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Effects of Lead on the Renal Response to Extracellular Volume Expansion (42019)

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Abstract. Subacute lead exposure has been observed to inhibit the natriuretic response to isotonic saline expansion in adult female rats. Three-week exposure to 0.5% lead acetate in drinking water resulted in a moderately high blood lead concentration of 57 μg/100 ml and up to 60% inhibition of the natriuretic response to extracellular volume expansion. This ability of lead to inhibit natriuresis following volume expansion (an induced stress) may be a more sensitive index of lead poisoning than alterations of renal function in nonstressed animals. Lead exposure had no effect on GFR or plasma aldosterone concentrations, and in the presence of large doses of DOCA (a mineralocorticoid) this inhibitory effect of lead was still persistent. Amiloride completely blocked the antinatriuretic effect of lead in volume-expanded lead-poisoned animals, causing a twofold increase in water and electrolyte excretion while having minimal effects on volume-expanded controls. It is concluded that lead interferes with the action of a "third factor," controlling natriuresis.

Most indices of lead-induced nephropathy, such as a decrease in glomerular filtration rate (GFR), aminoaciduria, phosphaturia, glycosuria, and structural alterations are observed only after prolonged exposure to lead—probably a reflection of severely damaged kidneys. Therefore, a more sensitive measure of renal damage following lead exposure, reflecting more subtle early alterations, is desirable.

Johnson and Kleinman reported on the effects of lead exposure on renal function in young Sprague–Dawley rats 35 to 50 days old with a mean blood lead of 120 μg/100 ml (1, 2). Lead-exposed rats showed a decreased ability to concentrate their urine. Also, when the extracellular fluid space was expanded with isotonic saline, the lead-treated rats excreted significantly more sodium than did control animals. It is important to note that only in the stressed (volume expansion and dehydration) animals was a lead effect observed. In a study of adult rats we could not confirm the natriuretic effect of lead; instead, an inhibition of natriuresis was observed. The present paper explores the mechanism of this action.

Materials and Methods. Female Sprague–Dawley rats weighing 200–300 g were maintained on Purina Rat Chow and tap water *ad libitum*. Lead acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ (Fisher Scientific Company), was added to the drinking water at a concentration of 0.5%.

After a 3-week period, the animals, now showing an average blood lead of 57 ± 6 μg/100 ml, were anesthetized with 100 mg/kg ip Inactin (BYK Gulden Konstanz, West Germany). The trachea, carotid artery, jugular vein, and urinary bladder were cannulated. A primer of 25 μCi [*methoxy-*^3H] inulin/0.3 ml (New England Nuclear) isotonic saline was injected iv followed by a constant iv infusion of isotonic saline (0.9% NaCl) containing 2.5 μCi [^{3}H]inulin/ml at a rate of 0.02 ml/min for 60 to 90 min prior to a 1-hr volume expansion period and 6 hr post-volume expansion. Animals were volume expanded by iv infusion of a volume of isotonic saline equal to 5.0% of their body weight over a 1-hr period. Urine collection periods were 30 min, with arterial blood samples taken at midpoint. Glomerular filtration rate was calculated as clearance of [^{3}H]inulin. Fractional electrolyte excretion was calculated as clearance divided by GFR.

Sodium and potassium were measured on an Instrumentation Laboratory Model 143 Flame Photometer; chloride was determined on an American Instrument Company Chloride Titrator Model J-4-4418. A Wescor 51003 Vapor Pressure Osmometer was used to determine urine osmolality. A Packard

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Tri-Carb 3255 Liquid Scintillation Spectrometer was used to assay [^{3}H]inulin. Titratable acid and NH_4 were measured using the method of Chan (3). Blood lead and erythrocyte protoporphyrin assays were performed by Environmental Sciences Associates.

In the acid loading test, at the end of a 3-week lead exposure period, animals received 1.0% NH_4Cl in drinking water *ad libitum* for 3 days. A 24-hr urine sample was then collected from each animal using stainless steel metabolism cages; food and tap water were supplied *ad libitum*. To measure urine concentrating ability animals were placed into metabolism cages without food or water. The first 6-hr urine sample was discarded and the ensuing 24-hr urine sample was collected to evaluate urinary concentrating ability.

