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EFFECTS OF ETHANOL AND LEAD INGESTION ON URINARY SODIUM EXCRETION AND RELATED ENZYME ACTIVITY IN RAT KIDNEY

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Abstract—The present study was undertaken to examine lead–ethanol interaction with respect to previously reported lead enhancement of urinary Na^+ excretion [Y. Suketa, S. Hasegawa and T. Yamamoto, *Toxic. appl. Pharmac.* **47**, 203 (1980)]. Simultaneous administration of ethanol reduced the enhancement due to lead alone. A decrease in serum Na^+ concentration followed lead administration and the decrease appears to be less when ethanol was administered simultaneously. Renal ($Na^+ + K^+$)ATPase activity was suppressed after lead administration, but it was stimulated after ethanol administration. The suppression of renal ($Na^+ + K^+$)ATPase activity after lead administration was diminished after simultaneous administration of ethanol and lead. The diminution of the suppression appeared to be associated with a corresponding reduction in urinary Na^+ excretion.

Lead is an occasional contaminant of illicit, or moonshine whiskey. Sandstead *et al.* [1] reported that most humans who exhibit signs of lead intoxication after long-term ingestion of moonshine whiskey show diminished renin activity, aldosterone secretion, and plasma Na^+ concentration. Such changes, however, cannot be attributed solely to the toxic effect of lead or ethanol.

Little attention has been given to the relation between changes in urinary Na^+ excretion and changes in renal enzyme activity after an acute dose of ethanolic lead. Thus, we decided to study the effect of ethanol on urinary Na^+ excretion and renal ($Na^+ + K^+$)ATPase activity, in rats concomitantly treated with large doses of lead orally.

METHODS

Treatment. Male Wistar albino rats, weighing 95–105 g, were used. They were housed in an air-conditioned room at 22° and were fed a basal diet (MF; Oriental Yeast Co., Tokyo, Japan) and water *ad lib.* for a minimum of 1 week. All animals were fasted 24 hr before the experiments to minimize the effects of electrolyte absorption from the bowel and to stabilize the urinary excretion of electrolytes. The rats were killed at 0, 12, or 24 hr after a single dose of 0, 5, 50 or 200 mg Pb/kg as lead acetate had been administered orally in 10 ml (per kg body weight) of water or 25% (v/v) ethanol solution by means of stomach tube. The animals were housed two to a cage and the urine was collected.

Preparation of microsomes. The animals were anesthetized with ether and killed by cardiac puncture. The kidneys were removed and immediately placed in ice-cold 0.25 M sucrose. Renal microsomes were prepared in ice-cold 0.25 M sucrose according to the method of Jørgensen [2]. The heavy microsomal fraction was used in this experiment. The preparations (1 mg protein/ml of 0.25 M sucrose) were stored in a freezer (−20°).

Analyses. Creatinine and urea-nitrogen in urine and serum were determined according to the routine laboratory methods. Concentrations of Na^+ and K^+ were estimated in urine and serum by the method of Willis [3] using a Hitachi model 518 digital atomic absorption spectrophotometer. Renal ($Na^+ + K^+$)ATPase activity was assayed according to the method of Jørgensen [2]. Renal acid and alkaline phosphatase activities were determined according to the method of Linhardt and Walter [4] using *p*-nitrophenylphosphate as the substrate.

Materials. Adenosine-5′-triphosphate (ATP) (disodium salt) was from P-L Biochemicals, Inc. (New York, NY, U.S.A.) Ouabain was obtained from Merck & Co. (Darmstadt, Germany). Disodium ATP was converted to the Tris salt by passage through a chilled column of Dowex 50-X8 cation exchange resin in the Tris form.

Statistical analysis. Differences between control and experimental groups of animals were analyzed by Student's *t*-test.

