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Decreased aortic contractile reaction to 5-hydroxytryptamine in rats with long-term hypertension induced by lead (Pb^{2+}) exposure

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ABSTRACT

Male Wistar rats were exposed to 100 ppm Pb^{2+} in drinking water for 10 months. Tail blood pressure, serum 5-hydroxytryptamine (5-HT), the expression of 5-HT_{2B} receptor in the aorta, the aortic response to 5-HT, and the pathologic changes of aorta were examined. The systolic blood pressure of Pb^{2+} exposed group was significantly increased after 2 months of Pb^{2+} exposure. After 10 months of Pb^{2+} exposure, aortic contractile response to 5-HT was significantly decreased. There was no significant difference in the levels of serum 5-HT and the expression of 5-HT_{2B} receptor between these two groups. The aortic media and the media-lumen ratio of Pb^{2+} exposed group were significantly increased. These data suggest that long-term Pb^{2+} exposure can increase blood pressure, and can alter the function and structure of aortic of rats. The decreased aortic response to 5-HT has little relation to the expression of 5-HT_{2B} receptor and the serum level of 5-HT, maybe is a result of the aortic structural alteration.

1. Introduction

Chronic exposure to low levels of Pb^{2+} is related to increased blood pressure or hypertension in human and laboratory animals (Fenga et al., 2006; Marques et al., 2001; Navas-Acien et al., 2007; Skoczynska et al., 2001; Webb et al., 1981). It was reported by an epidemic study that the blood Pb^{2+} was able to significantly increase cardiovascular mortality, even at level as low as 0.48 μM (0.1 ppm) in blood (Menke et al., 2006).

Abnormities of vascular structure and function, including increased wall-lumen ratio, increased vascular responsiveness to vasoconstrictors and/or decreased responsiveness to vasodilators, are important characteristics of laboratory animal hypertension and human genetic hypertension (Gohlke et al., 1993; Intengan and Schiffrin, 2001; Mulvany, 1992). In the case of Pb^{2+} induced hypertension, abnormal vascular responsiveness was reported by many researchers, such as increased vascular reactivity to catecholamines (Skoczynska et al., 2001; Victery, 1988; Webb et al., 1981), reduced relaxation to both acetylcholine (ACh) and sodium nitroprusside (SNP) (Marques et al., 2001). However, it is not reported till now whether there are vascular structural abnormities or not in Pb^{2+} induced hypertension.

5-Hydroxytryptamine (5-HT) is an autacoid with a myriad of actions in the cardiovascular system. The contractile response to 5-HT was found increased in many kinds of experimental and/or genetic hypertensive animal models, such as, in genetic and renal hypertensive rats (McGregor and Smirk, 1970), in portal hypertensive rats (Cummings et al., 1986), in mineralocorticoid hypertensive rats (Thompson and Webb, 1987; Watts et al., 1995), two kidney-two clip hypertensive rats (Roson et al., 1990), in hypertensive pigs induced by chronic inhibition of endothelium-derived nitric oxide synthesis (Ito et al., 1995), in deoxycorticosterone acetate-salt hypertensive rats (Banes and Watts, 2003; Watts et al., 1996; Watts and Fink, 1999), and in N_{ω}-L-arginine hypertensive rats (Russell et al., 2002). Recently, we also found that 1 ppm Pb^{2+} was able to enhance the contractile response of in vitro cultured rat aorta to 5-HT (Zhang et al., 2005). However, the mechanism was different from those of DOCA-salt hypertensive rats and N_{ω}-L-arginine hypertensive rats (Banes and Watts, 2002; Russell et al., 2002), such as in the participation of the endothelium and the expression of 5-HT_{2B} receptor. Since the in vitro culture environment is different from the internal environment, whether these differences also exist in the hypertensive rats induced by Pb^{2+} exposure needs further study.