Effects of amiloride on the antinatriuretic actions of lead. Following the previously described surgical procedure, amiloride [3,5-diamino-6-chloropyrazinoylguanidine hydrochloride; Merck, Sharpe and Dohme], was injected iv (1 mg/kg, over 15 min) and continuously infused (2.4 mg/kg/hr) except during the 1-hr volume expansion period (4, 5).

All data were statistically analyzed by two-tailed Student's *t* test (6, 7). Statistical comparison was made always between lead treated animals and non-lead-treated controls. Both tabular and graphic data are expressed as mean plus or minus standard error of the mean.

Results. Three-week exposure to 0.5% lead had no effect on body weight, but a significant ($P \leq 0.05$) increase in kidney weight was observed. The mean blood lead value of the treated group was 56.9 ± 6.4 μg/100 ml compared to a control value of 3.3 ± 0.3 μg/100 ml. The treated erythrocyte protoporphyrin was 55.5 ± 3.1 μg/100 ml and the control 25.3 ± 0.9 μg/100 ml.

As shown in Fig. 1, pre-volume expansion urine flow rate ($\dot{V}$) was 5 to 6 μl/min in both control and lead-treated animals. After VE, $\dot{V}$ rose maximally to 66 ± 16 μl/min in controls as compared to only 30 ± 7 μl/min in treated animals. This attenuated response in urine flow represents a 55% inhibition ($P \leq 0.05$) by lead exposure. Hematocrit and plasma electrolyte values were unaffected by

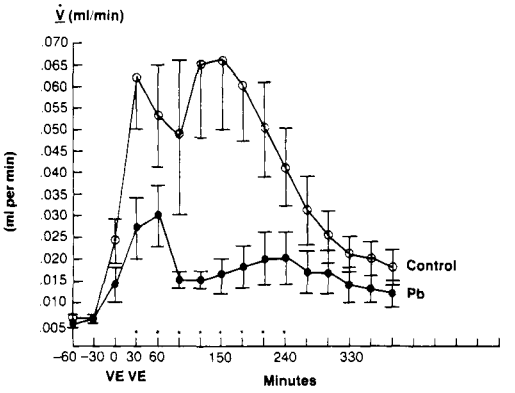

FIG. 1. Urine flow ($\dot{V}$) after 3 weeks exposure to 0.5% lead acetate (intravenous volume expansion). Urine flow rate in control and lead-treated rats, at 30 and 60 min prior to volume expansion, during the 1-hr volume-expansion period (VE), and 6 hr post-volume-expansion. Asterisks denote significant differences from control. Data expressed as mean ± SEM. Control $N = 4$; treated $N = 8$.

lead and remained constant in both groups during the experiment. Changes in sodium excretion rate ($U_{Na} \times \dot{V}$) resembled those seen in $\dot{V}$ (Fig. 1): There was no effect of lead on sodium excretion prior to VE; the maximum response to VE in controls was 11.3 ± 0.9 μeq Na/min, compared to 7.8 ± 1.9 μeq Na/min in the lead-treated animals; this difference represents a 31% inhibition ($P \leq 0.05$) of the natriuretic response in the lead-treated group. At 90–120 min post-VE, the inhibitory effect of lead on the natriuretic response was 63% ($P \leq 0.05$). At no point, either pre- or post-VE, was a difference in GFR seen between the two groups.

Role of steroids. To determine whether lead interferes with the natriuretic response to VE by altering the concentration of mineralocorticoid in plasma, both control and lead-exposed animals received 25 mg/kg of DOCA 24 hr prior to volume expansion. The difference in maximum urine flow ($\dot{V}$) was similar to that seen in Fig. 1, i.e., approximately a 63% inhibition ($P \leq 0.05$) of the diuretic response to VE by lead exposure. Similarly, the difference in maximum sodium excretion rate between control and treated groups (13.87 and 7.84 μeq Na/min, respectively) reflects a 44% inhibition ($P \leq 0.05$)

of the natriuretic response in the lead-exposed animals. Lead itself did not alter the endogenous mineralocorticoid levels: 20.5 ± 2.41 ng/dl in control animals and in treated animals 22.4 ± 4.1 ng/dl.

