Influence of Lead on Rat Thoracic Aorta Contraction and Relaxation

Stanislav A. Shelkovnikov and Harvey C. Gonick

Low levels of lead, but not high levels, produce hypertension. This mystery has not yet been resolved. In this study we compared the in vitro vasoresponsiveness in rat thoracic aorta to low dose (10^{-8} mol/L) and high dose (10^{-5} mol/L and 10^{-4} mol/L) lead acetate. In addition to the direct response to lead, we examined reactivity to norepinephrine, acetylcholine, isoproterenol, phorbol ester, and calcium in the presence and absence of lead. Neither low-dose nor high-dose lead directly affected aortic contractile or relaxant responses. However, lead, only at the highest concentration (10^{-4} mol/L), increased the contractions to calcium at all submaximal calcium concentrations. We conclude that low-dose lead must increase blood pressure indirectly through a humoral effect. The reasons for the failure of high-dose lead to influence blood pressure remain to be explored. Am J Hypertens 2001;14:873–878 © 2001 American Journal of Hypertension, Ltd.

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It has been found that chronic exposure to low, but not high, levels of lead consistently produces hypertension in humans and animals.1–11 Several mechanisms have been proposed to explain lead-induced hypertension: 1) chronic long-term effect, including decreased formation or decreased bioavailability of the relaxing factor EDRF;10,11 and an alteration of blood pressure regulation via increased release of vasoactive factors such as the Na-K-ATPase-inhibiting natriuretic factor, endothelin-3, and reactive oxygen species;6–9,11,12 and 2) acute short-term effects on blood pressure regulation, such as a direct effect of lead on blood vessel contraction.2,13–16 However, none of these explanations accounts for the absence of hypertension after high lead exposure. In addition, the data concerning a direct effect of lead on blood vessel contraction are contradictory. Indeed, the study by Purdy et al.9 on the mechanism of chronic low level lead toxicity revealed no evidence of altered vasoconstrictive or vasodilatory responses in isolated vessels, but the possibility remains that lead was washed away during the preparation of the vessels. Thus, the present study was designed to explore the acute effects of both high (10^{-5} mol/L and 10^{-4} mol/L) and low (10^{-8} mol/L) doses of lead on contraction and relaxation in isolated thoracic aorta.

Methods

Experiments were performed on Sprague Dawley rats of both sexes and 250 to 350 g body weight. Animals were anesthetized by isoflurane and killed by decapitation. Thoracic aorta were carefully excised and placed into Krebs solution. After separation of connective tissues, transverse rings 2 mm in width were cut with parallel razor blades. The contractile activity of the arteries was evaluated both with and without endothelium (rubbed or treated with the detergent, CHAPS 0.5% for 45 sec at a constant pH of 7.4). The vascular tissue baths contained 10 mL of Krebs bicarbonate, gased with 95% O2 and 5% CO2, of the following composition, in mmol/L: NaCl 118; KCl 4.8; CaCl2 2.5; MgSO4 1.2; KH2PO4 1.2; NaHCO3 24; D-glucose 10. The Krebs solution was warmed to 37°C by an equitherm heating circulation system.

The aortic ring was mounted between two stainless steel hooks in the organ bath. One hook was attached to the bottom of the bath. The other hook was connected to an isometric transducer Fort 10. The preload was approximately 1 g. At the start of experiments on blood vessels, the tension was gradually increased so as to obtain maximum contractile force in response to KCl (80 mmol/L). Isometric contractions were recorded electronically using Fort 10 strain gauges connected to an amplifier transbridge TBM 4 (World Precision Instruments, Sarasota, FL) and a Barnstead-Thermolyne linear recorder model 1201 (Dubuque, IA).

The contractile response to lead acetate was examined over a range of 10^{-10} to 10^{-4} mol/L, washing out between each concentration of lead. Norepinephrine concentration...
response curves were obtained by the cumulative addition of norepinephrine. Cumulative vasodilatory responses for acetylcholine and isoproterenol were obtained after the maximal effect of norepinephrine at a concentration of 1 μmol/L. The substances were added to the bath in a volume ≤ 0.1 mL. The interval between complete relaxation and the next contraction was 20 min. Agonist EC50 values were obtained from full concentration-effect curves. Lead acetate in concentrations of 10⁻⁸, 10⁻⁵, and 10⁻⁴ mol/L was introduced into Krebs solution 20 min before the addition of norepinephrine or KCl, and this dosage was maintained continuously throughout the addition of further increments of norepinephrine. After washout, each experiment was replicated. The data were based on five experiments. The contractions evoked by calcium were studied in Ca-free Krebs solution after preliminary depolarization of smooth muscle by KCl (50 mmol/L).