The present study aimed to investigate whether Pb^{2+} was able to enhance the aortic contractile response of long-term Pb^{2+} exposed rats to 5-HT. Furthermore, to study the mechanism of the effects of Pb^{2+} on the 5-HT induced contraction, the expression of 5-HT_{2B}

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receptor, the serum level of 5-HT, and the aortic structure of long-term Pb^{2+} exposed rats were studied.

2. Materials and methods

2.1. Drugs and reagents

Lead acetate, norepinephrine bitartrate, acetylcholine chloride (ACh), CHAPS and serotonin (5-hydroxytryptamine, 5-HT) creatinine sulfate complex were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). *o*-Phthaldialdehyde (OPA) was purchased from Fluka Chemical Corporation (Switzerland). All other reagents were of analytical grade. All chemicals were made fresh on the day of use. The amount of solvent added into the tissue bath did not exceed 1/100 of the solution. And the addition of these chemicals did not alter the pH of the solution in it.

2.2. Animals and treatment

Male Wistar rats, 160 ± 20 g, obtained from Laboratory Animal Center in Beijing Institute of Pharmacology and Toxicology, were randomly assigned to Pb^{2+} exposed group and control group (n = 10 for each). The Pb^{2+} exposed group was fed with regular rat chow and distilled water supplemented with 100 ppm Pb^{2+} (0.48 mM lead acetate) for 10 months. The control group was provided with regular rat chow and distilled water without any treatment throughout the observation period. The concentration of Pb^{2+} chosen in this study as 100 ppm was based on previous similar studies (Ding et al., 2001; Karimi et al., 2002; Vaziri et al., 1999, 2001). Systolic blood pressure was measured with the tail-cuff method monthly. At the end of study, rats were fasted overnight, and killed by exsanguination through celiac aorta puncture. Sera were separated for the measure of the concentration of 5-HT. Thoracic aortae were harvested, cleaned in 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). The arch section of each aorta was cut off and fixed in 10% PBS-buffered formaldehyde. Below arch section, 1.5 cm of each descending thoracic aorta was collected and cut into 3 mm long rings for vascular responses measurement. The left part of each aorta was frozen in liquid nitrogen, and then stored at −80 °C until processing for Western blot.

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).

2.3. Measurement of blood pressure

The blood pressure of conscious rats was measured with a rat tail sphygmomanometer (Natsume-KN209, Natsume Co., Japan). A minimum of 3 consecutive blood pressure measurements were obtained and averaged.

2.4. Determination of Pb^{2+} content in blood

Pb^{2+} content of whole blood was measured using an atomic absorption spectrophotometer (Analyst 600 with graphite furnace, PerkinElmer Co., USA). Whole-blood Pb^{2+} values were expressed as micrograms per liter.

2.5. Measurement of serum 5-HT with fluorophotometry

Serum 5-HT was measured with fluorophotometry, following the method of Zhang (Zhang et al., 1994) who made some emendations to Miller's method (Miller et al., 1970). Briefly, 1.8 ml acidified *n*-butanol (adding 0.85 ml 12 M HCl per liter *n*-butanol saturated with NaCl) was added to 0.2 ml serum, vortexed for 5 min, centrifuged at 3000 rpm for 10 min. 1.5 ml supernatant was collected, added with 1.5 ml *n*-heptane and 0.5 ml 0.1 M HCl, vortexed for 5 min, centrifuged at 3000 rpm for 5 min. After the supernatant phase (mainly *n*-heptane) was discarded, 0.25 ml aqueous phase, drawn from the bottom, was mixed with 0.05 ml 82.4 mM (10 g/l) L-cystine and 0.75 ml 60 mg/l *o*-phthaldialdehyde (OPA, prepared with 10 M HCl), and kept in boiling water for 10 min then put into icy water to stop reactions. Fluorescence was measured in a spectrophotofluorometer (1420 Mutilabel HTS, PerkinElmer, USA) using 96-well plate. Excitation and emission wavelengths were 355 and 460 nm, respectively. The standard line was made using serotonin creatinine sulfate complex dissolved in 0.01 M HCl following the same procedure above.

