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Influence of lead (Pb^{2+}) on the reactions of in vitro cultured rat aorta to 5-hydroxytryptamine

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Abstract

Although several studies demonstrated that lead induced abnormal vascular responses in low level lead exposed animals, investigations of the direct effects of lead on blood vessels are limited. In this study we tested the hypothesis that lead was able to directly affect the contractile reactivities of vessels. Male Wistar rat aortae were removed and cultured in PMRI 1640 with 1 ppm lead acetate for 0.5, 6, 12, 24 and 48 h, and then their responses to norepinephrine bitartrate (NE) and serotonin (5-hydroxytryptamine, 5-HT) were examined. The contractile responses to 5-HT of lead exposed aortae were significantly increased when the aortae were cultured for 24 and 48 h. Denudation of endothelium was able to abolish the increased contractile response completely. Diphenyleneiodonium (DPI), an inhibitor of the NAD(P)H oxidase, could abolish the increased contractile response to 5-HT. However, Vitamin C (VC) enhanced the contractile response of both groups to higher dosages of 5-HT. The expression of 5-HT_{2B} receptor was not significantly altered by incubation with 1 ppm lead for 24 h. These data suggest that exposure to low levels of lead can directly increase the contraction of aorta to 5-HT. This effect is endothelium dependent, which is not mediated by increased expression of the 5-HT_{2B} receptor. The increased contraction to 5-HT may be related to increased production of superoxide ($O_2^{\bullet -}$) induced by lead exposure.

Keywords: Lead toxicity; Aorta; Vascular reactivity; 5-HT; 5-HT_{2B} receptor; Superoxide

Abbreviations: 5-HT, 5-hydroxytryptamine; ACh, acetylcholine chloride; ANOVA, analysis of variance; CHAPS, 3-[3-(cholamidopropyl) dimethylammonio]-1-propanesulfonate; DMSO, dimethyl sulfoxide; DPI, diphenyleneiodonium chloride; K–H solution, Krebs–Henseleit solution; NE; norepinephrine bitartrate; $O_2^{\bullet -}$, superoxide; PBS, phosphate buffered saline; PVDF membrane, polyvinylidine difluoride membrane; ROS, reactive oxygen species; SOD, bovine erythrocyte superoxide dismutase; VC, Vitamin C

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1. Introduction

Chronic exposure to low levels of lead is related to increased blood pressure or hypertension in human and animals (Webb et al., 1981; Skoczynska et al., 1986, 2001; Victery, 1988; Schwartz, 1991; Purdy et al., 1997; Cheng et al., 2001; Marques et al., 2001; Shelkovnikov and Gonick, 2001; Nomiyama et al., 2002; Kopp et al., 1988).

Abnormity of structure and function of vessels, including the increased wall-lumen ratio, increased vascular responsiveness to vasoconstrictors and/or decreased responsiveness to vasodilators, can be found in several kinds of animal hypertension models and genetic hypertension (Mulvany, 1992; Gohlke et al., 1993; Intengan and Schiffrin, 2001). In the case of lead induced hypertension, although the structural abnormity is not reported till now, abnormal vascular reactivities have been found, including increased vascular reactivity to catecholamines (Webb et al., 1981; Skoczynska et al., 1986, 2001; Victery, 1988); significantly longer period of time required to reach half-maximal relaxation in tail artery strips from lead treated rats (Webb et al., 1981); and reduced relaxation to both acetylcholine (ACh) and sodium nitroprusside (SNP) in lead treated rats (Marques et al., 2001).

Numerous mechanisms of lead induced hypertension have been proposed, which can be summarized into three levels: (a) abnormal central and peripheral neurogenic components, such as central sympathetic hyperactivity, barofeflex hyposensitivity, vagal parasympathetic hypotone (Boscolo and Carmignani, 1988; Carmignani et al., 2000); (b) abnormal humoral components, such as increased plasma level of catecholamines and decreased plasma level of bradykinin (Carmignani et al., 2000), and increased activity of renin-angiotensin-aldosterone axis (Campbell et al., 1985; Victery et al., 1982); (c) abnormal local vessel components, such as altered both contraction and relaxation response to vasoactive agents (Webb et al., 1981; Skoczynska et al., 1986, 2001; Victery, 1988; Marques et al., 2001); inhibition to the repair of the damaged endothelial cell (Fujiwara et al., 1997); stimulated proliferation of vascular smooth muscle cell (Dorman and Freeman, 2002), and so on.

However, there are still many questions needing to study. For example, are the abnormal vascular reactivities induced, at least partly, directly through interaction between lead and vessels or only indirectly through abnormal neurogenic components or/and abnormal humoral components induced by lead? Another question is whether lead exposed arteries have significantly increased responsiveness to 5-hydroxytryptamine (5-HT), and whether the effect is mediated through upexpressed 5-HT_{2B} receptor, for significant increase in vascular responsiveness to 5-HT and in 5-HT_{2B} receptor expression were found in several hypertensive animal models (Thompson and Webb, 1987; Roson et al., 1990; Watts et al., 1995, 1996; Watts and Fink, 1999; Russell et al., 2002). And whether the increased vascular responsiveness to 5-HT is related to increased production of superoxide ($O_2^{\bullet -}$) by lead exposure is also worth to study, since in vivo studies demonstrated that superoxide trapping agent was able to significantly lower arterial pressure of animals with lead-induced hypertension (Vaziri et al., 2001, 2003); and in vitro study found that VC was able to reduce $O_2^{\bullet -}$ production and COX-2 expression in lead (1 ppm)-exposed aortic segments (Courtois et al., 2003).