Effects of amiloride on the antinatriuretic action of lead. The ability of the weak diuretic, amiloride to reverse the previously observed ability of lead to inhibit natriuresis is illustrated in Fig. 2 which shows the fractional excretion of sodium (FE_{Na}) during the course of the experiment. Values for the control groups (with and without amiloride) are almost identical. In contrast, Fe_{Na} in the lead group without amiloride (the lowest line on the graph) fell significantly ($P \leq 0.05$) below the values for the lead group with amiloride, indicating that the drug compensated for the inhibitory effects of lead on natriuresis.

The dose of amiloride used sufficed to produce the expected decrease in fractional potassium excretion (maximum FE_K for either control or lead-treated groups in the presence of amiloride was 8.1 ± 1.9%, compared to 72.5% in the absence of amiloride).

Figures 3 and 4 best illustrate the enhanced effect that amiloride had on the lead-treated animals. Figure 3 shows total urine volume in response to extracellular volume expansion both without amiloride present and with amiloride present. The left side of the graph which shows urine volume in response to VE without amiloride illustrates the fact that the total urine excreted after VE was significantly lower in lead-treated animals than controls. But in the presence of amiloride (right side of graph) there is no difference between control and treated urine volume. While urine volume decreased slightly in the expanded control group in the presence of amiloride, compared to expanded controls without amiloride, the difference was not significant. However, the urine volume in the treated group with amiloride was significantly greater ($P \leq 0.05$) than the urine volume in the treated group without amiloride. Thus, amiloride had no significant effect on urine volume in expanded control animals, but it significantly increased urine volume in expanded lead-treated animals. Figure 4 depicts a similar comparison of net sodium excretion in response to volume expansion with and without amiloride. Note that without amiloride (left side or graph) the treated group excreted only 50% of the administered sodium load as compared to 108% in the control group. Comparing the values observed in the presence of amiloride (right side of graph)—there was no difference between the two groups. Again the presence of amiloride had no effect on sodium excretion by control animals but substantially increased (approximately twofold) sodium excretion in treated animals.

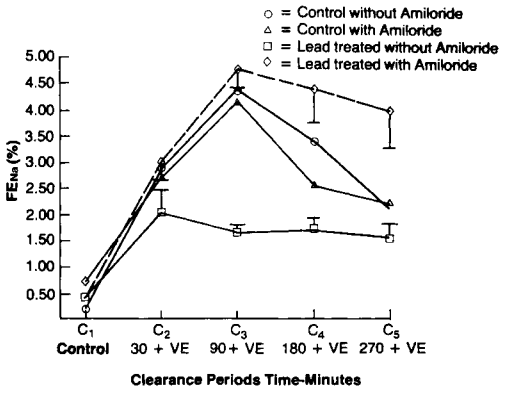

FIG. 2. Fractional excretion of sodium (FE_{Na}) with and without amiloride after 3 weeks exposure to 0.5% lead acetate (intravenous volume expansion). FE_{Na} in control without amiloride, $N = 4$; control with amiloride, $N = 8$; lead-treated without amiloride, $N = 8$; and lead-treated with amiloride, $N = 8$ at one pre-VE clearance period and four post-VE clearance periods. Data expressed as mean ± SEM.

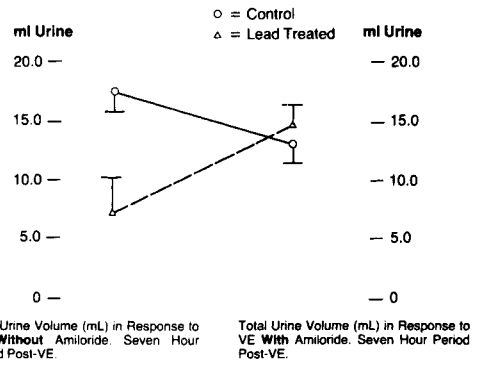

FIG. 3. Total urine volume (ml) in response to volume expansion with ($N = 8$) and without ($N = 7$) amiloride during 7-hr post-VE period. Data expressed as mean ± SEM.

