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Plasma Renin Is Increased in Young Rats Exposed to Lead *in Utero* and during Nursing[1] (41517)

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Abstract. Rats were exposed continuously to Pb *in utero* and after birth by giving their mothers, during pregnancy and lactation, drinking water containing 0, 5, 25, 100, or 500 ppm Pb (as Pb acetate); they were sacrificed at 1 month of age, at which time their mean blood Pb concentrations were, respectively, approximately 3, 9, 19, 30, and 70 μg/dl. All Pb-exposed groups sacrificed by decapitation had elevated mean plasma renin activities (PRA), relative to controls. Pentobarbarbital–anesthesia and laparotomy markedly increased PRA in the 0, 100, and 500 ppm groups, but the increase was significantly less in the 100 ppm group. Renal renin concentration was normal in the 5 and 25 ppm groups, but was significantly increased in the 100 and 500 ppm groups. The ratio of plasma angiotensin II to PRA was normal in the 100 ppm group but significantly reduced in the 500 ppm group. We conclude that exposure of rats *in utero* and during lactation to doses of Pb which produce blood Pb concentrations similar to those generally present in human populations stimulates basal renin secretion in 1-month-old rats, but partially inhibits the response to renin-releasing stimuli. The highest dose reduces plasma angiotensin II at any given PRA. These results, taken with previous publications, emphasize that the effects of lead on plasma renin even within a single species are greatly affected by the timing of the exposure.

We have previously demonstrated (1, 2) that the renin–angiotensin system was significantly altered in adult rats which had been exposed continuously to Pb *in utero* and after birth by giving their mothers, during pregnancy and lactation, drinking water containing 5–500 ppm Pb and then continuing these regimens after weaning. Rats exposed to 25 and 100 ppm Pb manifested significant decreases in basal plasma renin activity (PRA) and renal renin concentration, findings indicative of inhibition of renin secretion; at 500 ppm, the PRA values were essentially normal. This dose–response is consistent with reports that Pb-exposed persons manifest either a significant reduction in their PRA (3, 4) or no change (5). In other studies (6, 7) rats whose exposure to 500 ppm was begun at 5 weeks of age rather than *in utero* manifested a significantly elevated basal PRA. These findings, taken together, document that even in a single species PRA can be elevated, depressed, or normal, depending upon the experimental protocols used.

The aim of the present study was to determine whether the effects of Pb exposure begun *in utero*, on the renin–angiotensin system in adult rats, are also present within the first month after birth. Such information is not only valuable for elucidating the early effects of Pb exposure but also should contribute to the analysis of the sequences of events leading ultimately to the effects seen in adults.

Methods. All experiments were performed on offspring of 7-day or 14-day timed pregnant rats obtained from Charles River, (Portage, Michigan), housed individually and placed on Teklad Rat/Mouse Chow (4% fat content). The pregnant rats of the protocol designated as I were given drinking water solutions made with deionized demineralized water and containing 5 ppm Pb (as acetate), 25 ppm Pb (as acetate), or sodium acetate equimolar to the acetate in the Pb

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solutions. The pregnant rats of the protocols designated as II received equimolar acetate solutions containing 0, 100, or 500 ppm Pb. The extra sodium ingested in the drinking water by protocol I rats amounted to approximately 0.1% of total dietary sodium, and that by protocol II rats was approximately 3%; we and others (8) have shown that these amounts of sodium exert no effect on plasma renin.

After parturition, all mothers were maintained on their respective drinking solutions throughout the period of nursing. All litters were weaned on the 21st day of age and separated by sex. The drinking water and diet of the offspring were the same as those previously given to the mothers. Approximately 1 week later, the female animals of protocol I were sacrificed by decapitation. These animals had been conditioned previously on 3 separate days by placing their necks in the guillotine. All decapitations were performed between 7:30 and 10:00 AM to avoid the spontaneous afternoon rise in PRA seen in rats on a day–night light–dark cycle. Trunk blood was collected for 10–15 sec into prechilled tubes containing 7.6 g ammonium EDTA/dl as anticoagulant (approximately 10 μl/ml blood); immediately after collection, 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 the remaining blood was centrifuged at 4°, after which the plasma was separated and frozen.

