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Lead Exposure, Begun *in Utero*, Decreases Renin and Angiotensin II in Adult Rats[1] (41398)

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Abstract. Male rats were exposed continously to Pb *in utero* and after birth by giving their mothers, during pregnancy and lactation, drinking water containing 0, 5, or 25 ppm Pb (as Pb acetate) and then continuing this regimen after weaning for approximately 5 months. At the time of sacrifice (5 months) the 5- and 25-ppm groups had mean blood Pb concentrations of 5.6 and 18.2 μg/dl, respectively. No differences in systolic blood pressure occurred between groups. Rats exposed to 25 ppm manifested a significant decrease in basal plasma renin activity (PRA) but a significant increase in PRA during stimulation of renin release by acute volume depletion. In this latter state, the ratio of angiotensin II to PRA was significantly reduced in the 25-ppm group. Groups exposed to 5 and 25 ppm both had significant decreases in renal renin concentration. We conclude that chronic exposure of rats to doses of Pb which produce blood Pb concentrations similar to those generally present in urban human populations does not induce hypertension but does inhibit renin synthesis and release, as well as reducing plasma angiotensin II concentration at any given PRA, either by inhibiting conversion of AI to AII or by enhancing AII catabolism.

We have previously demonstrated (1) that rats developed stable persistent hypertension as adults after they were exposed continuously to Pb *in utero* and after birth by giving their mothers, during pregnancy and lactation, drinking water containing 100 ppm Pb and then continuing this regimen after weaning. These rats also showed significant decreases in plasma renin activity (PRA) and angiotensin II (AII) concentration. Their blood Pb concentration averaged 40 μg/dl, and the present studies were designed to determine whether similar effects could be produced by lower doses of Pb.

Materials and Methods. Fifteen 14-day timed pregnant rats (Charles River; Portage, Mich.) were obtained, housed individually, and placed on Teklad Rat/Mouse Chow (4% fat content). Of the fifteen, five animals were placed on sodium acetate-containing drinking water prepared in deionized demineralized water, five on 5 ppm Pb (as acetate), and five on 25 ppm Pb (as acetate); all solutions contained acetate equimolar to the 25-ppm Pb solution.[3] Two of the 25-ppm mothers were subsequently found not to have been pregnant, which accounts for the use of only three litters in this treatment group. There were no differences in the numbers of offspring in the litters of the three treatment groups nor in the average birthweights of the newborn animals. After parturition all three groups of mothers were maintained on their respective drinking solutions throughout the period of nursing. All litters were reduced to eight on Day 4, weaned on the 21st day, and separated by sex at that time. The male offspring were then reduced to 20, 20, and 13 total animals for the three treatment groups (control, 5, and 25 ppm, respectively) by randomly removing equal numbers of animals from each litter. Drinking water

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[3] The sodium drunk by the control animals (0.02 m*M*) increased total sodium intake by approximately 0.2%, an amount much too low to exert any effect on plasma renin.

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and diet were not changed but were continued throughout the remaining 5 months of the experiment. All data reported are for the male offspring only; at 1 month of age, the female offspring were sacrificed for hormone measurements to be reported elsewhere.

Blood pressure recordings were first obtained when the animals had reached 2 months of age (approximately 200-g weight), then again at 3 months and weekly thereafter. Indirect pressure readings by tail cuff method were obtained on each rat in the unanesthetized state, using a Narco Programmed Electrosphygmomanometer PE 300, as described previously (1). At 5 months of age, the animals were killed by decapitation. The rats had been conditioned on 3 separate days in advance by placing their necks in the guillotine. All decapitations were performed between 7:30 AM and 10 AM to avoid the afternoon rise in PRA. Three hours prior to the decapitation, approximately half the animals in each treatment group had been injected (21-gauge needle) intraperitoneally (1 ml/100 g body weight) with a 20% solution of polyethylene glycol (PEG) in isotonic saline. This was done for two reasons: (i) to study the effects of Pb on PRA when renin release is strongly stimulated (by plasma volume reduction secondary to PEG-induced accumulation of ascites fluid); (ii) to elevate plasma AII concentrations into the range over which our AII assay is more accurate.