RESULTS

Effect of ethanolic lead ingestion on urinary sodium excretion. Urinary Na^+ excretion after ethanolic lead ingestion was compared to that after aqueous lead ingestion. Urinary Na^+ excretion was elevated proportionally by logarithmically increasing doses of the aqueous lead solution, but when ethanolic lead was ingested the elevation by lead was significantly less, as shown in Fig. 1. In contrast urinary K^+ excretion was only slightly increased by logarithmically

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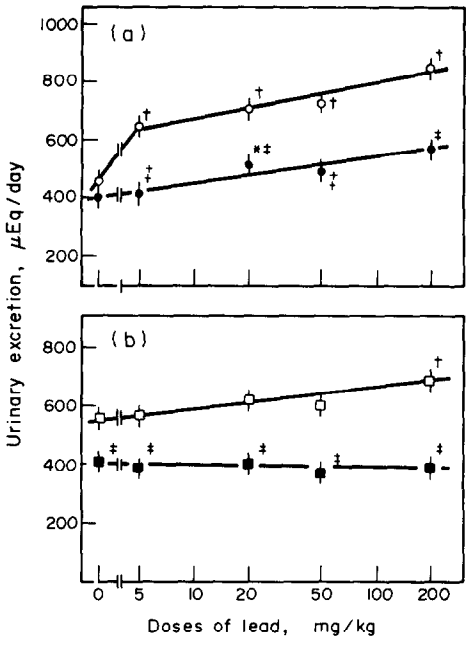

Fig. 1. Responses of urinary Na^+ and K^+ to aqueous or ethanolic lead ingestion. Lead acetate (0–200 mg Pb/kg) was administered orally at time 0 as an aqueous or 25% (v/v) ethanolic solution [200 mg Pb/kg (p.o.)] = 1/20 MLD (p.o. as Pb) for lead acetate [5]]. Urine was collected from 0–12 hr and from 12–24 hr after aqueous or ethanolic lead administration. Triplicate determinations were done for each pair of rats. The values are average data from three pairs of rats (Panel N = six rats per group); standard errors are indicated by bars. Panel A: Urinary Na^+ excretion of rats treated with aqueous lead (○) or with ethanolic lead (●). Panel B: Urinary K^+ excretion of rats treated with aqueous lead (□) or with alcoholic lead (■). The asterisk and the single daggers indicate values significantly different from the respective control (without lead). (*) $P < 0.05$ and (†) $P < 0.02$. The double daggers indicate values that were significantly different from the values for the corresponding doses of aqueous lead: (‡) $P < 0.02$.

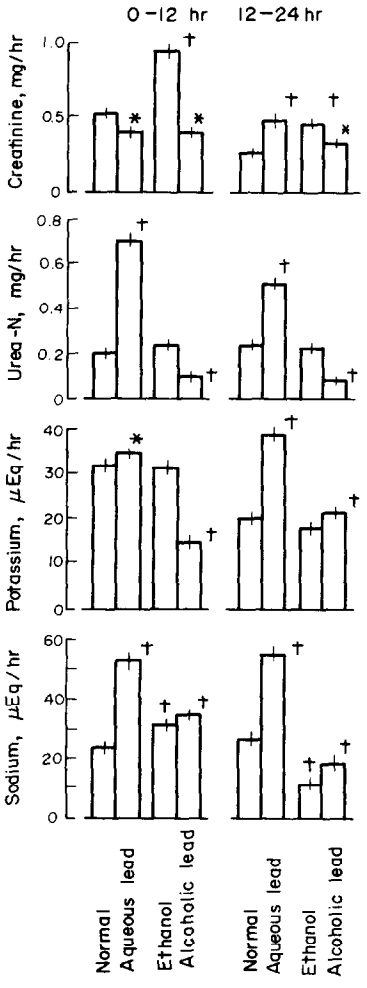

Fig. 2. Effects of aqueous of ethanolic lead administration on urinary excretion of Na^+, K^+, urea-nitrogen and creatinine. Lead acetate (200 mg Pb/kg) was administered orally at time 0 as an aqueous or 25% (v/v) ethanolic solution. Values are average data from three pairs of rats (N = six rats per group); standard errors are indicated by bars. Asterisks and daggers indicate values significantly different from normal: (*) $P < 0.05$ and (†) $P < 0.02$.

increasing doses of aqueous lead solution, and after ethanolic lead ingestion, elevation of urinary K^+ did not occur.

The relationship between kidney function and changes in urinary Na^+ excretion after ingestion of lead and/or ethanol solution (25%, v/v) was examined by measuring the urinary excretion of urea-nitrogen and creatinine. As shown in Fig. 2, ethanol did not change urinary urea-nitrogen. After aqueous lead ingestion, urinary urea-nitrogen markedly increased, in association with an increase of urinary Na^+ excretion. Elevations of the urinary excretion of Na^+ and urea-nitrogen after lead ingestion were suppressed significantly by the simultaneous administration of ethanol. Urinary Na^+ excretion was decreased slightly, compared to the normal level, 12–24 hr after ethanolic lead administration. The urinary Na^+ excretion for 1 day in rats given ethanolic lead, however, did not change in comparison to the normal level, but it was slightly enhanced compared to ethanol-treated control rats, as shown in Fig. 1.