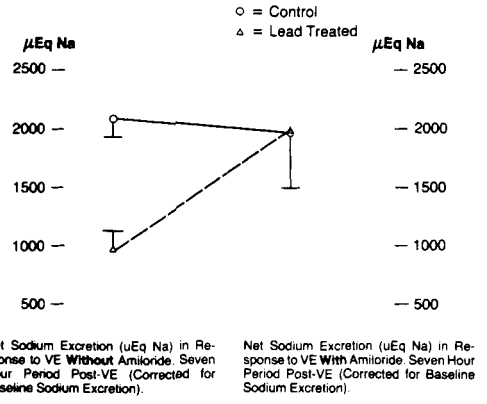

FIG. 4. Net sodium excretion (μeq Na) in response to volume expansion with (N = 8) and without (N = 7) amiloride during 7-hr post-VE period (corrected for baseline sodium excretion). Data expressed as mean ± SEM.

Urinary concentrating ability and acid load handling. To provide measures of functional renal integrity, the effects of lead on the renal response to acid loading were determined (Table I). There was no significant difference between the two groups in urinary titratable acid, ammonium, or total acid excretion. Maximum urinary concentrating ability was also measured following a 24-hr water deprivation period. There was no difference between the two groups regarding urine or plasma osmolality.

Discussion. The primary aim of this work was to study the effects of short-term exposure to lead on natriuresis in adult animals. Three-week exposure to 0.5% lead acetate in drinking water resulted in a moderately high blood lead concentration of 57 μg/100 ml and up to a 50% inhibition of the natriuretic response to volume expansion in adult animals. In contrast, Johnson and Kleinman (1, 2) reported that lead stimulated the natriuretic response in young rats exposed to the metal from birth. In neither study did lead have an effect on resting renal function, i.e., effects of the metal on sodium excretion were only observed in the stressed animal. However, there were several differences between the current study and that of Johnson and Kleinman; these include age of the animals and exposure regimen. It is possible that lead exerts a different effect on a mature kidney than on the developing kidney. This possibility is supported by the observation of Aviv *et al.* (8) that exposure to 1.0% lead acetate during development in rats results in chronic renal damage.

Previous investigators have also observed that lead can alter sodium excretion. Mouw *et al.* reported on the acute effects of lead on plasma renin activity (PRA) and sodium excretion (9). With GFR remaining constant, an acute infusion of 0.1 mg/kg (0.1 ppm) lead acetate resulted in an increased excretion of sodium, potassium, calcium, and water apparently by decreasing tubular reabsorption of those substances. The PRA was also increased. With 0.1 ppm of lead administered, a blood lead concentration of less than 5 μg/100 ml was obtained.

TABLE I. RESPONSE TO ACID LOADING AND URINARY CONCENTRATING ABILITY FOLLOWING 3 WEEKS EXPOSURE TO 0.5% LEAD ACETATE IN DRINKING WATER

	Excretion of acid load (meq/24 hr)			Urine osmolality (mOsm/kg H_2O)	
	TA[b]	NH_4[b]	TE[b]	Normal	Maximum[c]
Control (N = 7)					
$\bar{x}$	28	71	99	1330	1761
SEM	11	21	25	165	132
Lead (N = 9)					
$\bar{x}$	54	48	102	1405	1739
SEM	12	6	17	142	93

[a] All data expressed as mean ± SEM, no significant differences from control.
[b] TA = titratable acid; NH_4 = ammonium; TE = total acid excretion.
[c] Following 24 hr water deprivation.