Results
Effect of Lead on Norepinephrine Contraction
There was no direct effect of lead on contraction in doses ranging from 10⁻¹⁰ and 10⁻⁴ mol/L. The dose-response curves of aorta rings to norepinephrine before and in presence of lead in concentrations of 10⁻⁸ and 10⁻⁴ mol/L are shown in Fig. 1. Although these results pertain to de-endothelized blood vessels, the same was noted in blood vessels with the endothelium intact. The results with 10⁻⁵ mol/L lead acetate are shown in Table 1. In both experiments with endothelium and without endothelium, lead did not change the contraction evoked by norepinephrine (Table 1). The mean contractions to 1 μmol/L norepinephrine were 76% ± 0.2 in control and 74% ± 0.2 in the presence of lead at 10⁻⁸ mol/L and 73% ± 0.2 in the presence of lead at 10⁻⁵ mol/L. Drugs used in the study were acetylcholine iodide, norepinephrine bitartrate, isoproterenol, and phorbol 12-myristate 13-acetate (Sigma Chemical Company, St Louis, MO), lead acetate (Fisher ChemAlert Guide, Fair Lawn, NJ), and isoflurane (Abbott Laboratories, North Chicago, IL).

Table 1. pD₂ (−log EC₅₀, mol/L) values for the actions of agonists norepinephrine, acetylcholine, isoproterenol, and calcium before and in presence of lead on rat thoracic aorta rings

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Control</th>
<th>Lead, 10⁻⁸ mol/L</th>
<th>Lead, 10⁻⁵ mol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine, with endothelium denuded</td>
<td>7.5 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>Acetylcholine, with endothelium</td>
<td>7.3 ± 0.2</td>
<td>7.3 ± 0.2</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td>Isoproterenol, denuded</td>
<td>7.5 ± 0.2</td>
<td>7.5 ± 0.2</td>
<td>7.5 ± 0.2</td>
</tr>
<tr>
<td>Calcium, denuded</td>
<td>4.4 ± 0.2</td>
<td>4.4 ± 0.2</td>
<td>4.5 ± 0.2</td>
</tr>
</tbody>
</table>

Data are means ± SEM of five experiments on five different rats.
epinephrine in all tissues were $1.3 \pm 0.15$ g in control rings and $1.2 \pm 0.2$ g in the presence of $10^{-4}$ mol/L lead acetate.

**Effect of Lead on Vasodilation**

The dose-response curves to acetylcholine in aorta rings with endothelium precontracted with norepinephrine in a concentration $1 \mu$mol/L in control and in the presence of lead are shown in Fig. 2. The acetylcholine dose-response curves, $EC_{50}$ and maximal relaxation in aorta rings in control and in the presence of lead completely overlap (Fig. 2, Table 1). The relaxation dose-response curves to isoproterenol in aorta rings without endothelium, precontracted with norepinephrine in a concentration of $1 \mu$mol/L, in the presence and absence of lead are shown in Fig. 3. $EC_{50}$ and maximal relaxation evoked by isoproterenol in control rings and in the presence of lead are the same (Fig. 3, Table 1).

**FIG. 2.** Cumulative concentration-response curves for the relaxing activity of acetylcholine on rat thoracic aorta ring with endothelium in the absence (open squares) or presence of lead at concentrations of $10^{-8}$ mol/L (open circles) and $10^{-4}$ mol/L (open triangles), precontracted with $10^{-6}$ mol/L norepinephrine. Abscissa shows negative logarithm of the concentration of acetylcholine. Ordinate shows percent of maximum relaxation. Individual points represent means ± SEM. The three curves are superimposed.

**FIG. 3.** Cumulative concentration-response curves for the relaxing activity of isoproterenol on rat thoracic aorta ring without endothelium in the absence (open squares) or presence of lead at concentrations of $10^{-8}$ mol/L (open circles) and $10^{-4}$ mol/L (open triangles), precontracted with $10^{-6}$ mol/L norepinephrine. Abscissa shows negative logarithm of the concentration of isoproterenol. Ordinate shows percent of maximum relaxation. Individual points represent means ± SEM. The three curves are superimposed.
Response to Calcium

The dose-response curves to Ca\(^{2+}\) in calcium-free Krebs solution after smooth muscle depolarization by 50 mmol/L KCl in control rings and in the presence of lead are shown in Fig. 4. Potassium caused a 10% to 20% contraction in the absence of calcium. In high concentration (10\(^{-4}\) mol/L), but not in lower concentrations, ie, \(\leq10^{-5}\) mol/L, lead shifted the dose-response curve upwards from the KCl baseline at low concentrations of calcium (from 10\(^{-4}\) to 10\(^{-3}\) mol/L). The maximal contraction evoked by Ca\(^{2+}\) in the presence of lead did not change.

