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Full Length Research Paper

Vascular Effect of Lead on Rabbit Aortic Smooth Muscle

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ABSTRACT

Several reports have demonstrated a positive link between lead exposure and hypertension. It has also been suggested that alterations in vascular reactivity is one of several mechanisms by which lead induces hypertension. There are conflicting reports concerning the effect of lead on vascular reactivity. Some authors have reported an increase or decrease in vascular reactivity to phenylephrine after lead exposure. The goal of the present study was to investigate and characterize, using pharmacological methods the in-vitro vascular effects of lead exposure on isolated rabbit aortic smooth muscle. Rabbit aortic rings were isolated and mounted between two L shaped stainless steel holders in a 20ml organ bath containing PSS. Isometric contractions were recorded on a grass model 79D four channel polygraph. Dose response to Phenylephrine (PE) was examined both in the absence (control, n=8) and following 20mins exposure to 10^{-4}M lead acetate (n=8) in normal PSS. Acetylcholine (Ach) relaxation following 10^{-7}M PE pre-contraction was also examined both in the absence (control, n=7) and following 20mins exposure to lead acetate (10^{-4}M, n=7). The results showed that lead acetate significantly increased vascular reactivity to PE in rabbit aortic rings ($P<0.05$). The results also showed that lead acetate significantly decreased the relaxation response induced by Ach after PE pre-contraction ($P<0.05$). The depressed relaxation response to acetylcholine following lead exposure suggests impairment of endothelium-derived relaxant factor (EDRF). The present study reinforces the concept that the association between lead poisoning and hypertension is related, at least in part, to enhanced vasocontractile response to phenylephrine as well as attenuated endothelium-dependent relaxation.

Keywords; Lead acetate, phenylephrine, acetylcholine, aorta

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INTRODUCTION

Lead is a common environmental toxin that causes numerous acute and chronic illnesses (Vaziri *et al*., 2008) and is a major environmental pollutant with, great risk to public health (Menke *et al*., 2006). Lead is one of the most extensively used metals in the industrial sector of several countries. Occupational and environmental exposure to lead occurs during the manufacturing of a wide range of products, including: batteries, plastics, sheet lead, solder, ceramic glazes (Healy, 2009) and via drinking water from rivers located around manufacturing industries and mines (which utilize lead in their manufacturing process).

Several studies have linked occupational and/or environmental exposure to lead with increased arterial blood pressure (Schwartz, 1995; Vaziri *et al*., 1999; Navas *et al*., 2007). Vaziri *et al*. (1999) reported that treatment with 100ppm of lead via drinking water for 12 weeks induces hypertension in Sprague Dawley rats.

The precise mechanism by which lead induces hypertension is not clear. Several mechanisms have been suggested, including: alterations in vascular reactivity (Aviv *et al*., 1980); However, there are conflicting reports concerning the effects of lead exposure on contractile responses by vasoactive agents. Several authors have reported an increase in vascular reactivity to phenylephrine (PE) following treatment with lead (Karimi *et al*., 2002; Heydari *et al*., 2006; Silveira *et al*., 2010) whereas some others have reported a decrease in vascular reactivity (Zhang *et al*., 2009 and Fiorim *et al*., 2010). Chronic treatment with 100ppm of lead in drinking water increases vascular reactivity to PE (Karimi *et al*, 2002; Heydari *et al*, 2006; Silveira *et al*, 2010). In contrast to the above reports, Fiorim *et al*. (2010) and Zhang *et al*. (2009) all reported a decrease in vascular reactivity to PE and 5-HT respectively in isolated rat aorta treated with lead

acetate. In view of the conflicting reports concerning the effects of lead exposure on contractile responses induced by vasoactive agents, the goal of the present study was to further examine the effects of lead exposure on isolated rabbit aortic smooth muscle.

MATERIALS AND METHODS

Tissue Preparation: Segments of the aorta were obtained from New Zealand rabbits, sacrificed by stunning and bleeding. The tissues were placed in Physiological salt solution (PSS), carefully dissected free of all adhering connective tissues and cut into 2-3mm rings. The rings were suspended between 2 L-shaped wire loops in 20ml organ baths containing PSS. The upper loop was attached to a Grass Model FT03 force transducer connected to a Grass Model 7P polygraph (Grass Instruments Co., Quincy, MA, USA) while the lower loop was fixed to the base of the organ bath. The composition of the PSS was (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 24.9, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.6, glucose 11.5. The PSS was bubbled throughout with 95% O_2 - 5% CO_2 gas mixture with the pH and temperature maintained at 7.4 and 37°C respectively. High-K^+ PSS was prepared by equimolar replacement of K^+ with Na^+. Aortic rings were given a resting tension of 1g. An equilibration period of 90 minutes was allowed.