In this study we hypothesized that lead was able to directly affect the reactivities of vessels, the expression of the 5-HT_{2B} receptor was upregulated, and $O_2^{\bullet -}$, the major reactive oxygenic series (ROS) in blood vessel induced by lead (Griendling et al., 2000; Vaziri et al., 2001), was involved in this change. Since neurogenic and humoral components could be important confounding factors, which were able to induce abnormal reactivities of vessels, we removed normal male rat aortae and cultured in vitro with or without 1 ppm lead acetate for different periods of time to explore these questions.

2. Materials and methods

2.1. Preparation of rat aorta

Healthy male Wistar rats, 250–300 g, obtained from Experimental Animal Laboratory of Peking University Health Science Center, were housed in wire-mesh cages at temperature room with 17–27 °C, a 12:12 h light–dark cycle supplemented with food and water ad libitum.

After anesthetized by pentobarbital sodium (50 mg/kg), the rats were killed by decapitation. Thoracic aortae were carefully excised and placed

into icy Krebs–Henseleit (K–H) solution (containing in mM: NaCl, 118; KCl, 4.7; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11.1; and EDTA-Na_2, 0.026) or into D-Hanks with 100 U/ml penicillin and 100 U/ml streptomycin. After being cleaned free of fat and other connective tissues, the thoracic aortae were cut into 3 mm long rings, then suspended into culture solution (RPMI 1640 containing 10% fetal bovine serum) with or without 1 ppm lead acetate at 37 °C for 0.5, 6, 12, 24 or 48 h. The lead concentration of 1 ppm in the present study was chosen on the basis of published in vitro studies: 1 ppm lead was able to upregulate endothelial nitric oxide synthase expression in rat and human cultured endothelial cells, respectively (Ding et al., 2000; Vaziri and Ding, 2001); 0.1–1 ppm lead were able to significantly reduce sGC-β1 subunit expression in a concentration dependent manner, and the maximal reduction in sGC-β1 subunit expression was achieved in rat aortic rings incubated with 1 ppm lead for 24 h (Courtois et al., 2003).

The studies were conducted in accord with the principles and procedures outlined in the NIH guide for the Care and Use of the Laboratory Animals (National Research Council, 1996).

2.2. Isolated tissue bath protocol

After incubation, the reactivities of the aortic rings were evaluated in the vascular tissue baths. The baths contained 8 ml K–H solution bubbled with a mixture of 95% O_2 and 5% CO_2, and warmed to 37 °C by an equitherm heating circulation system. The aortic rings were mounted on a pair of stainless-steel Δ shaped hooks, one of which was fixed to an L-shaped rod inside the chamber and the other to an isometric force transducer (Xinhang Mechanical and Electronic Inc., Gaobeidian, Hebei Province, PR China) which was connected to a polygraph (Meiyi technological Inc., Nanjing, Jiangsu Province, PR China). Tissues were allowed to equilibrate under an optimum final force of 2.0 g for a period of 60 min, renewing the buffer every 15 min. After stabilization, the preparations were contracted twice with 40 mM KCl and the second contraction was taken as the reference value for analysis. The presence of functional endothelium was tested by the relaxation response to 1 μM ACh in norepinephrine precontracted rings. The aortic rings of lead exposure and control used in each experiment were removed from the same rat, and each ring was used only once.

Contractile responses of incubated aortic rings with intact endothelium were assessed by adding cumulative concentration of NE (0.1 nM to 10 μM) and 5-HT (10 nM to 10 mM). Since the contractile response to 5-HT was significantly enhanced in lead exposed aortae incubated over 24 h, and the contractions of aortae incubated 24 h were larger than those of 48 h, the following 5-HT concentration–contraction curves of aortae were further studied only using aortae incubated with or without lead for 24 h, including: (1) endothelium denuded preparations: after the aortae were incubated for 24 h, the endothelia were denuded by two methods: rubbing with cotton thread or treating with 0.5% CHAPS for 45 s at a pH of 7.4 (Shelkovnikov and Gonick, 2001); (2) preparations incubated for 24 h with 30 μM VC; and (3) preparations incubated for 24 h, then incubated with 50 μM DPI for 0.5 h.

2.3. Western analysis of aortic 5-HT_{2B} receptor expression

Aortic 5-HT_{2B} receptor expression was determined by Western blot. After in vitro cultured, aortae were ground to powder in liquid nitrogen. Four volumes (4 μl/mg tissue) of ice-cold homogenization buffer were added (50 mM Tris–Cl pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1% nonidet P-40, 1% sodium deoxycholate and 1 μg/ml leupeptin). The homogenate was vortexed, sonicated briefly on ice, and centrifuged at 14,000 × *g* for 20 min at 4 °C. The supernatant was removed and total protein was measured using the bicinchoninic acid method kit (Beyotime Biotech Co. Ltd., Hangzhou, Zhejiang Province, PR China). Fifty micrograms of total protein was separated on 10% SDS-polyacrylamide gels and transferred onto polyvinylidine difluoride (PVDF) membranes. Membranes were blocked for 1 h in TBST (20 mM Tris–hydrochloride, pH 7.6, 120 mM NaCl, 0.05% Tween-20) containing 5% nonfat milk powder and probed overnight or 1.5 h with anti-5-HT_{2B} receptor polyclonal antibody at 1:100 (Wuhan Bolster Biological Technology Ltd., Hubei Province, PR China). Blots were rinsed in TBST for five minutes three times and incubated with the horseradish