2.6. Preparation and contraction measurement of aorta

The contractile reactivity of the aortic rings was 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 with an equitherm heating circulation system. Each aortic ring was 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, PR China) which was connected to a polygraph (Meiyi Technological Inc., Nanjing, 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. The presence of functional endothelium was tested by the relaxation response to 1 μM ACh after precontracted with 1 μM norepinephrine. Contractile responses of aortic rings with or without endothelium were assessed by adding cumulative 5-HT (10 nM to 10 mM). The endothelium was denuded with 0.5% CHAPS (pH 7.4) for 45 s (Shelkovnikov and Gonick, 2001; Zhang et al., 2005).

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

Aortic 5-HT_{2B} receptor expression was determined with Western blot. After aortae were ground to powder in liquid nitrogen, 4 volumes (4 μl/mg tissue) of ice-cold homogenization buffer (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) were added. The homogenate was vortexed, sonicated briefly on ice, and centrifuged at 14,000 × *g* for 20 min at 4 °C. The supernatant was collected and total protein was measured using the bicinchoninic acid method kit (Beyotime Biotech Co. Ltd., Hangzhou, PR China). 50 μg 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–Cl, pH 7.6, 120 mM NaCl, 0.05% Tween-20) containing 5% nonfat milk powder and probed for 1.5 h or overnight with anti-5-HT_{2B} receptor polyclonal antibody at 1:1000 (Wuhan Bolster Biological Technology Ltd., PR China). Blots were rinsed in TBST for 5 min 3 times and incubated with the horseradish peroxidase conjugated secondary antibody at 1:2000 (Wuhan Bolster Biological Technology Ltd., PR China) for 1 h. After rinsed in TBST for 5 min 5 times and then rinsed twice in TBS (20 mM Tris–Cl, pH 7.6, 140 mM NaCl), Blots were 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 1:1000.

2.8. Histomorphometric changes in aortae of rats long-term exposed with Pb^{2+}

To assess the structural changes in the aortae of rats long-term exposed with Pb^{2+}, after fixed in 10% PBS-buffered formaldehyde, processed and embedded in paraffin, the aortic segments were cut into 4 μm sections with microtome. The sections were stained with hematoxylin and eosin. The internal diameter and media thickness were estimated at magnification 5×. The collagen in the aortae was stained with Mason's trichrome and estimated at magnification 100×. The internal diameter, calculated as the mean value of the major and minor axis, the media thickness, and the percentage of the area of collagen (stained blue) in the transverse section of each aorta were measured with Image-Pro Plus 5.0.2 (Media Cybernetics, Inc., MD, USA).

2.9. Data presentation and analysis

The aortic contractile responses to various concentration of 5-HT were expressed as the percentage of the maximal contraction of each aortic ring to the second exposure of 40 mM KCl. The maximal responses (R_{max}) and the concentration of 5-HT exhibiting 50% of the R_{max} (EC_{50}) were calculated with the curve-fitting tool of Microcal Origin 7.0 (Microcal Software, Inc., MA, USA). Each experiment was repeated 4–10 times. All data are expressed as means ± S.D. Unpaired *t*-tests were performed and a $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Blood pressure and blood Pb^{2+} concentration

The blood pressure of conscious rats was measured with a rat tail sphygmomanometer. Compared with the control group, there was a significant increase in mean blood pressure of rats treated with 100 ppm Pb^{2+} in their drinking water since the end of the second month of Pb^{2+} exposure (control group: 114.3 ± 5.7 mmHg, Pb^{2+} exposed group: 122.1 ± 4.3 mmHg, $p < 0.05$) and this significant increase persisted to the end of this study (at the end of the tenth month, control group: 122.4 ± 3.3 mmHg; Pb^{2+} exposed group: 137.7 ± 4.9 mmHg, analyzed with unpaired *t*-test, $p < 0.05$, Fig. 1).