Experimental Protocols: At the start of all experiments, the functional integrity of the endothelium was assessed by examining the relaxation response induced by 10^{-5}M of acetylcholine following pre-contraction induced by 1 x 10^{-7}M PE in normal PSS. Only aortic rings that elicited 60% relaxation responses (or more) to response to Ach were considered to have functional endothelium (Silveira *et al*., 2009).

Response to high-K^+ PSS: Following equilibration, aortic rings were made to contract twice with 80mM K^+, at 20-minute interval. The average of these contractions represented the maximum (100%) against which subsequent contractions were evaluated (Ebeigbe and Cabanie, 1992).

Effect of Lead acetate on baseline tension: Lead acetate (10^{-7} – 10^{-3}M) was added to the bath cumulatively, to examine the possible contractile effect of the compound.

Dose-Response to Phenylephrine
Phenylephrine was added to the bath cumulatively; a higher concentration was added when the response to the previous concentration has stabilized. The contractions were matched against the reference (100%) contraction induced by high-K^+ (80mM) depolarizing solution The effect of lead exposure on the PE dose-response was examined by exposure of the aortic rings for 20 minutes to 10^{-4}M lead acetate.

Role of the endothelium: The relaxation responses to Ach in control and lead-treated rings were examined following pre-contraction with EC_{70} (10^{-7}M) concentration of phenylephrine in endothelium-intact as well as endothelium-denuded rings. Endothelium removal was effected by gently rubbing the internal surface of the rings with a roughened glass rod (Ebeigbe and Cabanie, 1992). The effectiveness of the denudation process was confirmed by the failure of 10^{-7}M Ach to elicit relaxation in endothelium-denuded rings.

Analysis of data: Data are presented as means ± SEM. Statistical analysis was by means of MicroCal Origin software and Student's *t*-test. A *p* value less than 0.05 was considered statistically significant, while *n* values denote number of animals from which vessels were obtained. Tests were carried out on at least six vessel preparations. EC_{50} and EC_{70} (concentrations producing 50 and 70% of maximal contraction) values were derived graphically.

RESULTS

Responses to high-K^+ PSS and Phenylephrine: The maximum contraction induced by 80mM K^+ was 1967.50 ± 43.4 mg (n=10). Phenylephrine elicited concentration-dependent contractile responses in all experiments (n=10). The maximum contraction induced by PE was 1832±37.6mg while the EC_{70} (M) of PE contraction is 1.02 x 10^{-7}M.

Effect of Lead acetate on baseline tension: In unstimulated rabbit, aortic rings, cumulative increases in the concentration of lead acetate did not produce any observable response in all experiments (n = 10).

Effect of Lead acetate on dose-response to PE*::* In other to investigate the acute effect of lead acetate on the vascular reactivity to PE, phenylephrine dose dependent contractile response was observed in the absence (control, N=8) and presence of lead acetate (Pb^{2+}, N=8) in endothelium intact rings. Cumulative increases in the concentration of phenylephrine resulted in graded contractions (Fig. 1).

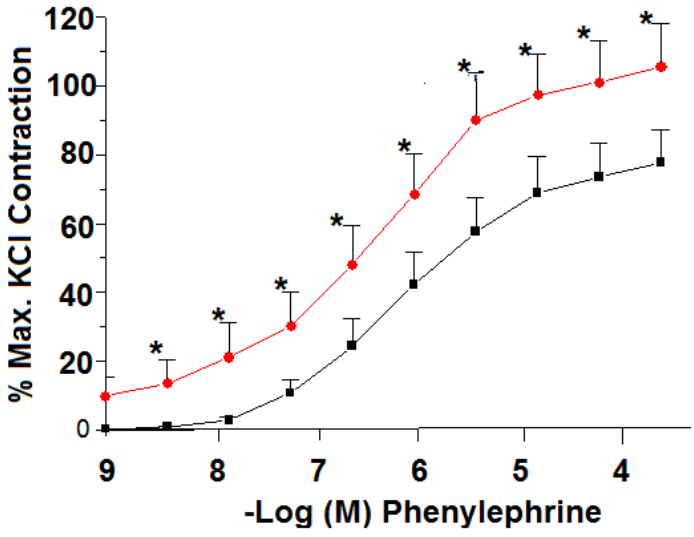

Fig. 1.
Effect of 10^{-4}M lead acetate on concentration-dependent contractions induced by acetylcholine in rabbit aortic rings (n = 7). Values are Means ± SEM for control (square) and lead-treated (circle) rings. Asterisks denote significant difference ($p<0.01$).

