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Increase in the Blood Pressure of Rats Chronically Fed Low Levels of Lead

by H. Mitchell Perry, Jr.,* Margaret W. Erlanger,* and Elizabeth F. Perry*

Groups of 15 to 18 female weanling Long-Evans rats fed a rye-based diet low in lead (0.25 ppm) were exposed to 0.1, 1.0, and 5.0 ppm lead in drinking water. No suggestion of clinical lead toxicity was recognized. Systolic pressures were measured at 3-month intervals after weaning. The groups of lead-exposed animals had consistently and significantly higher average pressures than control animals, the increase approximating 15 mm Hg. With the lowest lead exposure (0.1 ppm), the increase in average pressure was gradual, being half minimal at 3 months and requiring 1 year to become maximal. After 1 year, half of these rats had pressures from 0 to 10 mm Hg above the control average; 40, 20, and 10% had pressures that were 20, 30, and 40 mm Hg, respectively, above the control average. Thus, rats exposed to lead in amounts comparable to the environmental exposure of many Americans had an average elevation in systolic pressure comparable to that of human beings with essential hypertension.

Introduction

Lead has long been blamed for a wide range of toxic manifestations (*1*). As early as 1886, high blood lead was associated with increased risk of high blood pressure (*1*). Until recently, the reports of lead intoxication have involved heavy exposure, which was usually occupational in origin. Accurate data on cumulative dose and body burden of lead are difficult to obtain, but blood lead levels associated with the most serious chronic toxic manifestations, nephropathy and encephalopathy, generally range from 100 to 120 μg/dL (*2,3*) and certainly reflect heavy exposure. When hypertension has been associated with lead exposure, nephropathy has usually been present, making it likely that hypertension is of renal origin.

In addition to renal hypertension induced by heavy lead exposure, it has now been suggested that mild to moderate hypertension may be associated with long-term, perhaps lifelong, exposure of a much larger segment of the population to much lower levels of environmental lead. Such exposure could result in widespread but asymptomatic lead toxicity manifested by the complications of hypertension, particularly strokes and heart attacks, during middle and later life. Two independent lines of evidence suggest an association between low-level lead exposure and some essential hypertension.

First, long-term exposure of rats to small amounts of lead in drinking water has induced increased blood pressure that resembles human essential hypertension in that there are no obvious associated manifestations, toxic or otherwise (*4–6*). The second line of evidence involves human epidemiologic data, particularly the recently reported relationships between blood lead and blood pressure for blood lead levels ranging from 6 to 30 μg/dL (*7*), well below the levels that have previously been considered toxic. An increase in strokes, myocardial infarction, and total mortality has been reported for blood lead levels in this range (*8*).

The postulated low-dose lead toxicity manifested by essential hypertension would be a different phenomenon with a different pathogenesis from the previously described and more familiar high-dose occupational toxicity manifested by renal hypertension. This report deals only with the increase in systolic pressure of rats exposed to low levels of lead. Systolic pressure is the parameter of choice to follow because, unlike diastolic pressure, it can be repeatedly measured without injuring the animal. The lead-induced increase in pressure is reproducible, statistically significant, and similar to human essential hypertension in generally being mild to moderate in degree although some animals have larger increases in pressure.

The data presented here are from two different experiments. In both, groups of rats had increases in

*Hypertension Division, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110, and Hypertension Section, VA Medical Center, St. Louis, MO.

their average systolic pressure of about 15 mm Hg following exposure to low levels of lead under our standard, carefully controlled, and low contamination conditions. The lead exposure ranged from 0.1 to 5.0 ppm dissolved in drinking water. The methodologic differences between the experiments involved anesthesia (experiment 1) or lack of it (experiment 2) during measurement of the blood pressure. The results of the first year of experiment 1 have already been reported (*2*) and so are presented here only briefly.

Methods

Weanling female Long-Evans rats were individually marked, randomly assigned in groups of 14 to 18 animals to stainless-steel cages, and given deionized water fortified with five essential metals *ad libitum*. They were also given a metal-poor, rye-based diet *ad libitum* that contained 0.25 mg lead and 39 mg calcium per kg of food and other metals as described (*9*). The rats were maintained under standard low contamination conditions, including filtered air (*10*); constant temperature of 21°C and relative humidity of 45%; and a 12-hr light-dark cycle. Beyond daily feeding and watering and weekly cage cleaning, the animals were not manipulated or disturbed except for periodic weighings and blood pressure measurements.