Trunk blood was collected for 10–15 sec into prechilled tubes containing 7.6 g ammonium EDTA per deciliter as anticoagulant (approximately 10 μl/ml blood); immediately after collection, a 1-ml aliquot was transferred to a tube containing the inhibitors required for AII analysis (50 μl of a solution of EDTA, 7.6 g/dl, 0.5% *O*-phenanthroline, and 0.2% neomycin sulfate), and a 100-μl aliquot of blood was placed into an equal volume of 5% Triton X-100 for blood lead determination. A microsample for hematocrit was then taken, and both the remaining blood and the sample for AII were centrifuged at 4°, after which the plasma was separated and frozen. A kidney was rapidly removed and frozen in isotonic saline for later measurement of renin content.

Methods for measurement of PRA, plasma AII, renal renin concentration, plasma sodium and potassium concentrations, and plasma creatinine have all been described previously (1–4). Blood Pb was measured by graphite furnace atomic absorption (Varian Instruments, Model 375: CRA90) using methods of addition.

All grouped data are presented as mean ± one standard error. Student's *t* test was used for computing the significance of the differences between Pb-exposed animals and controls, using Schefé allowances since two Pb groups were being compared to a single control group.

Results. As summarized in Fig. 1, systolic blood pressures of the three groups remained relatively stable throughout the experiment. At no time was there a significant difference between the control and either of the Pb-treated groups.

Figures 2 and 3 summarize the PRA data for the rats at time of sacrifice. The mean basal PRA (Fig. 2) is significantly lower for the 25-ppm group than for the control group. The PEG-treated rats (Fig. 3) showed just the opposite pattern, i.e., a significantly greater PRA in the 25-ppm group.

The mean AII concentrations for the

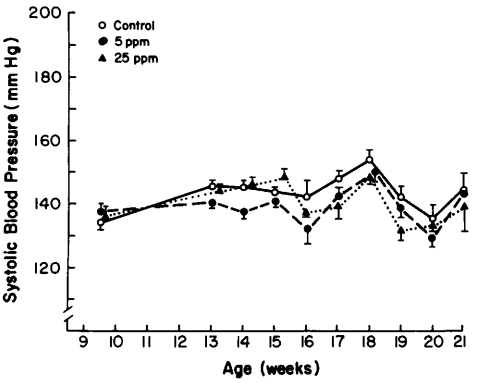

FIG. 1. Lack of effect of Pb on systolic blood pressure in unanesthetized male rats.

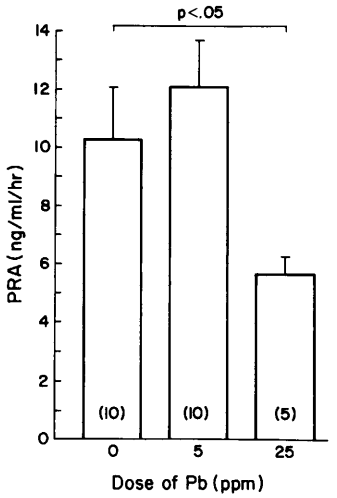

FIG. 2. Effects of Pb on basal plasma renin activity (PRA). Blood samples were collected by decapitation.

three PEG groups (control, 5 ppm, 25 ppm, respectively) were (in pg/ml): 113 ± 20.5, 111 ± 23.1, and 71.6 ± 8.6. The difference between the control and 25-ppm groups was not statistically significant. However, since the PRA of the 25-ppm group was significantly elevated, the AII should also have been higher were Pb to have had no influence on the generation or catabolism of AII. Therefore, the evaluation of any Pb effect on AII independent of its effect on

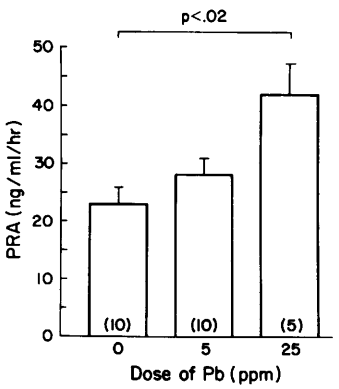

FIG. 3. Effects of Pb on plasma renin activity (PRA) in rats given polyethylene glycol (PEG) intraperitoneally to stimulate renin release. Blood samples were collected by decapitation 3 hr after the PEG injection.

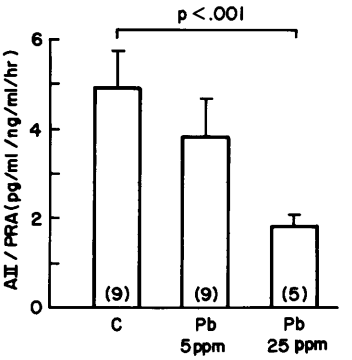

FIG. 4. Effects of Pb on the ratio of plasma angiotensin II concentration to plasma renin acitivity in rats given polyethylene glycol intraperitoneally 3 hr earlier to stimulate renin release.