After 10 months of Pb^{2+} exposure, the mean blood Pb^{2+} concentration of Pb^{2+} treated rats, measured with an atomic absorption spectrophotometer, was 28.4 ± 3.6 μg/l, while the blood Pb^{2+} concentration of control rats was below the detection limit of 1 μg/l. There was no significant difference in the behavior, food intake

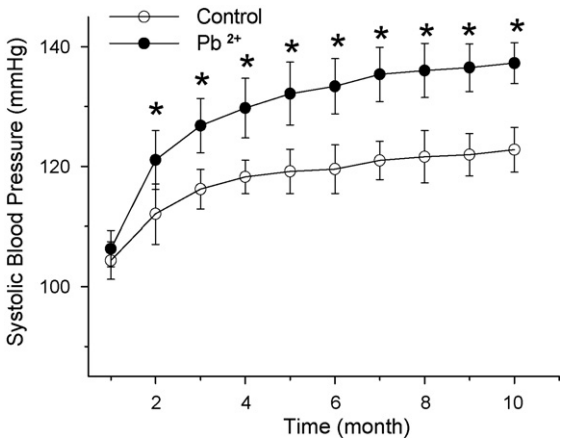

Fig. 1. Changes of the systolic blood pressure of rats fed 100 ppm Pb^{2+} in the drinking water for 10 months. Vertical bars represent means ± S.D. (n = 8–10). Control: control group; Pb^{2+}: Pb^{2+} exposed group. *, statistically significant differences ($p < 0.05$) from control group.

and body weight between the two groups (analyzed with unpaired t-test, $p < 0.05$, data not shown).

3.2. Contractile responses of aortae to 5-HT

The contractile reactivity of the aortic rings to 5-HT was evaluated in the vascular tissue baths. Based on the results of previous studies both by others (Purdy et al., 1997) and us (Zhang et al., 2005), and on the result that there was no significant difference between Pb^{2+} exposed group and control group in the second maximal contraction to 40 mM KCl (1.15 ± 0.12 g and 1.28 ± 0.10 g, unpaired t-test, $p > 0.05$), and to decrease the systematic errors induced by individual variation of aortic rings, length variation of aortic rings cut and different contractile status by only one preload of 2 g (each ring should have its own optimum preload to produce its own maximal contraction), the contractions induced by 5-HT were presented as the percentage of the second maximal response to 40 mM KCl of each aortic ring.

EC_{50}s (the concentration of 5-HT exhibiting 50% of the R_{max}) were calculated with the curve-fitting tool of Microcal Origin 7.0. The aortic rings of Pb^{2+} exposed group showed a slightly higher EC_{50} (not statistically different) and much lower R_{max} (unpaired t-test, $p < 0.05$, Fig. 2(A) and Table 1) to 5-HT. These results demonstrated that the maximal contractile response to 5-HT was decreased in long-term Pb^{2+} induced hypertensive rats, while the sensitivity to 5-HT was similar to that of the control group.

The EC_{50}s to 5-HT of aortic rings denuded of endothelium of control and Pb^{2+} exposed group were both significantly lower than those of the rings with endothelium respectively (unpaired t-test, $p < 0.05$, Fig. 2(B) and Table 1), which meant that denudation of endothelium was able to enhance the sensitivity of the aortic responses to 5-HT.

3.3. Pathomorphological changes of the aortae of long-term Pb^{2+} exposed rats

The transverse sections of aortae were stained with hematoxylin and eosin, or Mason's trichrome. The internal diameter, the media thickness, and the percentage of the area of collagen (stained blue) of each aorta were measured with an image analyzing software (Image-Pro Plus 5.0.2). Compared with the control group, the aortic media thickness of Pb^{2+} exposed rats was increased significantly (unpaired t-test, $p < 0.05$, Table 2 and Fig. 3). The collagen (stained blue) in the media was significantly increased in the Pb^{2+} exposed rats (unpaired t-test, $p < 0.05$, Table 2 and Fig. 3).

