ouabain-induced hypertensive rats. J. Hypertens. 27, 83–91.
Callera, G.E., Yogi, A., Tostes, R.C., Rossoni, L.V., Bendhack, L.M., 2004. Ca^{2+} activated K^+ channels underlying the impaired acetylcholine-induced vasodilation in 2K-1C hypertensive rats. J. Pharmacol. Exp. Ther. 309, 1036–1042.
Chatterje, A., Catravas, J.D., 2008. Endothelial nitric oxide (NO) and its pathophysiologic regulation. Vascul. Pharmacol. 49, 134–140.
Cheong, A., Quinn, K., Dedman, A.M., Beech, D.J., 2002. Activation thresholds of K_V, BK and ClCa channels in smooth muscle cells in pial precapillary arterioles. J. Vasc. Res. 39, 122–130.
Ding, Y., Vaziri, N.D., Gonick, H., 1998. Lead-induced hypertension. II response to sequential infusions of L-arginine, superoxide dismutase and nitroprusside. Environ. Res. 76, 107–113.
Eichhorn, B., Dobrev, D., 2007. Vascular large conductance calcium-activated potassium channels: functional role and therapeutic potential. Naunyn Schmiedebergs Arch. Pharmacol. 376, 145–155.
Farmand, F., Ehdaie, A., Roberts, C.K., Sindhu, R.K., 2005. Lead-induced dysregulation of superoxide dismutases, catalase, glutathione peroxidase, and guanylate cyclase. Environ. Res. 98, 33–39.
Félétou, M., 2009. Calcium-activated potassium channels and endothelial dysfunction: therapeutic options? Br. J. Pharmacol. 156, 545–562.
Félétou, M., Vanhoutte, P.M., 2006. Endothelium-derived hyperpolarizing factor: where are we now? Arterioscler. Thromb. Vasc. Biol. 26, 1215–1225.
Félétou, M., Vanhoutte, P., 2009. EDHF: an uptape. Clin. Sci. 117, 139–155.
Félétou, M., Köhler, R., Vanhoutte, P., 2010. Endothelium-derived vasoactive factors and hypertension: possible roles in pathogenesis and as treatment targets. Curr. Hypertens. Rep. 12, 267–275.
Fiorim, J., Ribeiro Júnior, R.F., Silveira, E.A., Padilha, A.S., Vescovi, M.V., de Jesus, H.C., Stefanon, I., Salaices, M., Vassallo, D.V., 2011. Low-level lead exposure increases systolic arterial pressure and endothelium-derived vasodilator factors in rat aortas. PLoS One 6 (2), e17117.
Forstermann, U., Munzel, T., 2006. Endothelial nitric oxide synthase in vascular disease. Circulation 113, 1708–1714.
Glenn, S.B., Bandeen-Roche, K., Lee, B.K., Weaver, V.M., Todd, A.C., Schwartz, B.S., 2006. Changes in systolic blood pressure associated with lead in blood and bone. Epidemiology 17, 538–544.
Gonick, H.C., Ding, Y., Bondy, S.C., Ni, Z., Vaziri, N.D., 1997. Lead induced hypertension. Interplay of nitric oxide and reactive oxygen species. Hypertension 30 (6), 1487–1492.
Gupta, S., McArthur, C., Grady, C., Ruderman, N.B., 1994. Stimulation of vascular Na^+–K^+-ATPase activity by nitric oxide: a cGMP-independent effect. Am. J. Physiol. 266 (35), H2146–H2251.
Harlan, W.R., 1988. The relationship of blood lead levels to blood pressure in the U.S. population. Environ. Health Perspect. 78, 9–13.
Kempe, A.S., Lang, P.A., Eisele, K., Klarl, B.A., Wieder, T., Huber, S.M., Duranton, C., Lang, F., 2005. Stimulation of erythrocyte phosphatidylserine exposure by lead ions. Am. J. Physiol. Cell Physiol. 288, C396–C402.
Ko, E.A., Han, J., Jung, I.D., Park, W.S., 2008. Physiological roles of K^+ channels in vascular smooth muscle cells. J. Smooth Muscle Res. 44 (2), 65–81.
Kosnett, M.J., Wedeen, R.P., Rothenberg, S.J., Hipkins, K.L., Materna, B.L., Schwartz, B.S., Hu, H., Woolf, A., 2007. Recommendations for medical management of adult lead exposure. Environ. Health Perspect. 115 (3), 463–471.
Ledoux, J., Werner, M.E., Brayden, J.E., Nelson, M.T., 2006. Calcium-activated potassium channels and the regulation of vascular tone. Physiology 21, 69–76.
Levin, S.M., Goldberg, M., 2000. Clinical evaluation and management of lead exposure construction workers. Am. J. Ind. Med. 37, 23–43.
Marín, J., Redondo, J., 1999. Vascular sodium pump endothelial modulation and alterations in some pathological processes and aging. Pharmacol. Ther. 84, 249–271.
Navas-Acien, A., Guallar, E., Silbergeld, E.K., Rothenberg, S.J., 2007. Lead exposure in cardiovascular disease — a systematic review. Environ. Health Perspect. 115, 472–482.