Animals were weighed monthly because growth rate was considered a sensitive index of toxicity. At intervals of 3 months, indirect systolic pressure in the tail artery was measured three times in rapid succession. The median of the three pressures was used for obtaining group averages and for plotting pressure distributions of individual rats. Pressure measurement was with a Narco Bio-Systems pneumatic transducer and a Sanborn Recorder, using a modification of the technique described by Friedman and Freed (*11*). The statistical significance of the differences between the average pressure for control and lead-exposed animals was assessed using Student's *t*-test.

Two experiments are considered here. The first lasted 18 months and involved 89 control rats and 74 experimental rats that from the time of weaning received lead, as the acetate, in their fortified drinking water. There were five groups of experimental animals: one received water with a final concentration of 0.1 ppm lead; two received water with 1.0 ppm lead; two received water with 5.0 ppm lead (Table 1). For the indirect systolic pressure measurements, the rats were lightly anesthetized with pentobarbital (0.25 mg/kg body weight).

The confirmatory experiment lasted 12 months and involved 69 control and 71 experimental rats that received lead as in experiment 1. There were four groups of experimental animals, two received 0.1 ppm lead and two received 1.0 ppm lead (Table 1). For this experiment, indirect systolic pressure was measured in conscious, unanesthetized rats.

Results

Food and water intake was similar for all groups of rats in both experiments during the first year of observation and averaged 6.60 kg of food and 7.05 L of water per rat. From their food, the control animals obtained lead which was estimated to total 1.75 mg per rat during the first year. The lead-exposed animals had the same intake from food, but they also obtained lead from water. The 0.1 ppm lead animals ingested 0.71 mg from water during the first year, with the 1.0 and 5.0 ppm animals ingesting 10 and 50 times this amount, respectively. At the end of the first year, the animals with the heaviest lead exposure weighed 5% less than the control animals; the difference was not statistically significant, and no clinical evidence of lead toxicity was recognized.

Table 1 cites the average systolic pressure at 3-month intervals for the control rats that received no lead in their drinking water and the average increases above those control values for groups receiving 0.1, 1.0, and

Table 1. Average increase in systolic pressure with chronic low lead exposure.

		Increase in systolic pressure, mm Hg[a]					
		Months of exposure					
Lead exposure, ppm	*n*	3	6	9	12	15	18
Experiment 1, anesthetized rats[b]							
0	89	103	99	101	102	102	110
0.1	15	5	10*	12	17†	16†	16†
1.0	30	12†	13†	11†	12†	15†	13†
5.0	29	16†	15†	13†	10*	19†	12
Experiment 2, unanesthetized rats[c]							
0	69	116	117	118	120		
0.1	36	9†	12†	7*	15†		
1.0	35	11†	13†	10†	14†		

[a]Average systolic pressure is given for control animals.
[b]SD varied from 11 to 14 mm Hg, except at 18 months, when it was 27 mm Hg for the 5.0-ppm group.
[c]SD was 6 to 7 mm Hg.
*Significant difference from control, $p < 0.05$.
†Significant difference from control, $p < 0.001$.

Table 2. Distribution of systolic pressure in experiment 2 rats as a function of time.

		Percent of group with systolic pressure in indicated range					
Lead intake	*n*	< 120	> 120	> 130	> 140	> 150	> 160
Control[a]	69	65	35	4	0	0	0
0.1 ppm	36	13	87	41	19	8	2
3 Months		22	78	28	3	0	0
6 Months		14	86	44	16	3	0
12 Months		4	96	52	37	22	7

[a]Time breakdown measurements are not given for control rats because there was little increase over time; only 4% of pressures in control rats exceeded 130 mm Hg.

5.0 ppm lead in their drinking water. During the first year, the average systolic pressure of the anesthetized control animals (experiment 1) remained essentially constant at about 100 mm Hg, although there may have been some increase at 18 months. During the first year, the average systolic pressure of the unanesthetized control animals (experiment 2) ranged from 116 to 120 mm Hg; this increase over the unanesthetized control level is what has been regularly observed in the absence of anesthesia. Pressure-wise, the control group was very homogeneous, with 80% of the individual values being from 110 to 130 mm Hg.