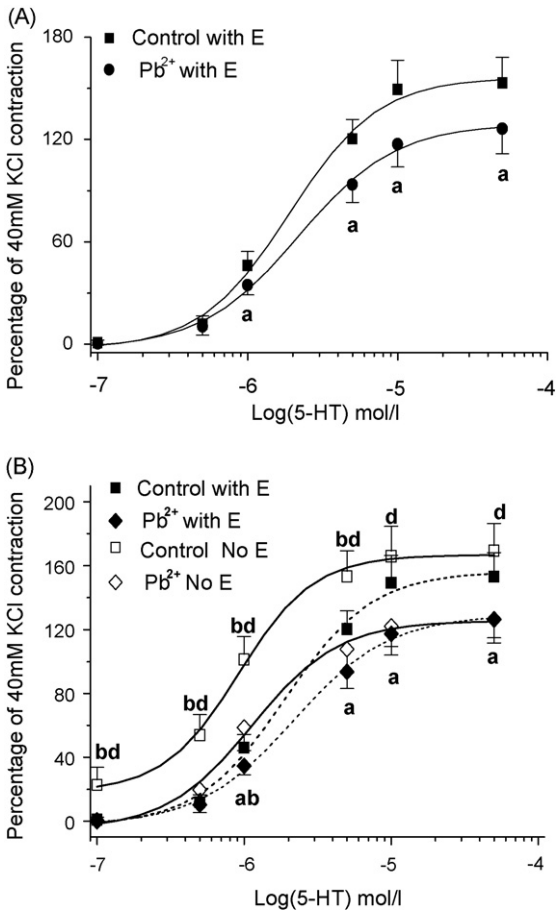

Fig. 2. Contractile responses to 5-HT of aortae with endothelium (A) and denuded of endothelium (together with the responses of rings with endothelium in dot lines, B). Control with E: aortic rings with endothelium from control group; Pb^{2+} with E: aortic rings with endothelium from Pb^{2+} exposed group; Control No E: aortic rings without endothelium from control group; Pb^{2+} No E: aortic rings without endothelium from Pb^{2+} exposed group. Points represent means ± S.D. (n = 6–8). The contractions are expressed as percentages of the second maximal response of 40 mM KCl. a: statistically significant differences ($p < 0.05$) between control with E group and Pb^{2+} with E group; b: statistically significant differences ($p < 0.05$) between control with E group and Control No E group; c: statistically significant differences ($p < 0.05$) between Pb^{2+} with E group and Pb^{2+} No E group; d: statistically significant differences ($p < 0.05$) between Control No E group and Pb^{2+} No E group.

Table 1
Characteristics of the contractile responses induced by 5-HT in isolated aortic rings of control and Pb^{2+} treated rats.

Group	R_{max}*	$-\log EC_{50}$**	EC_{50}**
Control with E	155.91 ± 16.78	5.73 ± 0.10	1.92 ± 0.44 μM
Pb^{2+} with E	129.55 ± 16.81[a]	5.67 ± 0.13	2.27 ± 0.73 μM
Control No E	167.47 ± 17.20	6.05 ± 0.08	0.90 ± 0.12 μM[b]
Pb^{2+} No E	125.61 ± 9.37[d]	5.94 ± 0.09	1.17 ± 0.14 μM[c]

* and **: the R_{max} (the maximal response to 5-HT, % of the second maximal response of 40 mM KCl) and EC_{50} values (the concentration of 5-HT to produce a half R_{max}) were calculated with the curve-fitting tool of Microcal Origin 7.0 (Microcal Software, Inc., Northampton, MA, USA). Each experiment was repeated 6–8 times. Control with E: aortic rings with endothelium from control group; Pb^{2+} with E: aortic rings with endothelium from Pb^{2+} exposed group; Control No E: aortic rings without endothelium from control group; Pb^{2+} No E: aortic rings without endothelium from Pb^{2+} exposed group. a: statistically significant differences ($p < 0.05$) between Control with E group and Pb^{2+} with E group; b: statistically significant differences ($p < 0.05$) between Control with E group and Control No E group; c: statistically significant differences ($p < 0.05$) between Pb^{2+} with E group and Pb^{2+} No E group; d: statistically significant differences ($p < 0.05$) between Control No E group and Pb^{2+} No E group.