The protocol II rats (those exposed to 100 and 500 ppm Pb along with their controls) were sacrificed in two ways. In one batch of these animals, both males and females were sacrificed by decapitation as described above for the low-dose (protocol I) rats. In another batch of protocol II rats, only the females were killed, not by decapitation, but rather they were anesthesized with sodium pentobarbitol (50 mg/kg ip) and rapidly laparotomized to permit blood collection from the abdominal aorta; this procedure was used to study the effects of Pb upon PRA and angiotensin II during stimulation of renin release caused by anesthesia and surgery. Two samples, one for angiotensin II (collected into 50 μl of a solution of EDTA, 7.6 g/dl, 0.5% *O*-phenanthroline, and 0.2% neomycin sulfate) and the other (collected with EDTA as anticoagulant) for all other measurements, were collected simultaneously with two syringes connected via a three-way stop-cock.

The males of protocol I and the second batch of protocol II were not sacrificed and are the animals whose data as adults were previously reported (1, 2).

Methods for measurement of PRA, plasma AII, renal renin concentration, and plasma sodium and potassium concentrations have all been described previously (1, 2, 6, 7); Pb has been shown not to interfere with the methods used for PRA and AII (6, 9). 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 ± 1 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. *Protocol I.* Figure 1 depicts the PRAs for protocol I animals (0, 5, and 25 ppm Pb). Both Pb-exposed groups of this protocol had significantly elevated mean PRAs, relative to that of the control. There were no differences among the groups for body weight, kidney weight, hematocrit, and plasma concentrations of sodium or potassium. Renal renin concentrations also were not different (control = 1006 ± 35 μg AI/g wet wt; 5 ppm = 1092 ± 50; 25 ppm = 1015 ± 48). Blood Pb concentrations in the three groups were, respectively, 2.8 ± 0.4, 9.0 ± 0.8, and 18.6 ± 1.6 μg/dl.

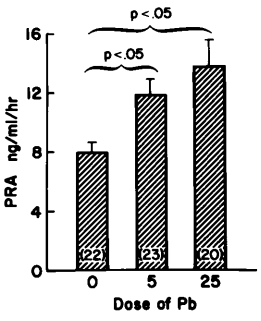

FIG. 1. Effects of lead (5 and 25 ppm in drinking water) on basal plasma renin activity (PRA) in 1-month-old female rats sacrificed by decapitation.

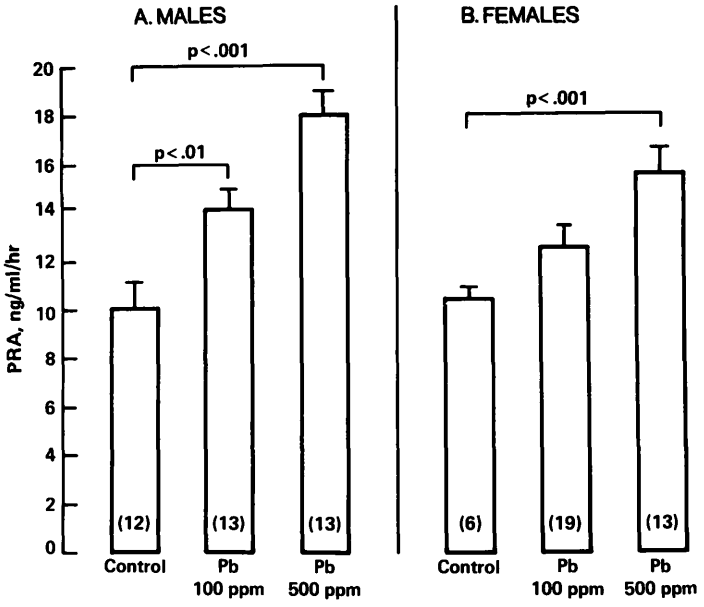

FIG. 2. Effects of lead (100 and 500 ppm in drinking water) on plasma renin activity (PRA) in 1-month-old male (panel A) and female (panel B) rats sacrificed by decapitation.