peroxidase conjugated secondary antibody at 1:2000 (Wuhan Bolster Biological Technology Ltd., Hubei Province, PR China) for 1 h. After rinsed in TBST for five minutes five times and then rinsed twice in TBS (20 mM Tris–hydrochloride, pH 7.6, 140 mM NaCl), Blots incubated with enhanced chemiluminescence reagents to visualize bands. In addition, prestained protein markers (New England Biolabs) were used for molecular mass determinations. To compare the expression of 5-HT_{2B} receptor with the expression of another protein, we analyzed the expression of actin in a parallel gel with 15 μg samples and with the same experimental procedure. The first antibody for actin was actin polyclonal antibody (Santa Cruz, sc-1616) at 1:500.

2.4. $O_2^{\bullet -}$ generation in the aortic segments

The amount of $O_2^{\bullet -}$ generated in the vascular wall was determined by measuring the SOD-inhibitable reduction of cytochrome *c*, following the published method (Landmesser et al., 2003). In brief, after incubation and treatment, aortic rings were washed with K–H solution twice, transferred into incubation solution (K–H solution containing 1 U/ml catalase, 1% fetal bovine serum and 50 μmol/l cytochrome *c*) at 37 °C for 0.5 h with or without 125 U/ml SOD, then put on ice to stop reactions. The supernatants were collected and cytochrome *c* reduction was calculated using absorbance at 550 nm corrected for background reading at 540 and 560 nm. Superoxide production over 0.5 h was quantified in picomoles/mg aorta from the difference between absorbance with or without SOD.

To examine whether lead could increase the production of $O_2^{\bullet -}$ in incubated lead exposed aortae, and whether DPI, the inhibator of NAD(P)H oxidase, and antioxidant Vitamin C (VC) were able to decrease the production of $O_2^{\bullet -}$ in incubated lead exposed aortae, we set up six groups: (a) control group, aortae incubated without any treatment for 24 h; (b) lead group, aortae incubated with 1 ppm lead for 24 h; (c) DPI group, aortae incubated for 24 h then exposed to 50 μM DPI for 0.5 h; (d) lead + DPI group, aortae incubated with 1 ppm lead for 24 h then exposed to 50 μM DPI for 0.5 h; (e) VC group, aortae incubated with 30 μM VC for 24 h; (f) lead + VC group, aortae incubated with 1 ppm lead and 30 μM VC for 24 h.

2.5. Chemicals

Drugs used in the present experiment were lead acetate, norepinephrine bitartrate, Serotonin creatinine sulfate complex, acetylcholine chloride, Vitamin C, diphenyleneiodonium chloride, CHAPS, bovine erythrocyte superoxide dismutase, catalase, cytochrome *c*, all of which were purchased from Sigma–Aldrich Corporation, St. Louis, MO, USA. All chemicals were made fresh on the day of use. All chemicals, except for DPI, were dissolved in distilled water, and the amount of solvent added did not exceed 1/100 of the solution. DPI is dissolved in DMSO, the amount of which did not exceed 5/1000 of the solution. The addition of these chemicals to K–H solution did not alter the pH of the solution. RPMI 1640 and fetal bovine serum were purchased from Gibco (Grand, NY, USA).

2.6. Statistical analysis

Contraction to the second exposure of 40 mM KCl was taken as 100%, and the contractile responses to NE, 5-HT were expressed as a percentage of this contraction. Each experiment was repeated 6 times, except Western blot, which was repeated 4 times. All data are expressed as mean ± S.E.M. Paired and unpaired *t*-tests, or one-way ANOVA, followed by either the Student–Newman–Keuls or Turkey–Kramer test were performed and a *p*-value smaller than 0.05 was considered to be significant.

3. Results

3.1. Effect of incubation on the aortic contractile responses

After stabilization, the magnitude of contraction to the second and all subsequent exposures to 40 mM KCl were uniform in each tissue, and these findings were consistent with published results (Purdy et al., 1997). Fig. 1 depicted the effects of different periods of incubation on the aortic maximal contractile responses to KCl, NE and 5-HT. The contractions induced by NE and 5-HT were expressed as percentages of the second contraction of 40 mM KCl of each ring. During the 48 h incubation, no significant differences among the maximal contractile responses to KCl were found