Table 2
The media thickness, lumen diameter, media-lumen ratio and the percentage of collagen in the aortic transverse section of the control and Pb^{2+} treated rats.

Group	Media thickness (μm)	Lumen diameter (μm)	Media-lumen ratio	Percentage of the area of collagen (%)
Control	49.8 ± 4.8	1565.5 ± 132.0	0.03 ± 0.001	25.6 ± 2.3
Pb^{2+} exposed	69.8 ± 6.4*	1630.1 ± 147.4	0.04 ± 0.001*	34.7 ± 3.4*

* Statistically significant differences ($p < 0.05$) between control group and Pb^{2+} exposed group ($n = 4$).

3.4. Level of the serum 5-HT of long-term Pb^{2+} exposed rats

At the end of the observation period, the serum 5-HT levels were measured with fluorophotometry. There was no significant difference between control and Pb^{2+} exposed group (control group: 5.56 ± 1.26 μM; Pb^{2+} exposed group: 5.48 ± 2.04 μM, $n = 6$, unpaired t-test, $p > 0.05$).

3.5. 5-HT_{2B} receptor expression in the aortae of long-term Pb^{2+} exposed rats

Aortic 5-HT_{2B} receptor expression was determined with Western blot, which was performed on aortic homogenates from control and long-term Pb^{2+} exposed rats to ascertain whether 5-HT_{2B} receptor 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 were not observed when the primary antibody was removed from the experiment. Immunoreactive bands were photographed with a transilluminator (Vilber Lourmat, Cedex, France). The densitometry of the bands was measured with the BiocaptMW Version 10.02 software for Windows (Vilber Lourmat, Cedex, France) then tested with unpaired t-test. There was no significant difference in the 5-HT_{2B} receptor immunoreactive bands between Pb^{2+} exposed group and control (Fig. 4). These results demonstrated that the expression of 5-HT_{2B} receptor in the aortae of rats exposed Pb^{2+} 10 months was not significantly different from that of the control rats. Therefore, the decreased contractile response induced by 5-HT was not likely induced by the different expression of the 5-HT_{2B} receptor.

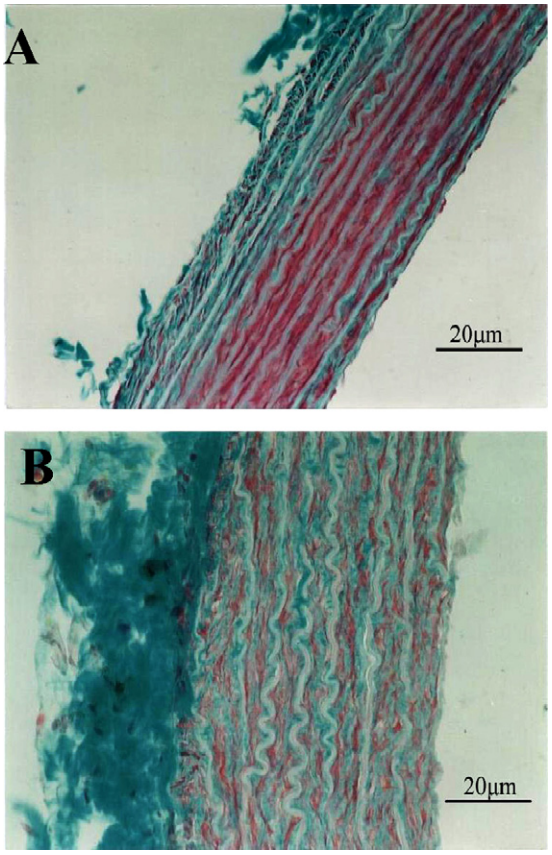

Fig. 3. Morphological changes of the aortae. Transverse section of rat aortae at the arch, Masson staining. A: Control group; B: Pb^{2+} exposed group. In Pb^{2+} exposed group, aortic internal membrane and media thickness and the collagen content increased significantly.