Urinary creatinine excretion was elevated in both 12 hr periods after ethanol ingestion (Fig. 2). It was depressed significantly in the first 12 hr after aqueous lead ingestion and, then, increased slightly. Simultaneous administration of ethanol markedly diminished the increment in urinary creatinine excretion that followed ingestion of ethanol alone. After aqueous or ethanolic lead ingestion, changes in urinary K^+ excretion were not associated with changes in urinary sodium excretion.

Effect of ethanolic lead ingestion on serum Na^+ *concentration*. Serum Na^+ concentration was determined in association with changes in urinary Na^+ excretion. Serum Na^+ concentration decreased linearly with logarithmically increasing doses of aqueous lead, but after ethanolic lead ingestion this decrease diminished (Fig. 3). The decreases in serum Na^+ concentration, after either an aqueous or an

ethanolic lead doses (Fig. 3) were associated with elevation of urinary sodium excretions (Fig. 1).

Moreover, creatinine and urea-nitrogen in serum were determined to examine the relationship between kidney function and the decrease in serum Na^+ caused by aqueous or ethanolic lead. As shown in Fig. 4, a change in serum creatinine at 24 hr after aqueous lead ingestion was associated with a change in serum sodium, but serum creatinine was not altered very much by aqueous, and not at all by ethanolic, lead administration at 12 or 24 hr after administration. It appears, therefore that the glomerular filtration apparatus was not damaged by these doses. Serum urea-nitrogen, however, increased markedly 24 hr after aqueous or ethanolic lead administration. The augmentation was, perhaps, due to suppression of urea-nitrogen reabsorption by the proximal tubules which may have been damaged by the aqueous or ethanolic lead. The profile of serum Na^+ concentrations 24 hr (but not 12 hr) after the various doses was similar to the corresponding profile of serum K^+ concentration.

Relationship between renal ($Na^+ + K^+$)*ATPase activity and urinary* Na^+ *excretion in rats after ethanolic lead ingestion.* ($Na^+ + K^+$)ATPase activity is known to play a very important role in the transport of sodium ions. Lead is reported to be an inhibitor of ($Na^+ + K^+$)ATPase [6] and K^+-phosphatase [7, 8]. This suggests that the elevation of urinary Na^+ excretion, after lead ingestion, may have been due to a decrease in ($Na^+ + K^+$)ATPase activity.

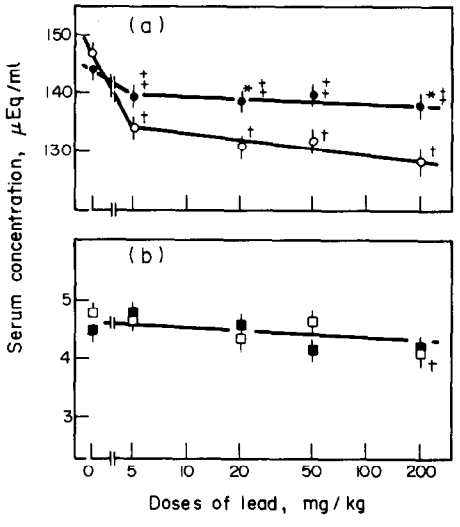

Fig. 3. Responses of serum Na^+ and K^+ to aqueous or ethanolic lead administration. Lead acetate (0–200 mg Pb/kg) was administered by stomach tube at time 0 as an aqueous or 25% (v/v) ethanolic solution. The rats were killed 24 hr later. Values are average data of six rats; standard errors are indicated by bars. Panel A: Serum Na^+ of rats treated with aqueous lead (○) or with ethanolic lead (●). Panel B: Serum K^+ of rats treated with aqueous lead (□) or with ethanolic lead (■). An asterisk or a single dagger indicates a value significantly different from the respectively control (without lead). (*) $P < 0.05$ and (†) $P < 0.02$. A double dagger indicates a value significantly different from the corresponding dose of aqueous lead: (‡) $P < 0.02$.