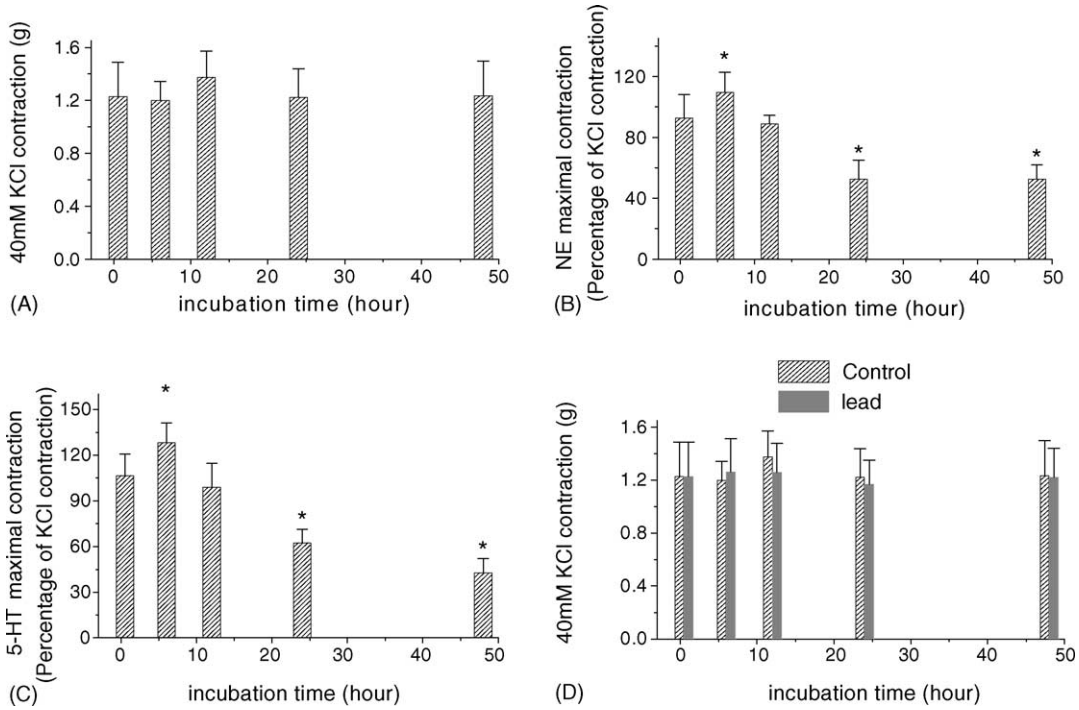

Fig. 1. Effects of incubation on the maximal contractile responses of aortae to KCl (A and D), NE (B) and 5-HT(C). Vertical bars represent mean ± S.E.M. ($n = 6$). The maximal contractions of NE and 5-HT are expressed as percentages of the second 40 mM KCl contraction. When the incubation time was less than 6 h, incubation promoted the contraction to NE and 5-HT; while when incubation time was more than 6 h, it decreased the maximal contractile responses to NE and 5-HT. (★) Statistically significant differences ($p < 0.05$) from the contraction of the aortae incubated for 0.5 h.

(Fig. 1A), and lead had no significant effects on the maximal contraction induced by KCl (Fig. 1D). Therefore, the contractions to 40 mM KCl can be used as reference values to the contractions induced by NE and 5-HT of aortae with or without lead exposure.

Incubation, however, had significant effects on the maximal contraction of aortae to NE and 5-HT. When the incubation time was less than 6 h, incubation promoted the contraction to NE and 5-HT. While when incubation time was more than 6 h, incubation decreased the maximal contractile responses to NE and 5-HT. When aortae were incubated for 24 h or 48 h, their maximal contractile responses to NE and 5-HT were significantly reduced (Fig. 1B and C). The maximal contractions of aortae incubated for 24 and 48 h to NE, compared with the contractions of aortae incubated for 0.5 h, were reduced about 42% and 45%, respectively, and those to 5-HT were reduced about 41% and 60%, respectively.

3.2. Effect of lead on the contractile responses of aortae to NE and 5-HT

No matter whether the incubation period was short or long (from 0.5 to 48 h), there was no significant difference in contractile responses to NE between control and lead exposed aortae (results not shown). And there was also no significant difference in contractile responses to 5-HT between control and lead exposed aortae when the aortae were incubated for 0.5–12 h. However, when the incubation periods were 24 and 48 h, the contractions induced by 5-HT were significantly increased in lead exposed aortae compared with their controls (Fig. 2). For example, when incubated 24 h, the contraction of lead exposed aortae reached 75.14 ± 6.98% at the 5-HT dosage of 10^{-5} M, while control group was only 52.73 ± 11.59%, $P < 0.01$. Since the responses of aortae incubated for 24 h to 5-HT was larger than those incubated for 48 h