Fleischer *et al.* reported on the chronic effects of lead on plasma renin and sodium excretion (10). Rats that had been exposed to 0.5 mg/ml (500 ppm) lead acetate in drinking water for 6 weeks with a mean blood lead concentration of 30 μg/100 ml showed a decrease in PRA. After 5 months of lead exposure PRA was elevated in the treated animals. However, when the animals were placed on a sodium-free diet, the renin response (the expected rise in renin to facilitate sodium retention) was blunted in the lead-treated animals. The treated animals also could not sufficiently reduce sodium excretion when placed on a sodium-free diet. Therefore, both acute and chronic lead exposure have been observed to alter sodium metabolism and PRA.

The ability of lead to alter renal function in stressed animals (rats subjected to extracellular volume expansion) appears to be a more sensitive index of lead poisoning than alterations of renal function in nonstressed animals. Of the more "classical" renal function tests—measurement of GFR, renal plasma flow, extraction of paraminohippurate, fractional excretion of aminoacids, and electrolytes—most have been reported to be influenced only after several weeks' exposure to lead, with blood lead values exceeding 100 μg/100 ml. Apparently, in animals stressed by volume expansion subtle alterations of renal function can be noticed at lower lead levels.

The antinatriuretic effects of lead were seen at constant GFR, and in the presence of excess mineralocorticoids; in addition lead exposure had no detectable effect on plasma aldosterone values. Lead induced alterations of the so-called first and second factors controlling natriuresis, i.e., GFR and aldosterone secretion can thus be ruled out as mechanisms of action for the observed antinatriuretic effects of lead (11–13). However, it is possible that lead alters some other aspect of aldosterone metabolism, i.e., binding sites, binding capacity, and efficiency or aldosterone receptor concentrations.

By definition, therefore, lead acts to inhibit the natriuretic response to saline expansion by interfering with third factor processes. In an attempt to determine the site of action of lead, the studies were repeated in the presence of amiloride, a diuretic blocking distal sodium reabsorption and potassium secretion. Amiloride abolished the antinatriuretic effect of lead, suggesting that lead exerts its actions in the amiloride sensitive region of the nephron.

If it is assumed that: (1) amiloride acts primarily at the level of the cortical distal nephron and (2) the ability of amiloride to block this natriuretic inhibitory effect of lead is a drug specific effect, as opposed to a more general diuretic or natriuretic effect, then a distal tubular site of action for the antinatriuretic effect of lead may be proposed.

At least two possible mechanisms may be operant. First, lead may bind to sodium entry sites in some region of the distal nephron and thus enhance passive sodium flux from lumen to cell. More sodium would then be available for the active sodium transport mechanism, resulting in an increase in sodium reabsorption. Since the distal cortical nephron (post macula densa) is normally a low-volume area, baseline sodium excretion would be unaltered. However, during extracellular volume expansion a much greater sodium load is presented to the post macula densa region and consequently a decrease in sodium excretion becomes detectable. Also, calcium is capable of binding to the entry sites (14–17). Since lead and calcium compete for binding sites under many physiological conditions, it is conceivable that lead binds to the sodium entry sites in the distal nephron and in some way facilitates sodium reabsorption (16). The presence of amiloride would block these sodium entry sites and return sodium excretion to normal.

A second possibility is that a lead-induced increase in protein synthesis results in a greater concentration of sodium entry sites, resulting in increased sodium reabsorption (18). This possibility is strengthened by the observation that lead treatment increased the dry kidney weight/body weight ratio by 17%. Cuthbert has observed that a stimulation of protein synthesis results in a corresponding increase in the quantity of sodium entry sites (14, 15). Amiloride would then block all sodium entry sites and return sodium excretion to normal. In any case, and by definition, as lead does not exert its antinatriuretic action

through effects on GFR or mineralocorticoids it must affect a "third factor" process; this suggests a distal site of action of third factor.

In order to determine whether the natriuretic inhibitory action of lead on the distal tubule is specific, effects on renal handling of acid loads and on urinary concentrating ability were also measured. As shown in Table I, lead did not significantly alter these two parameters, indicating that the ability of lead to inhibit natriuresis is a specific rather than a general renal effect.

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