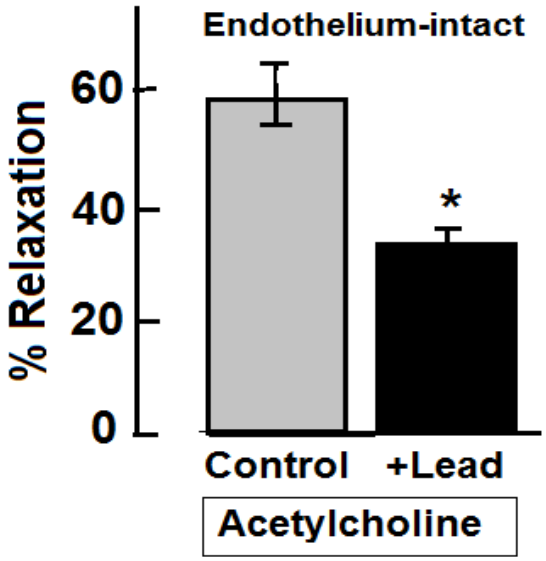

Fig. 2.
Summary of data showing the relaxation-response induced by 10^{-5}M Acetylcholine following 10^{-7}M phenylephrine pre-contraction, in control (n=7; □) and 10^{-4}M lead-treated (n=7; ■) endothelium-intact rabbit aortic rings. Values are Means ± SEM; $p<0.05$.

There was a left-ward shift of the phenylephrine dose-response curve in aortic rings exposed to lead acetate (n = 8), in comparison with controls (n = 8). The respective EC_{50} values for phenylephrine contractions in the absence and presence of 10^{-4}M lea acetate are: 1.8 x 10^{-6} and 2.3 x 10^{-7}M. The maximal contractile response induced by PE in the absence or presence of Pb^{2+} was 77.67± 9.70% (N=8) and105.42±12.68 (N=8), respectively ($P < 0.05$).

Role of the endothelium:
In all experiments (n=7) Ach did not elicit relaxation in endothelium-denuded rings. A concentration-dependent acetylcholine-induced relaxation was observed in endothelium intact rings (control, n=7). Table 1 shows the relaxation response induced by acetylcholine in the absence and presence of lead acetate. The respective mean magnitudes of relaxation responses induced by acetylcholine (Fig. 2) in control rings (n=7) and in rings exposed for 20mins to 10^{-4}M lead acetate (pb^{2+}, n=7) were: 58.50±4.69 and 33.14 ± 2.46%; the difference was statistically significant ($p<0.05$).

DISCUSSION

One of the major finding of the present study was that acute exposure to 10^{-4}M lead acetate increases the reactivity of rabbit aortic rings to phenylephrine. This observation is in-line with the findings of several other investigators (Silveria *et al*., 2010; Karimi *et al*., 2002, Heydari *et al*., 2006). The increase in vascular reactivity has been suggested to be endotheliu-dependent since the effects were abolished in the absence of endothelium (Silveria *et al*., 2010). Silveira *et al*. (2010) reported that acute exposure to 100µM of lead acetate increases the reactivity of the rat tail artery to phenylephrine; however, our findings are in contrast with the findings of some other researchers (Zhang *et al*., 2009; Fiorim *et al*., 2011). Fiorim *et al*. (2011) reported a decrease in the reactivity to phenylephrine in aortic rings after administering lead acetate to wistar rats for seven days via drinking water. In their study, this decrease was suggested to be due, probably, to enhancement of endothelial function. The various discrepancies in results as regard the actions of lead on vascular reactivity induce by various vasoactive agents have been suggested by Vaziri *et al*. (2008) to be due to the extent and the duration of exposure, the nature of the blood vessels and perhaps the animal species.

The present study also shows that lead acetate significantly decreases relaxation responses induced by acetylcholine in endothelium intact rings (Figure 2). Our findings are in line with those of Marques *et al*., (2001), who reported a significant reduction of vasodilatory response to acetylcholine in wistar rats fed with lead acetate (5ppm in drinking water) for a duration of four weeks. Our findings are also comparable to the findings of Oishi *et al*. (1996), who reported a significant reduction of endothelium dependent vaso-relaxation of the mesenteric artery to acetylcholine and suggested that lead acetate may impair endothelium-dependent hyperpolarization in the tissue.

The data presented in this study are however, not in line with those of Fiorim *et al*., (2011) and Grizzo *et al*., (2008). Fiorim *et al*. (2011) reported that relaxation induced by acetylcholine and sodium nitroprusside were not altered after seven days' exposure to lead acetate.
Acetylcholine induces relaxation of the vascular smooth muscle by stimulating nitric oxide (NO) release from the vascular endothelium. The depressed relaxation response induced by acetylcholine following lead exposure as observed in our study suggests impairment of endothelial function. It has also been reported by Mital *et al*. (1995) and Vaziri *et al*. (1997) that chronic lead exposure reduces the release or production of NO; however, further studies involving the use of NO synthesis inhibitors and endothelium-denuded rings will further characterize the nature of the endothelial factors.

The results from present study further reinforces the concept that the association between lead poisoning and cardiovascular disorder is related, at least in part, to enhanced vasocontractile response to phenylephrine as well as attenuated endothelium-dependent relaxation.

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