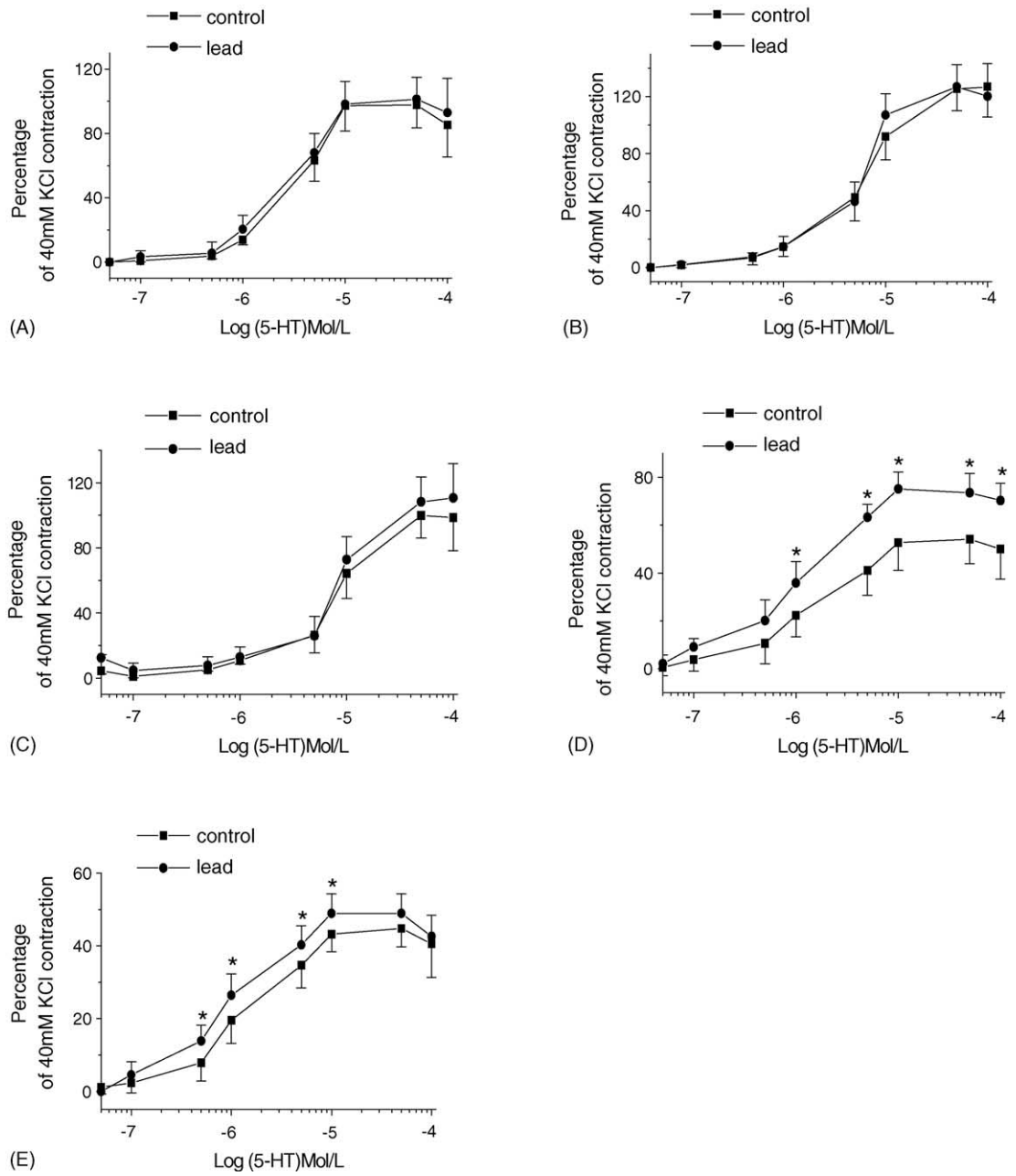

Fig. 2. 5-HT induced contraction of aortae incubated with or without 1 ppm lead for 0.5 h (A), 6 h (B), 12 h (C), 24 h (D) and 48 h (E). Points represent mean ± S.E.M. ($n = 6$). The contractions are expressed as percentages of the second 40 mM KCl contraction. (✱) Statistically significant differences ($p < 0.05$) between control and lead-exposed aortae.

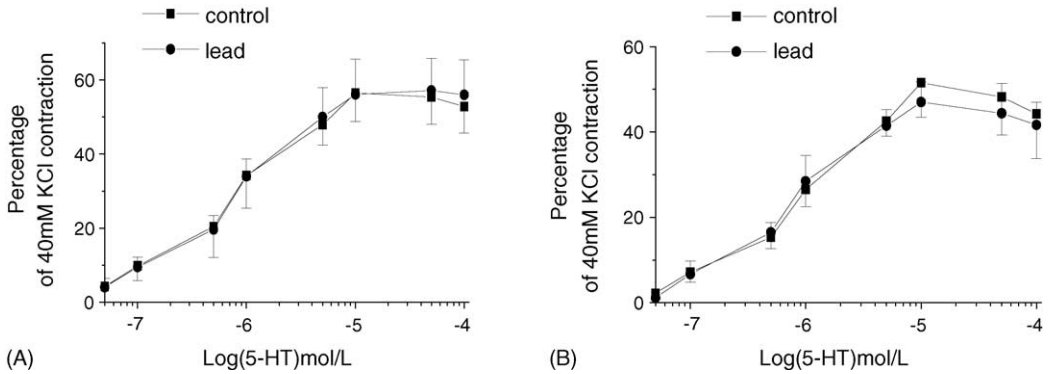

Fig. 3. Effects of endothelium denudation on the 5-HT induced contraction of aortae incubated with or without 1 ppm lead for 24 h. Points represent mean ± S.E.M. ($n = 6$): The contractions are expressed as percentages of the second 40 mM KCl contraction. (A) aortae denuded with rub and (B) aortae denuded with detergent.

(Fig. 1C), the experiments below were conducted with aortae incubated for 24 h only.

3.3. Effect of endothelium denudation on the contraction induced by 5-HT

To ascertain whether the endothelium of the lead exposed aortae, like in the DOCA-salt hypertensive rats and N_ω-L-arginine hypertensive rats (Watts et al., 1995; Banes and Watts, 2002; Russell et al., 2002), also has little relation to the enhanced response to 5-HT, we conducted the experiment with endothelium-denuded aortae rings. At first, we denuded the endothelia of aortae incubated for 24 h through rubbing with cotton thread. The result was that the significant difference in responses to 5-HT between control and lead exposed aortic rings disappeared (Fig. 3A). Since there was the possibility that rubbing off endothelium may damage the aortae mechanically, and to confirm this result, we repeated this experiment using aortic rings with endothelium denuded through incubating with 0.5% CHAPS, a detergent, for 45 s. The result was similar (Fig. 3B). These results proved that the mechanism of enhanced response of lead exposed aortae to 5-HT was likely different from that of DOCA-salt hypertensive rats and the N_ω-L-arginine hypertensive rats.