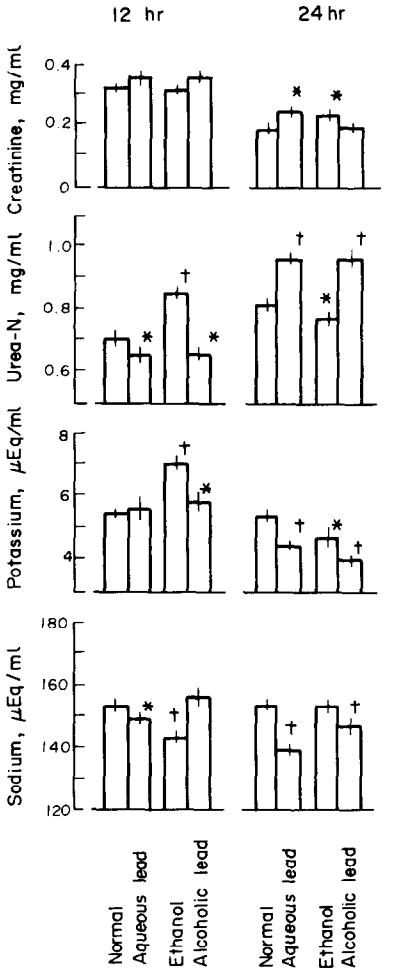

Fig. 4. Effects of aqueous or ethanolic lead on Na^+, K^+, urea-nitrogen, and creatinine in serum. Lead acetate (200 mg Pb/kg) was administered by stomach tube at time 0 as an aqueous or 25% (v/v) ethanolic solution. The rats were killed 12 and 24 hr later. Values are average data of six rats; standard errors are indicated by bars. Asterisks and daggers indicate values normal: (*) $P < 0.05$ and (†) $P < 0.02$.

Renal ($Na^+ + K^+$)ATPase activity was determined and compared with renal acid and alkaline phosphatase activities in rats that had ingested aqueous or ethanolic lead solution (Table 1). Renal ($Na^+ + K^+$)ATPase and acid phosphatase activities were changed significantly by ingesting the 25% ethanolic solution the ATPase activity having increased and the ACP activity having decreased. The renal ($Na^+ + K^+$)ATPase activity, alone among these enzyme activities, decreased markedly aqueous or ethanolic lead ingestion. Renal Mg^{2+}-ATPase activity was not changed significantly 12 and 24 hr after aqueous or ethanolic lead ($P > 0.05$).

The response of renal ($Na^+ + K^+$)ATPase activity to logarithmically increasing doses of lead was examined 12 and 24 hr after aqueous or alcoholic lead ingestion (Fig. 5). At 12 hr after ingesting aqueous lead renal ($Na^+ + K^+$)ATPase activity exhibited a linear decrease as dose increased logarithmically.

Table 1. Effects of lead or ethanol administration on renal $(Na^+ + K^+)$ATPase and phosphatase activities*

		$(Na^+ + K^+)$ATPase†	Mg^{2+}-ATPase†	ALPase‡	ACPase‡
Aqueous	Normal (N)	419 ± 7	291 ± 25	43.4 ± 2.3	22.3 ± 1.3
	Lead (L)	190 ± 25	279 ± 23	34.1 ± 2.0	23.8 ± 2.3
	L/N	0.45§	0.96	0.79§	1.07
Ethanolic	Control (C)	594 ± 66 (1.33)‖	299 ± 9 (1.03)	48.4 ± 3.8 (1.16)	17.3 ± 1.2 (0.78)‖
	Lead (L)	429 ± 63 (1.03)‖	306 ± 9 (1.05)	39.5 ± 2.4 (0.91)	22.4 ± 2.4 (1.06)
	L/C	0.72¶	1.02	0.82¶	1.29§

* Rats were killed 24 hr after an aqueous or ethanolic dose of lead (200 mg Pb/kg (p.o.) = 1/20 MLD (p.o.) as lead acetate [5] in water (10 ml/kg, p.o.) or 25% (v/v) ethanol (10 ml/kg, p.o.) respectively or of water or 25% ethanol as control. All values are averages of six rats ± S.E. Numbers in parentheses are relative values against normal rats.

† $(Na^+ + K^+)$ATPase and Mg^{2+}-ATPase activities are expressed as Pi liberated [nmoles·(mg protein)$^{-1}$·min^{-1}].

‡ ALPase (alkaline phosphatase) and ACPase (acid phosphatase) activities are expressed as *p*-nitrophenol liberated [nmoles·(mg protein)$^{-1}$·min^{-1}].