Lead-induced increases in pressure appeared within 3 months and persisted for the entire 18 months of follow-up. The average increases ranged from 5 to 19 mm Hg; three-fourths of the increases were highly statistically significant ($p < 0.001$). With the lowest exposure to lead, the average increase was initially small and tended to rise during the first year. In contrast, with the highest exposure, there was the suggestion of a reverse trend; the full increase was present at 3 months and tended to become smaller during the remainder of the first year. Figure 1 shows these trends during the first year of experiment 1.

In experiment 2, the average systolic pressure of the 0.1 ppm lead group increased successively from 125 to 129 and then to 135 mm Hg at 3, 6, and 12 months, respectively. Despite this increase in average pressure, 40 to 50% of the lead-exposed animals had systolic pressures between 120 and 130 mm Hg at all three time intervals (Table 2). Thus, most of the increase in average pressure was due to the remaining half of the rats developing more marked elevations in pressure. Whereas only 4% of control rats ever had systolic pressures above 130 mm Hg, 28, 44, and 52% of the lead-exposed rats had pressures above that cut point at 3, 6, and 12 months, respectively. Moreover, at 12 months, 37, 22, and 7% of the lead-exposed rats had systolic pressures over 140, 150, and 160 mm Hg (Fig. 2).

Discussion

Low-dose lead exposure is consistently pressor for rats under the rigid low-contamination conditions used in our experiments. Exposure to 0.1 ppm lead in all drinking water provided a weanling rat with 0.04 mg of lead during the first month and with 0.7 mg during the first year. The standard diet, which all rats received, contained 2.5 times as much lead as did the water, but apparently much of the dietary lead was relatively unavailable.

The average increase in systolic pressure for all lead-exposed rats approximated 15 mm Hg. In addition, the distribution of pressures was different for the control

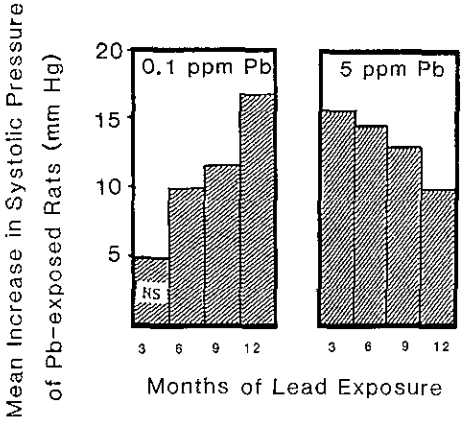

FIGURE 1. The change in pressor response (i.e., difference between the average systolic pressure of control and lead-exposed rats) is given as a function of time for the lowest and highest lead exposures in experiment 1. Except for bar marked "NS" (not significant), all increases were significant with $p < 0.05$.

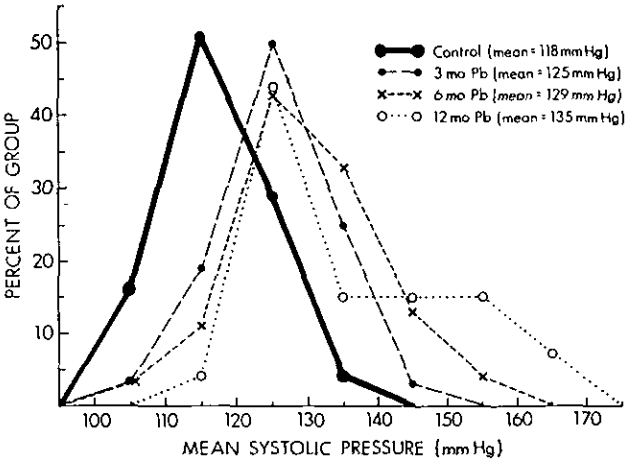

FIGURE 2. Distribution of all systolic pressures for 69 control rats from experiment 2 and separate 3-, 6-, and 12-month distributions for 36 minimally lead-exposed (0.1 ppm) from that same experiment.

rats and those with the lowest lead exposure. The first two lines of Table 2 indicate a much smaller range of pressures for the control than for the lead-fed animals. Eighty percent of the former had pressures between 110 and 130 mm Hg versus 50% of the latter, with 40, 20, and 10% of the latter having pressures greater than 130, 140, and 150 mm Hg. Thus, much of the lead-induced increase was due to a fraction of the animals with moderate and marked increases. Moreover, as indicated by the last three lines of Table 2, the distribution of pressures for the lead-fed rats differed increasingly from the distribution for the control rats, which changed very little with time. Roughly half of the lead-fed animals continued to have pressures in the 120 to 130 mm Hg range throughout the first year; however, increasing numbers had values over 130, 140, 150, and even 160 mm Hg thus producing a stepwise increase in mean pressure for the group.