Protocol II. Figure 2 summarizes the PRA data for protocol II male and female rats sacrificed by decapitation. For both sexes, the Pb-treated rats showed a dose-dependent rise in PRA. The patterns were similar in the two sexes, although there was a tendency for the Pb effect to be somewhat smaller in the females. Renal renin concentrations were measured only for the male animals, and were significantly elevated in both Pb-exposed groups (Fig. 3).

As shown in Fig. 4A, the pattern of PRAs was quite different when the animals (all female) were subjected to anesthesia and surgery for blood collection. As expected, mean PRA in the control group was markedly elevated (approximately 14-fold), relative to the decapitated value; however, PRA was significantly reduced, relative to controls, in the 100 ppm animals. The value for the 500 ppm animals was not significantly different from control. Renal renin was not measured in these animals.

As illustrated in Fig. 4B, plasma AII was significantly decreased relative to controls in a dose-dependent manner in both Pb-exposed groups of protocol II. Figure 5 illustrates the relationship between PRA and plasma AII in these animals. The 100 ppm data fit a single linear regression having a slope of 1.71, whereas the slope of the linear regression for the 500 ppm data was 1.03; at any given PRA, plasma AII was always lower in the 500 ppm animals than in the 100-ppm animals. The control data appear to be similar to those for the 100 ppm rats, but quantitative comparison between these groups is difficult because of the paucity and scatter of the control data.

Table 1 summarizes all measured data other than those for renin and angiotensin

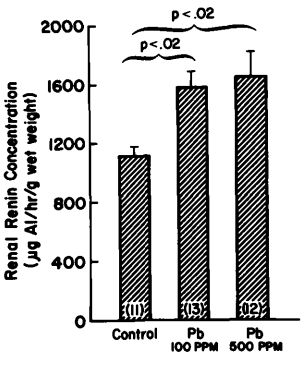

FIG. 3. Effects of lead (100 and 500 ppm in drinking water) on renal renin concentration in 1-month-old male rats.

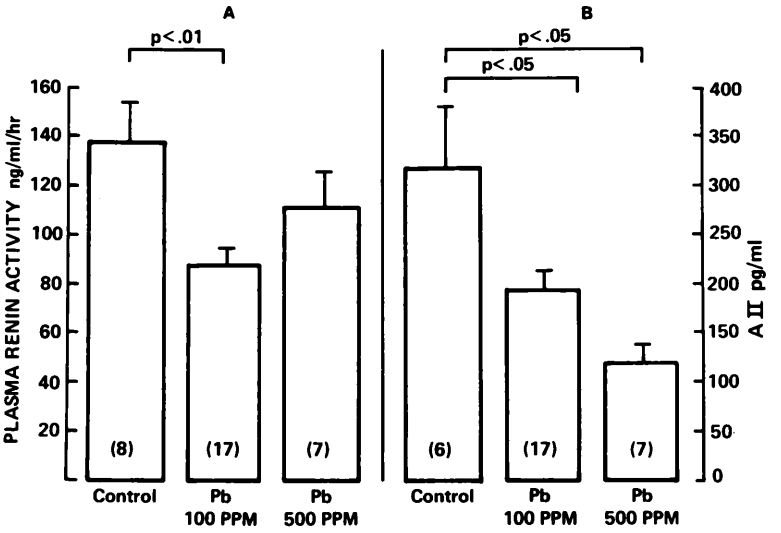

FIG. 4. Effects of lead (100 and 500 ppm in drinking water) on plasma renin activity (PRA, panel A) and plasma angiotensin II concentration (AII panel B) in 1-month-old female rats subjected to pentobarbital anesthesia and laparotomy. Blood was collected from the abdominal aorta.