§ Significantly different from the respective control, $P < 0.02$. The controls were the values for aqueous or ethanolic lead-administered rats.

‖ Significantly different from the corresponding aqueous dose, $P < 0.02$.

¶ Significantly different from the respective control, $P < 0.05$. The controls were the values for aqueous or ethanolic lead-administered rats.

The magnitude of the decrease in activity was reduced by ethanol (Fig. 5A). At 24 hr after aqueous lead ingestion, the effect on renal $(Na^+ + K^+)$ATPase activity returned gradually to normal, whereas the renal enzyme activities from ethanol, or ethanolic lead, ingesting rats were elevated above the normal level (without these treatments.) Thus, stimulation of renal $(Na^+ + K^+)$ATPase by ethanol diminished the enzyme activity due to lead [Fig. 5(A and B)]. Reductions in renal $(Na^+ + K^+)$ATPase activity at 12 hr but not 24 hr, after an aqueous or ethanolic lead dose were associated with corresponding elevations of urinary sodium excretion and corresponding depressions of serum Na^+ concentrations caused by the lead in the aqueous or ethanolic lead solutions. The elevation of enzyme activities 24 hr after ethanolic lead or ethanol alone is an interesting observation.

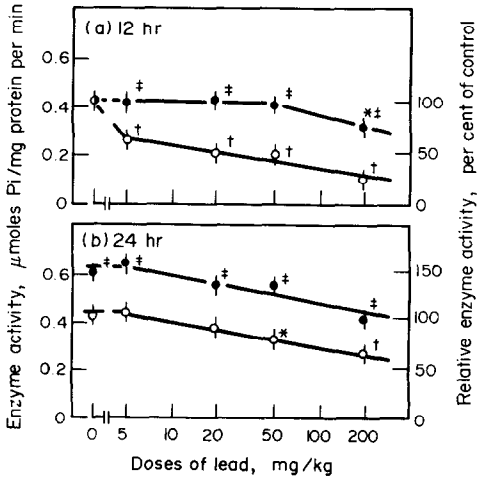

Fig. 5. Response of renal $(Na^+ + K^+)$ATPase activity to aqueous or ethanolic lead administration. Rats were killed 12 hr (A) or 24 hr (B) after a single dose of lead acetate (0–200 mg Pb/kg) as an aqueous or 25% (v/v) ethanolic solution. Values are average data for six rats; standard errors are indicated by bars. Key: renal $(Na^+ + K^+)$ATPase activity in rats treated with aqueous lead (○) or with ethanolic lead (●). As asterisk or a single dagger indicates a value significantly different from the respective control (without lead). (*) $P < 0.05$ and (†) $P < 0.02$. A double dagger indicates a value significantly different from the corresponding dose of aqueous lead: (‡) $P < 0.02$.

DISCUSSION

In this study the elevation of urinary Na^+ excretion caused by lead [9] was reduced by ethanol. Moreover, the increment in urinary K^+ excretion produced by lead was completely prevented by ethanol. The changes in urinary excretions after aqueous or ethanolic lead ingestion alterations in urinary urea-nitrogen excretion. These results suggest that lead damages the proximal tubules and that this damage was diminished by ethanol.

Renal $(Na^+ + K^+)$ATPase activity decreased after administration of lead alone, and this decrease was prevented by alcohol. Jørgensen [2] had indicated that renal $(Na^+ + K^+)$ATPase activity is reduced after adrenalectomy but returns to normal with aldosterone treatment. Ellis [10] has shown that ethanol administration causes stimulation of adrenocortical function. The possibility exists, therefore, that in our experiments ethanol ingestion caused stimulation of adrenocortical function, which resulted in elevation of renal $(Na^+ + K^+)$ATPase activity. Chronic ethanol administration produces an increase in $(Na^+ + K^+)$ATPase activity in the rat [11] and the cat [12] brain. Rangaraj and Kalant [13] recently suggested that the increase in $(Na^+ + K^+)$ATPase in rat brain may be a result of the stress of alcohol withdrawal rather than an adaptive response to chronic ethanol exposure.

On the other hand, Goyer [14] reported that lead administration caused hyperaminoaciduria. The hyperaminoaciduria was postulated to be due to damage of the proximal tubule by lead.

In the present study, it was demonstrated that urinary sodium excretion was associated with renal $(Na^+ + K^+)$ATPase activity after aqueous or ethanolic lead ingestion.

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