3.4. Effect of lead on 5-HT_{2B} receptor expression

Immunoblot analysis was performed on homogenates from control and lead exposed aortae incubated for 24 h to ascertain whether 5-HT_{2B} receptor protein expression was affected. Equivalent amounts of total protein were immunoblotted with a 5-HT_{2B} receptor antibody. This antibody recognized two bands at 55 and 110 kDa, respectively (Fig. 4), both of which are not observed when the primary antibody was removed from the experiment. There was no significant difference in the 5-HT_{2B} receptor

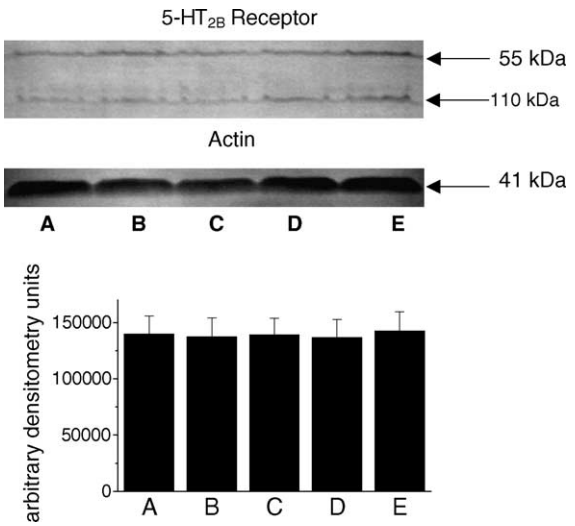

Fig. 4. Top, blot of 5-HT_{2B} receptor protein in aortae incubated with or without lead or VC for 24 h. This was the representative of four separate experiments. Bottom, arbitrary densitometry units of the 5-HT_{2B} receptor in aortic homogenates for the 55-kDa band. Bars represent mean ± S.E.M. ($n = 4$). A: aortae without incubation; B: aortae incubated for 24 h; C: aortae incubated with 1 ppm lead for 24 h; D: aortae incubated with 30 μM VC for 24 h; E: aortae incubated with 1 ppm lead and 30 μM VC for 24 h.

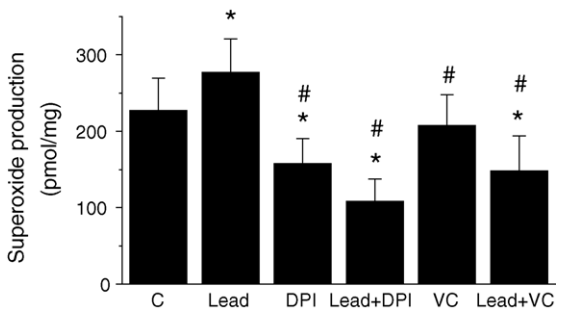

Fig. 5. Effects of lead, DPI and VC on the $O2^{\bullet -}$ production in aortae incubated for 24 h measured by the SOD-inhibitable cytochrome *c* reduction assay. C: control group, aortae incubated for 24 h without any treatment; lead: lead group, aortae incubated with 1 ppm lead for 24 h; DPI: DPI group, aortae incubated for 24 h then exposed to 50 μM DPI for 0.5 h; lead + DPI: lead + DPI group, aortae incubated with 1 ppm lead for 24 h then exposed to 50 μM DPI for 0.5 h; VC: VC group, aortae incubated with 30 μM VC for 24 h; lead + VC: lead + VC group, aortae incubated with 1ppm lead and 30 μM VC for 24 h. Vertical bars represent mean ± S.E.M. ($n = 6$). (✱) Statistically significant differences ($p < 0.05$) from control group. (#) Statistically significant differences ($p < 0.05$) from lead group.

immunoreactive bands between lead exposed aortae and control ones. Incubation with VC also had no significant effects on the expression of 5-HT_{2B} receptor. These results demonstrated that the increased contractile responses induced by 5-HT was not, like some kinds of hypertension models, mediated by up-regulation of the 5-HT_{2B} receptor protein.

3.5. Effects of lead, DPI and VC on the production of $O_2^{\bullet -}$ in the aortae incubated 24 h

Lead exposed aortae produced more $O_2^{\bullet -}$ than control ones ($p < 0.05$). DPI was able to significantly decrease the production of $O_2^{\bullet -}$ in aortae with or without lead exposure ($p < 0.01$, Fig. 5). VC was also able to decrease the production of $O_2^{\bullet -}$ in aortae with or without lead exposure. These results consisted with the findings published (Vaziri et al., 2001, 2003; Courtois et al., 2003). However, the difference between VC group and control group was not statistically different in this study.