for the rats of protocol II. There was a tendency for body weight to be lower in all the 500 ppm groups. Absolute kidney weight was significantly elevated, relative to controls, only in the 100 ppm males; however, kidney weight as a percentage of body weight was

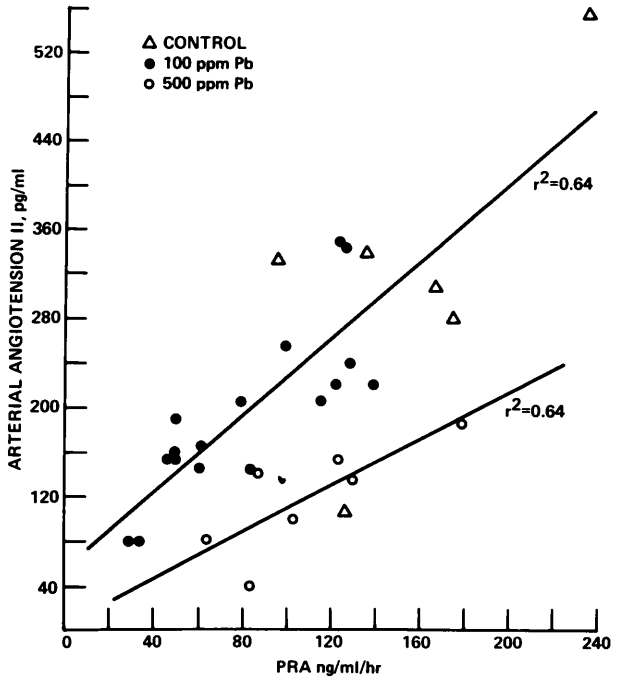

FIG. 5. Linear regression of AII on PRA for 1-month-old rats subjected to pentobarbital anesthesia and laparotomy. The regression lines are for the 100 ppm and 500 ppm Pb rats.

TABLE I. EFFECTS OF Pb ON VARIABLES OTHER THAN THE RENIN–ANGIOTENSIN SYSTEM IN PROTOCOL II RATS SACRIFICED AT 30 DAYS OF AGE

	Females			Males		
	Control	100 ppm Pb	500 ppm Pb	Control	100 ppm Pb	500 ppm Pb
Anesthesia and laparotomy	(n = 8)	(n = 17)	(n = 7)			
Body weight (g)	120.5 ± 6.0	111.1 ± 1.4	114.6 ± 0.9	—	—	—
Right kidney weight (g)	0.58 ± 0.032	0.58 ± 0.10	0.66 ± 0.014			
Right kidney weight/body weight × 100	0.48 ± 0.10	0.52 ± 0.007**	0.58 ± 0.021**	—	—	—
Decapitation	(n = 6)	(n = 19)	(n = 13)	(n = 13)	(n = 13)	(n = 13)
Body weight (g)	120.7 ± 4.8	115.1 ± 2.7	103.4 ± 3.6	143.6 ± 3.9	147.6 ± 0.1	123.1 ± 4.0**
Kidney weight (g)	1.17 ± 0.06	1.23 ± 0.02	1.28 ± 0.06	1.38 ± 0.03	1.57 ± 0.03**	1.34 ± 0.06
Kidney weight/body weight × 100	0.98 ± 0.025	1.07 ± 0.018*	1.23 ± 0.020**	0.96 ± 0.032	1.07 ± 0.022**	1.09 ± 0.02**
Hematocrit × 100	41.0 ± 0.72	41.9 ± 0.38	39.1 ± 0.58*	39.4 ± 0.45	38.3 ± 0.35	37.3 ± 0.32**
$[Na]_p$, mM	140.3 ± 2.2	142.3 ± 2.2	138.8 ± 1.4	138.8 ± 1.2	136.4 ± 0.74	137.3 ± 0.43
$[K]_p$, mM	8.32 ± 0.21	8.34 ± 0.37	7.73 ± 0.15	7.79 ± 0.22	7.73 ± 0.16	7.94 ± 0.16
$[Pb]_B$, μg/dl	—	30.0 ± 2.2 (n = 5)	78.2 ± 1.8 (n = 4)	—	32.6 ± 2.6 (n = 3)	65.8 ± 0.2 (n = 4)

Note. Only the right kidney weight was measured in the decapitated rats. Because of the use of decapitation, the values for $[K]_p$ are artifactually elevated. Subscripts p and B stand for plasma and whole blood, respectively. Note that $[Pb]_B$ was measured in only a fraction of the animals from each group.