Response to Phorbol Ester

Lead did not change the blood vessel contractions evoked by phorbol ester (phorbol-12-myristate-13-acetate) in vessels without endothelium. The contractions evoked by 1 \(\mu\)mol/L of phorbol-12-myristate-13-acetate in calcium-free Krebs solution after blocking calcium channels by verapamil (1 \(\mu\)mol/L) in control rings and in the presence of lead in concentrations of 10\(^{-8}\) and 10\(^{-4}\) mol/L were the same. Phorbol-12-myristate-13-acetate evoked contractions that were 70% ± 10% (SE) of the maximal produced by KCl (80 mmol/L). In the presence of lead at a concentration of 10\(^{-4}\) mol/L, phorbol ester evoked 72% ± 12% of the maximal effect produced by 80 mmol/L KCl.

Thus, lead in low and high concentrations did not change the contraction evoked by norepinephrine both in blood vessels with and without endothelium, or calcium and phorbol ester. Also, lead did not influence the blood vessel relaxation evoked by acetylcholine (with endothelium) and by isoproterenol (without endothelium) after precontraction by norepinephrine (1 \(\mu\)mol/L). The only influence of lead that could be documented was that the highest concentration of lead (10\(^{-4}\) mol/L) increased the blood vessel submaximal contractions evoked by calcium.

Discussion

Lead can directly influence blood vessel contraction via five pathways. First is the phospholipid messenger pathway of smooth muscle and contraction. This way is activated by norepinephrine.\(^{17,18}\) Because lead does not change the thoracic aorta contraction evoked by norepinephrine, it is unlikely that lead influences this pathway. Second is release of nitric oxide by endothelium and, consequent, alteration of cGMP levels by smooth muscle cells, opening of K\(^+\)-channels, and relaxation.\(^{19–21}\) This pathway is activated via the muscarinic receptor of endothelium by acetylcholine. Lead does not influence this pathway because it does not change the effect of acetylcholine. Third is the cAMP second messenger pathway and relaxation. This way is activated through \(\beta\)-adrenoreceptors by isoproterenol.\(^{22,23}\) It is unlikely that lead influences the cAMP level in smooth muscle because it does not change the relaxation effect of isoproterenol. Fourth is the influence on calcium and potassium channels.\(^{24,25}\) Only at the very high concentrations (10\(^{-4}\) mol/L) does lead increase the contraction evoked by a low concentration of calcium, indicating that lead has no influence on calcium channels at physiologic levels. Activation of potassium channels evokes relaxation, but blocking of potassium channels evokes contraction. Acetylcholine and isoproterenol open potassium channels of smooth muscle.\(^{26,27}\) Lead does not influence potassium channels because it does not change the relaxation effects of isopro-
terenol and acetylcholine. Fifth is the direct effect on contractile proteins. Lead does not influence contractile proteins. Lead does not influence contractile proteins because it does not change the contractile effect of norepinephrine. Phorbol ester evokes Ca-independent blood vessel contraction via activation of protein kinase C and consequently through calponin and contractile protein. Lead does not influence the effect of phorbol ester and thus does not influence Ca-independent contraction.

In a previous article concerning the in vitro effect of lead, Favalli et al demonstrated that $10^{-3}$ mol/L lead increased the contractions produced by calcium in potassium-depolarized rat tail artery. They also found that high lead concentrations caused an increase in intracellular calcium. Webb et al, in contrast to Purdy et al, found that feeding 100 ppm lead acetate to rats led to an enhanced maximal contractile response in the tail artery. In addition, Chai and Webb as well as Watts et al are more difficult to reconcile with the present results except to say that resistance blood vessels (ie, mesenteric arteries) were employed, which may have a different degree of responsiveness from that of the thoracic aorta, a conduit vessel. Irrespective of the acute effects of lead on blood vessel contractility in vitro, it is apparent from the lag period (1 to 2 months) in the development of hypertension in animals fed 100 ppm lead acetate that one or more vasoactive compounds must be produced.

Thus, lead has no direct effect on vasoconstriction either in vivo or in vitro. Short-term incubation of aorta rings with either high or low concentrations of lead failed to modify the response to different vasodilator or vasoconstrictor agonists. What, then, accounts for the observation of the demonstration by Long et al that lead in subnanomolar concentrations activates protein kinase C. Other findings are more difficult to reconcile with the present results except to say that resistance blood vessels (ie, mesenteric arteries) were employed, which may have a different degree of responsiveness from that of the thoracic aorta, a conduit vessel.

References