3.6. Effects of VC and DPI on the contractile response of lead exposed aortae to 5-HT

After we found that lead exposed aortae were able to produce more $O_2^{\bullet -}$, and DPI and VC could significantly decrease the production of $O_2^{\bullet -}$ in lead exposed ones, we examined the role of $O_2^{\bullet -}$ in the increased

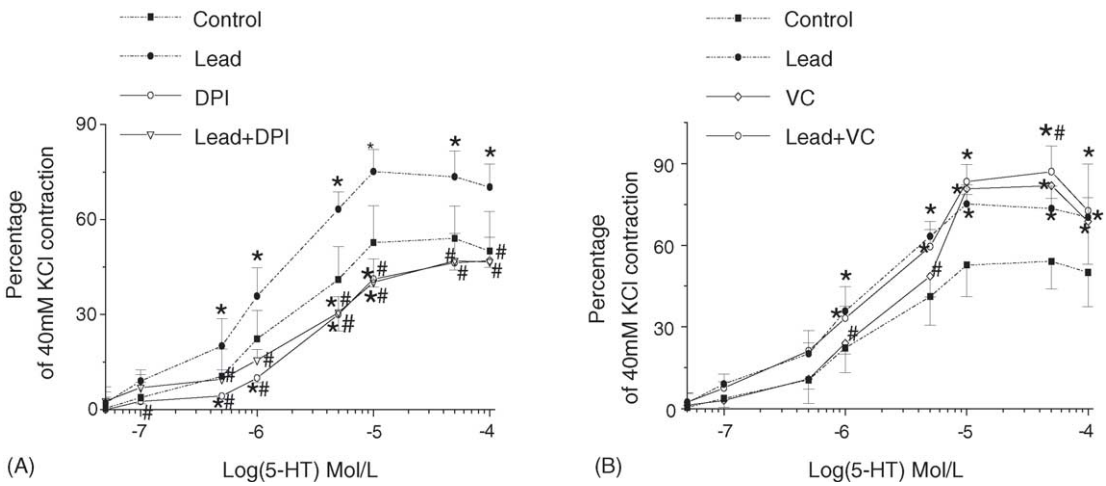

Fig. 6. Effects of DPI (A) and VC (B) on the 5-HT induced contractile response of aortae incubated with or without 1 ppm lead for 24 h. Points represent mean ± S.E.M. ($n = 6$). The contractions are expressed as percentages of the second 40 mM KCl contraction. Control: control group, aortae incubated for 24 h; lead: lead group, aortae incubated with 1 ppm lead for 24 h; DPI: DPI group, aortae incubated for 24 h then exposed to 50 μM DPI for 0.5 h; lead + DPI: lead + DPI group, aortae incubated with 1 ppm lead for 24 h then exposed to 50 μM DPI for 0.5 h; VC: VC group, aortae incubated with 30 μM VC for 24 h; lead + VC: lead + VC group, aortae incubated with 1 ppm lead and 30 μM VC for 24 h. (✱) Statistically significant differences ($p < 0.05$) from control group. (#) Statistically significant differences ($p < 0.05$) from lead group.

response to 5-HT with these two agents. 30 μM VC was added to lead exposed aortae and paired controls immediately after the addition of lead; and 50 μM DPI was added after the second contraction of KCl and then incubated for 30 min. We found that DPI was able to significantly decrease the contraction of lead exposed aortae and controls (Fig. 6A). However, VC demonstrated an apparently different effect on the contractile response of incubated aortae: it not only did not decrease the 5-HT induced contraction, but it enhanced the contractile response to higher dosages of 5-HT (Fig. 6B).

4. Discussion

Hypertension is one of the most prevalent disorders in the industrial world and is increasingly becoming a global issue. Results of numerous published studies supported the association between blood lead and hypertension in human and animals (Webb et al., 1981; Skoczynska et al., 1986, 2001; Victery, 1988; Purdy et al., 1997; Cheng et al., 2001; Marques et al., 2001; Shelkovnikov and Gonick, 2001; Nomiyama et al., 2002). Lead is a ubiquitous environmental and industrial pollutant that has been detected in almost all phases of environmental and biological systems. Therefore, it is of great significance to study the mechanism of lead inducing hypertension. Vascular abnormity, including the increased wall-lumen ratio, increased vascular responsiveness to vasoconstrictors and/or decreased responsiveness to vasodilators, can be found in almost all kinds of animal hypertension models and genetic hypertension patients (Mulvany, 1992; Gohlke et al., 1993; Intengan and Schiffrin, 2001). And vascular abnormity is the main cause of coronary heart disease and stroke. The importance of studying the mechanism of vascular abnormity of lead induced hypertension is obvious.

In the case of lead induced rat hypertension, the data concerning vascular reactivity are not consistent till now. Some investigators (Webb et al., 1981; Skoczynska et al., 1986, 2001; Victery, 1988; Marques et al., 2001) found abnormal responses to vasoconstrictors and/or to vasodilators. However, some (Purdy et al., 1997; Shelkovnikov and Gonick, 2001) failed to get similar findings. The cause(s) of these divergences need further investigation.

As to the question that whether lead can directly affect the response of vessels by the direct interaction of lead and vessels, nearly all published studies cannot answer, since nearly all these studies used hypertensive animal vessels, and under this condition lead can affect many neurogenic and humoral components (Boscolo and Carmignani, 1988; Carmignani et al., 2000), which were known to have the ability to change the vascular reactivities. In present study, to eliminate the confounding effects of neurogenic and humoral factors, we used in vitro cultured normal male rat aortae to explore this question. We found that exposure to 1 ppm lead did can directly increase the contraction of aorta to 5-HT.