* $P < 0.05$.

** $P < 0.01$.

significantly increased in a dose-dependent manner in all groups. There was a small but significant decrease in hematocrit in both male and female 500 ppm groups. There were no differences among groups in the plasma concentrations of sodium and potassium (the potassium values are not true plasma concentrations because of the decapitation method but should be adequate to reveal changes induced by experimental manipulation). The blood concentrations of lead in the 100 and 500 ppm groups were approximately 30 and 70 μg/dl, respectively.

Discussion. These experiments demonstrate that exposure to lead, beginning *in utero*, continued through the animals' nursing period, and in their drinking water until sacrifice, can produce, in rats, significant elevations of plasma renin activity at 1 month of age. Moreover, this effect is produced by Pb exposures which induce a mean blood Pb concentration of only 9 μg/dl; no attempt was made in these experiments to use lower doses of lead, but the fact that 5 and 25 ppm produced very similar effects suggests that even lower doses might have been effective. These results document, therefore, that the renin–angiotensin system of young animals is one of the most sensitive physiological processes in its response to Pb exposure; investigation of this interaction in human infants and children exposed to Pb would certainly seem warranted.

The Pb-induced elevation of PRA seen in these experiments is similar to that reported previously for animals exposed to 500 ppm Pb for the same number of weeks (5, 6) but beginning at 5 weeks of age rather than *in utero* (6, 7). However, it is very different from the results reported previously (1, 2) for the present animals' littermates, which were not sacrificed at 1 month but rather were studied as adult mature animals, the Pb exposure being maintained chronically. In contrast to the Pb-induced elevated plasma renins of the present young animals, the older animals manifested no change in plasma renin at 5 and 500 ppm, with reductions at 25 and 100 ppm, i.e., perturbation of plasma renin in the direction opposite to that seen in the present young rats. This reversal of the plasma renin response is associated with a similar reversal of renal renin concentrations, suggesting that the primary cause of the Pb-induced changes in plasma renin is alteration of renin secretion (rather than altered hepatic renin clearance (9)). However, it should be noted that the increased PRA of the present 5 and 25 ppm rats occurred in the absence of changes in renal renin; that this is still consistent with increased renin secretion is indicated by recent studies in our laboratory (manuscript in preparation) demonstrating Pb-induced increased *in vitro* secretion of renin by rabbit renal cortical slices despite normal renin content of the slices.

In contrast to the increased basal PRA induced by Pb exposure in the present experiments, the renin response to the intense stimuli of anesthesia and surgery was significantly inhibited by Pb exposure, at least in the 100 ppm group. This decreased response is consistent with previous reports for people (3, 4) and older rats (6). Therefore, perhaps the simplest explanation for all the present and previous findings is that Pb exerts dual effects on renin secretion, one inhibitory and one stimulatory, the magnitude of these effects on plasma renin reflecting the dose and precise timing of the exposure to lead as well as the physiological state of the animal.

In contrast to the reversal with time of the Pb-induced change in plasma renin, the effect of lead on the ratio of plasma angiotensin II to PRA seems to progress over time. The present 1-month-old rats exposed to 500 ppm Pb, manifested a decrease in the ratio, whereas a reduction was observed for older rats with doses as low as 25 ppm (2).

Thus, older rats whose Pb exposure was begun *in utero* not only manifest a greater tendency for reduced plasma renin levels but also have a relatively lower AII at any given plasma renin, presumably because of decreased AI conversion to AII as well as increased clearance of AII (manuscript in preparation). At present there exist no reports of the relationship between AII and PRA in Pb-exposed people or rats whose exposure was not begun in utero.

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