4. Discussion

In this study, we found that the blood pressure of the Pb^{2+} exposed rats was significantly elevated after 2 months of 100 ppm Pb^{2+} exposure from drinking water. After 10 months of Pb^{2+} exposure, compared with the control group, the aortic reaction of Pb^{2+} exposed rats to 5-HT was markedly decreased, and the media thickness and media-lumen ratio of Pb^{2+} exposed rats were significantly increased. The results of this study support the theory that Pb^{2+} exposure can induce hypertension or increase blood pressure. To our best knowledge, till now, in Pb^{2+} induced hypertensive animals, there is no paper reporting decreased vascular response to vasoconstrictors or significant abnormal morphologic changes in vessels.

In our previous study (Zhang et al., 2005), we found that the aortic contractile response to 5-HT was significantly increased in the aorta cultured in vitro with 1 ppm Pb^{2+} over 24 h with different mechanisms from those reported in both DOCA-salt hypertensive rats and N_ω-L-arginine hypertensive rats (Banes and Watts, 2002; Russell et al., 2002). In this study, we conceived that rats with hypertension induced by long-term Pb^{2+} exposure would also demonstrate an increased aortic contractile response to 5-HT, just like in many other hypertensive animal models. However, the results of this study demonstrated that the aortic contractile response to 5-HT was significantly decreased in long-term Pb^{2+} induced hypertensive rats. This result was quite out of our original conception, because, till now, only the portal-hypertensive animals, among all the hypertensive animal models, demonstrated decreased contractile response to vasoconstrictors, for example, decreased vessel responses to α_1-adrenoceptor agonists (Kiel et al., 1985) and angiotensin II (Murray and Paller, 1985).

The decreased contractile response to 5-HT could be induced by any abnormity/abnormities in the cascade of the vascular contrac-

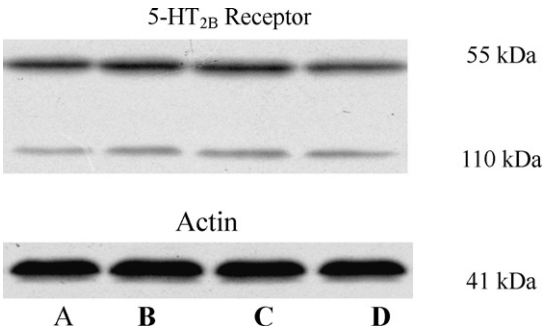

Fig. 4. The representative of 4 separate Western blot experiments. A and B: aortae from the control group; C and D: aortae from the Pb^{2+} exposed group. There was no significant difference between the control group and the Pb^{2+} exposed group.

tile response to 5-HT. In this study, based on the results of similar studies or our hypothesis, we tested the following factors.

The first one was the serum level of 5-HT. If Pb^{2+} exposure could significantly affect the serum concentration of 5-HT, the abnormal 5-HT levels would likely induce abnormal aortic contractile response to 5-HT. However, our results showed that the serum 5-HT level of the long-term Pb^{2+} exposed rats was at the similar level with their controls.

The second factor we examined was the expression of the receptor of 5-HT. If the expression of the receptor of 5-HT were decreased, the contractile response induced by 5-HT would also decrease.

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 (Banes et al., 1999), 5-HT_{2B} receptors (Thompson and Webb, 1987; Roson et al., 1990; Watts et al., 1995, 1996, 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 upregulated in both DOCA-salt hypertensive rats and N_ω-L-arginine hypertensive rats (Banes and Watts, 2002; Russell et al., 2002).