5-HT is an autacoid with a myriad of actions in the cardiovascular system. There are thirteen different mammalian G-protein coupled 5-HT receptor types identified by molecular cloning, which have been grouped in seven families (Hoyer et al., 1994). Among these seven families, 5-$HT_{1D\beta}$, 5-HT_{2A}, 5-HT_{2B}, 5-HT_4, 5-HT_7 receptor mRNA were found to be expressed in nearly all vessels, with 5-$HT_{1D\beta}$, 5-HT_{2B} and 5-HT_4 receptor mRNA expressed in endothelial cells, and 5-$HT_{1D\beta}$, 5-HT_{2A}, 5-HT_{2B}, 5-HT_7 receptor mRNA expressed in smooth muscle cells (Ullmer et al., 1995). Rat aorta can be contracted by 5-HT through 5-HT_{2A} receptors, 5-HT_{2B} receptors (Thompson and Webb, 1987; Roson et al., 1990; Watts et al., 1995, 1996; Watts and Fink, 1999; Russell et al., 2002) and 5-HT_{1B} receptors (Banes and Watts, 2003). Among these three receptors, 5-HT_{2A} receptor primarily mediates contraction in arteries from normotensive rats (Watts et al., 1995, 1996; Watts, 1998); 5-HT_{1B} receptors expression was found unchanged in DOCA-salt hypertension (Banes and Watts, 2003); 5-HT_{2B} receptor expression was found up-regulation in both DOCA-salt hypertensive rats and N_ω-L-arginine hypertensive rats (Banes and Watts, 2002; Russell et al., 2002). Therefore, there was a possibility that the enhanced contraction of lead exposure aortae induced by 5-HT was mediated by upregulated 5-HT_{2B} receptor expression. However, our results of Western blot did not support this hypothesis. There was no significant difference between the lead exposed aortae and their controls in 5-HT_{2B} receptor expression. Incubation with VC also was not able to alter the expression of 5-HT_{2B} receptor.

In vivo and in vitro studies proved that $O_2^{\bullet -}$ had close relation to lead induced hypertension: infusion of tempol, the superoxide trapping agent, or antioxidant

VC was able to significantly lower arterial pressure of animals with lead-induced hypertension, and the oxidative stress was associated with mild up-regulation of superoxide-generating enzyme, NAD(P)H oxidase (Vaziri et al., 2001, 2003; Marques et al., 2001); VC partially restored sGC-beta(1) subunit expression in lead (1 ppm)-exposed aortic segments, and reduced both COX-2 expression and $O_2^{\bullet -}$ production related to lead exposure (Courtois et al., 2003). Therefore, there was also a possibility that the enhanced contraction of lead exposure aortae induced by 5-HT was mediated by increased production of $O_2^{\bullet -}$. In this study, we found that lead was able to increase the production of $O_2^{\bullet -}$ in cultured aortae. Both DPI and VC were able to significantly decrease the production of $O_2^{\bullet -}$ in lead exposed aortae (Fig. 5). However, their effects on the 5-HT induced contraction were apparently different: DPI was able to significantly decrease the 5-HT induced contractile response of lead exposed aortae and their controls; on the contrary, VC did not decrease the 5-HT induced contraction, but it enhanced the contractile response to higher dosages of 5-HT (Fig. 6). Although there was a divergence between the result of DPI and VC, the hypothesis that $O_2^{\bullet -}$ was involved in the enhanced contraction response to 5-HT of lead exposed aortae still worth to support, since the effect of DPI can give us directly proof to support this hypothesis, and VC's effect is based on its ability to scavenge aqueous phase reactive oxygen species (ROS) by rapid electron transfer (Halliwell et al., 1987), it does not reduce the production of $O_2^{\bullet -}$directly. Therefore, there was a possibility that $O_2^{\bullet -}$ exerted its effects before it was scavenged, which needs further investigation. Another worthy to be studied question is why VC, which was found to be able to lower arterial pressure of animals with lead-induced hypertension, was able to promote the contraction of incubated aortae to higher dosages of 5-HT.

In present study, we found that intact endothelium was essential to the enhanced response of lead exposed aortae to 5-HT. At the beginning of this experiment, we denuded the endothelium by rubbing with cotton thread, just wanted to find that endothelium, like in the mineralocorticoid hypertensive rat and the N_ω-L-arginine hypertensive rat (Watts et al., 1995; Banes and Watts, 2002; Russell et al., 2002), had little relation to the increased contractile response to 5-HT. Surprisingly, denuding endothelium in our study completely abolished the increased contractile response to 5-HT in lead exposed aorta. To confirm this result, we repeated the experiment by denuding the endothelium with detergent—0.5% CHAPS, and the results were similar: the enhanced contractions were completely abolished (Fig. 6). These results indicated that lead exposure was able to induce certain change(s) in the endothelium, and the change(s) caused the enhanced contraction to 5-HT. There were published papers, which reported that endothelial dysfunction was able to augment responses to 5-HT (Heistad et al., 1995), and a good endothelial function was able to offer coronary artery protection against the action of certain vasoconstrictors including 5-HT (Yang et al., 1989). However, exactly what is (are) affected by lead exposure on the endothelium calls for further study.

In summary, exposure with 1 ppm lead can directly increase the contraction of rat aorta to 5-HT, the mechanism of which is not similar to that of the mineralocorticoid hypertensive rat and the N_ω-L-arginine hypertensive rat. The enhanced contraction is endothelium dependent, and has little relation to the expression of the $5\text{-}HT_{2B}$ receptor. Lead induced increased production of $O_2^{\bullet -}$ may be related to the enhanced contractile response to 5-HT.

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