PRA is better given by the ratio of AII to PRA. As illustrated in Fig. 4, there is a marked and significant decrease in this ratio.

Renal renin concentration, like PRA, was also reduced in the 25-ppm group (Fig. 5). Moreover, a significant reduction occurred in the 5-ppm group as well.

There were no significant differences between groups with regard to total body weights, kidney weights, hematocrit, or

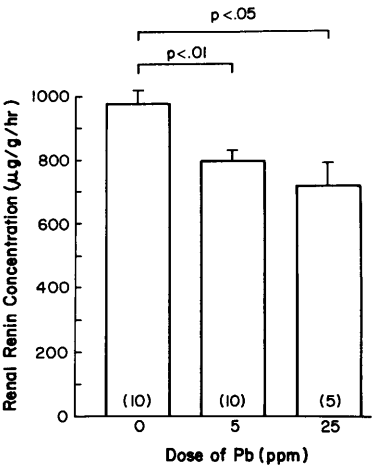

FIG. 5. Effects of Pb on renal renin concentration in "basal" rats sacrificed by decapitation. Renal renin was not measured for rats given polyethylene glycol.

plasma concentrations of sodium and potassium (rats given PEG were analyzed as separate groups). Blood Pb concentrations were (in μg/dl): control, 1.4 ± 0.3; 5 ppm, 5.6 ± 0.4; 25 ppm, 18.2 ± 1.5. Blood Pb was measured only in rats not given PEG.

Discussion. Perhaps the most important finding in this study is that reduction of basal PRA, reported previously for a 100-ppm dose of Pb (1) also occurs in animals exposed to 25 ppm. The mean blood Pb concentration of these rats was only 18.2 μg/dl, a value approximating that of most urban human American populations. Five parts per million did not reduce PRA although it did lower renal renin; thus a significant effect of Pb exposure on renin metabolism is seen with a dose which produces a blood Pb concentration of only 5.6 μg/dl. These data, taken together with our previous findings (1) round out the dose–response profile for Pb and basal PRA in chronic adult rats whose exposure is begun *in utero;* no change in PRA occurs at 5 ppm, a significant inhibition occurs at both 25 ppm (present data) and 100 ppm (1), but at 500 ppm, the PRA values are essentially normal (1). This dose response is consistent with reports that Pb-exposed persons manifest either a significant reduction in their PRA (5, 6) or no change (7). In contrast, a highly significant increase in both basal PRA and renal renin is produced in rats by 500 ppm Pb when exposure was begun later in life rather than *in utero* (4).

The most likely explanation for the decreased PRA seen at 25 and 100 ppm Pb is a decreased renal synthesis of renin, as evidenced by the decreased renal renin seen in all groups of rats whose exposure to Pb was begun *in utero,* regardless of dose.

The fact that PRA was not decreased at 500 ppm (1), despite a decrease in renal renin, indicates that at least one other factor must be operating at the higher dose; a likely possibility is decreased hepatic clearance of renin, a phenomenon which has been shown to occur during acute administration of lead to dogs (2), but no data are available on this question in chronically exposed animals.

Decreased clearance of renin could also explain why, in contrast to its effect on basal PRA, 25 ppm Pb caused the PRA response to volume depletion (induced by PEG) to be enhanced; a decreased clearance should cause a larger rise in PRA at any given increased rate of secretion. A second possibility concerns the feedback of AII on renin release; as PRA increases secondary to stimulation of secretion, the resulting rise in plasma AII normally feedbacks on the JG cells to inhibit secretion, but this process would be less prominent in the Pb-exposed rats because of the failure of their plasma AII to increase as much as normal.

The Pb effect on plasma AII reflects either an inhibition of AII generation or an enhancement of AII breakdown (2). The present experiments document that the lowest dose of Pb producing this effect lies between 5 and 25 ppm. These data therefore strongly suggest the need for evaluating AII/PRA ratios in Pb-exposed persons, since a change in this ratio might be a sensitive indicator of biologically important lead effects.

Finally, these experiments taken together with our previous findings (1) indicate that the minimal effective dose of Pb for producing hypertension, between 25 and 100 ppm, is greater than that for altering the renin–angiotensin system. However, it should be noted that control blood pressures are relatively high in our rats, presumably because of our lack of animal housing facilities rigidly controlled for noise, species intermixing, and other environmental factors (8), and this might impair the detection of subtle Pb-induced effects on blood pressure.

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