Based on the above studies, only 5-HT_{2B} receptor was reported to take part in the abnormal contractile response to 5-HT. There was also a possibility that the decreased aortic contraction of Pb^{2+} exposed rats to 5-HT was mediated by downregulated 5-HT_{2B} receptor expression. However, our results of Western blot did not support this hypothesis. There was no significant difference between the Pb^{2+} exposed group and their controls in the 5-HT_{2B} receptor expression.

After a more detailed study of the curves of the contractile response to 5-HT of these two groups, we found the EC_{50}s of the contractile curves of both groups were not significantly different, which meant that the aortic sensitivity 5-HT of the Pb^{2+} exposed group to was similar to that of the control group. The difference of the curves of the contractile responses of these two groups was mainly in the amplitudes. This result accorded with the result of 5-HT_{2B} receptor expression, because if the expression of 5-HT_{2B} receptor was abnormal in the aortae of Pb^{2+} exposed group, the sensitivity of the aortic contractile responses to 5-HT of Pb^{2+} exposed group would also be different from those of the controls.

At last, we examined whether there were any abnormal morphological changes in the aortae of the lead exposed rats, since abnormal structure also could affect the response of vessel. We found that the aortic media thickness and the collagen in the media were significantly increased in the Pb^{2+} exposed rats (Fig. 3). Since the increased deposition of collagen and other proteins of the extracellular matrix can change the visco-dynamic properties of the vessel wall in hypertension (Assoian and Marcantonio, 1997), the contractile responses of vessels with such morphological changes can be decreased, compared with the normal vessels. This hypothesis was further supported by the contractile responses of the aortae of Pb^{2+} exposed rats to NE which were also significantly decreased (Fig. 5).

However, this hypothesis cannot explain why the contractions induced by 40 mM KCl were at a similar level in these two groups. Moreover, the results got from the in vitro studies by us (Zhang et al., 2005) and by others (Courtois et al., 2003), such as increased production of superoxide anion, and the increased level of cAMP and/or increased basal vasoconstrictor level by superoxide anion, support an increased aortic contractile response to 5-HT. Therefore, further investigation is needed to study the effects of long-term Pb^{2+} exposure to these factors.

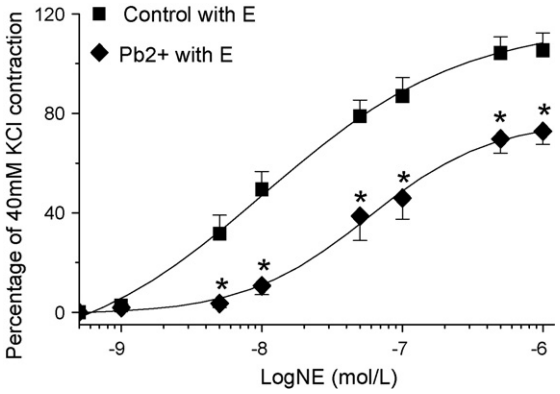

Fig. 5. Effects of lead on the NE induced contractile response of aortae of Pb^{2+} exposed rats and controls. Control with E: aortic rings with endothelium from control group rats; lead with E: aortic rings with endothelium from lead exposed group rats. Points represent means ± S.D. ($n = 6$). The contractions are expressed as percentages of the second 40 mM KCl contraction. *, statistically significant differences ($p < 0.05$) from control group.

In summary, these results demonstrated lead exposure was able to induce hypertension or increase blood pressure. After long-term hypertension induced by Pb^{2+} exposure, the structure of the aorta was altered apparently and the aortic contractile response to 5-HT was significantly decreased. The decreased aortic contractile response to 5-HT of the Pb^{2+} exposed rats could have some relation to the abnormal visco-dynamic properties of the aortic wall induced by significantly increased collagen, but had little relation to the serum level of 5-HT and the expression of 5-HT_{2B} receptor.

Conflict of interest

There is no competing interests.

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