Conditions were carefully standardized in these experiments. Although the standard diet is relatively low in calcium and contains some lead, it is known to support normal growth and continuing good health for the Long-Evans rats used here; moreover, the systolic pressure of the control animals is reproducible and well known. Female rats were used because, unlike males, groups of females can be housed in a single cage without fighting. Indirect systolic pressure was used because it can be repeatedly measured without injuring the animal. In our original experiments, low-dose pentobarbital anesthesia was used; however, because of unexpected effects from the anesthetic (*12*) we have begun to use trained, conscious rats without anesthetic. Withholding the anesthetic consistently results in an average increase of almost 20 mm Hg in the systolic pressure of control rats; however, at the same time, the standard deviation falls to half of the value observed in anesthetized rats. Finally, it is worth noting that in Schroeder's early experiments, using identical rats, diet, and water, but carried out in a less contaminated environment with clean filtered rural air and plastic cages, the control systolic pressures of anesthetized rats averaged 85 mm Hg (*13*).

The mild hypertension without overt toxicity induced by low lead exposure, as described here, would seem to have a different pathogenesis from the apparently renal hypertension associated with kidney damage from much higher lead exposure (*14*). The hypertension associated with low-dose lead resembles human essential hypertension in usually being mild, having no associated abnormalities, and being of unknown etiology. There has been great variability in the amount of lead exposure required to induce hypertension. At one extreme, we found 0.1 ppm to be pressor. At the other extreme, Victery and co-workers have found 100 ppm to be pressor (*15*). These animals had low renin hypertension and slight increases of body weight, suggesting that sodium retention might be responsible for the hypertension. Thus, doses that are far higher than our minimum dose may induce the essential type of hypertension by a mechanism that is unknown but which does not involve significant renal dysfunction. This could result from poor bioavailability of the lead and/or major differences in dietary constituents, e.g., calcium. This group also observed increased responsiveness to catecholamines in lead-exposed rats (*16,17*).

There are only limited data indicating how much lead is actually absorbed with different exposures. Many investigators have used blood lead levels to estimate lead intake. With chronic exposure, however, blood lead is a poor measure of body burden. Once absorbed, lead is slow to leave the body because it has a major reservoir in bone from which it can only be leached slowly. The levels of lead in critical tissues would seem to be better indices of its pharmacologic effects.

There are suggestive similarities between the lead-induced essential-type hypertension and the hypertension induced by comparable exposure to cadmium. The lead and cadmium pressor responses are similar in magnitude, in causing no overt toxic manifestations, and in some of the associated biochemical effects, e. g., both lowered tissue concentrations of ATP and phosphocreatine (*18*). It is possible that both nonessential metals compete with some essential metal, possibly calcium. With our standard experimental conditions, there is a limited low range of cadmium concentrations that are pressor (0.1 to 5.0 ppm), with less than 0.1 ppm having no effect and more than 5.0 ppm being vasodepressor (*10*).

Cottier observed the same phenomenon for parenteral lead, with repeated SC doses (20 mg lead phosphate) being pressor, while half and twice that dose were not pressor and even may have been depressor in the case of the higher dose. He also observed that less than half of the exposed rats had a significant pressor response to lead (*19*). The decreasing pressor effect with continued relatively high (5.0 ppm) lead exposure (Fig. 1) suggests that lead too may induce essential-type hypertension over a limited low range of exposures. In the case of cadmium, there is some evidence that the pressor response is associated with minimum sodium retention (*20*), perhaps enhanced by some direct vasoconstrictor activity (*21*). With cadmium-induced, but not lead-induced, hypertension, we observed some increase in renin activity (*4*); however, others using larger doses of lead have observed hyporeninemia (*16*).

Conclusion

Chronic ingestion by rats of small amounts of lead (0.1 to 5.0 ppm in drinking water) produced an increase in systolic pressure without obvious toxicity. The increase in pressure averaged about 15 mm Hg, but this average included some animals with little if any increase and some with large increases. After 3 months of exposure to the lowest dose (0.1 ppm), only one-quarter of the animals had even minimum hypertension, but after 1 year of exposure more than half

were hypertensive and almost one quarter had marked elevations. This low-exposure, lead-induced hypertension seems to differ from that induced by heavy occupational exposure to lead that is usually associated with significant renal dysfunction.

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