Nelson, M.T., Quayle, J.M., 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. 268 (37), C799–C822.
Ni, Z., Hou, S., Barton, C.H., Vaziri, N.D., 2004. Lead exposure raises superoxide and hydrogen peroxide in human endothelial and vascular smooth muscle cells. Kidney Int. 66, 2329–2336.
Patrick, L., 2006. Lead toxicity, a review of the literature. Part I: exposure, evaluation, and treatment. Altern. Med. Rev. 11 (1), 2–22.
Poreba, R., Poreba, M., Gać, P., Andrzejak, R., 2011. Ambulatory blood pressure monitoring and structural changes in carotid arteries in normotensive workers occupationally exposed to lead. Hum. Exp. Toxicol. 30, 1174–1180.
Rossoni, L.V., Salaices, M., Marín, J., Vassallo, D.V., Alonso, M.J., 2002. Alterations in phenylephrine induced contractions and the vascular expression of Na^+, K^+-ATPase in ouabain-induced hypertension. Br. J. Pharmacol. 135 (3), 771–781.
Silveira, E.A., Lizardo, J.H.F., Souza, L.P., Stefanon, I., Vassallo, D.V., 2010. Acute lead-induced vasoconstriction in vascular beds of isolated perfused rat tails in endothelium-dependent. Braz. J. Med. Biol. Res. 43 (5), 492–499.
Simões, M.R., Ribeiro-Júnior, R.F., Vescovi, V.A., de Jesus, H.C., Padilha, A.S., Stefanon, I., Vassallo, D.V., Salaices, M., Fioresi, M., 2011. Acute lead exposure increases arterial pressure: role of the renin–angiotensin system. PLoS One 6 (4), e18730.
Standen, S.B., Quayle, J.M., 1998. K^+ channel modulation in arterial smooth muscle. Acta Physiol. Scand. 164, 549–557.
Tammaro, P., Smith, A.L., Hutchings, S.R., Smirnov, S.V., 2004. Pharmacological evidence for a key role of voltage-gated K^+ channels in the function of rat aortic smooth muscle cells. Br. J. Pharmacol. 143, 303–317.
Triggle, C.R., Hollenberg, M., Anderson, T.J., Ding, H., Jiang, Y., Ceroni, L., Wiehler, W.B., Ng, E.S., Ellis, A., Andrews, K., McGuire, J.J., Pannirselvam, M., 2003. The endothelium in health and disease — a target for therapeutic intervention. J. Smooth Muscle Res. 39 (6), 249–267.
Urakami-Harasawa, L., Shimokava, H., Nakashima, M., Egashira, K., Takeshita, A., 1997. Importance of endothelium-derived hyperpolarizing factor in human arteries. J. Clin. Invest. 100, 2793–2799.
Vassallo, D.V., Lebarch, E.C., Moreira, C.M., Wiggers, G.A., Stefanon, I., 2008. Lead reduces tension development and the myosin ATPase activity of the rat right ventricular myocardium. Braz. J. Med. Biol. Res. 41, 789–795.
Vaziri, N.D., Ding, Y., 2001. Effects of lead on nitric oxide synthase expression on coronary endothelial cells: a role of superoxide. Hypertension 37 (2), 223–226.
Vaziri, N.D., Ding, Y., Ni, Z., 1999a. Nitric oxide synthase expression in the course of lead-induced hypertension. Hypertension 34, 558–562.
Vaziri, N.D., Liang, K., Ding, Y., 1999b. Increased nitric oxide inactivation by reactive oxygen species in lead-induced hypertension. Kidney Int. 56, 1492–1498.
Vaziri, N.D., Ding, Y., Ni, Z., 2001. Compensatory up-regulation of nitric oxide synthase isoforms in lead-induced hypertension; reversal by a superoxide dismutase-mimetic drug. J. Pharmacol. Exp. Ther. 298, 679–685.
Vaziri, N.D., Lin, C.Y., Farmand, F., Sindhu, R.K., 2003. Superoxide dismutase, catalase, glutathione, peroxidase and NADPH oxidase in lead-induced hypertension. Kidney Int. 63, 186–194.
Weeb, R.C., Bohr, D.F., 1978. Potassium induced relaxation as an indicator of Na^+/K^+ ATPase activity in vascular smooth muscle. Blood Vessels 15, 198–207.
Weiler, E., Khalil-Manesh, F., Gonick, H.C., 1990. Effects of lead and a low molecular-weight endogenous plasma inhibitor on the kinetics of sodium potassium-activated adenosine triphosphatase and potassium-activated p-nitrophenylphosphatase. Clin. Sci. 79, 185–192.
Wiggers, G.A., Peçanha, F.M., Briones, A.M., Pérez-Girón, J.V., Miguel, M., Vassallo, D.V., Cachofeiro, V., Alonso, M.J., Salaices, M., 2008. Low mercury concentrations cause oxidative stress and endothelial dysfunction in conductance and resistance arteries. Am. J. Physiol. Heart Circ. Physiol. 295 (3), H1033–H1043.