Effect of Cadmium on Sodium and Potassium Excretion and on Action of Hydrochlorothiazide in Rats

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Abstract □ Cadmium administered to rats decreased urinary sodium excretion. Single doses of 1.0 mg/kg or greater were equally effective. Doses of 0.5 mg/kg or less and 20 multiple doses of 0.5 mg/kg had no effect. A single dose was effective for only a limited time. The greatest decrease in sodium excretion occurred at a time that was 0–12 hr after injection, but an effect was still seen at 24 hr and up to 7 days. The action of hydrochlorothiazide was not altered by either a single 10-mg/kg dose or 20 0.5-mg/kg doses of cadmium. An effect of cadmium on potassium excretion was seen but was less evident than the effect on sodium excretion. The concentration of cadmium in the kidneys was not directly related to its effect on sodium excretion, indicating that the biochemical form and not the amount is the determinative factor. It is postulated that cadmium exerts its effect by an indirect mechanism and not by direct action on the nephron.

Keyphrases □ Cadmium—effect on sodium and potassium excretion and action of hydrochlorothiazide, rats, mechanism of action □ Hydrochlorothiazide—effect of single and multiple doses of cadmium, rats □ Sodium, excretion—effect of single and multiple doses of cadmium □ Potassium, excretion—effect of single and multiple doses of cadmium

Two major reviews (1, 2) within the last 2 years dealing with the heavy metal cadmium emphasized the growing hazard resulting from environmental contamination by this trace metal. Analyses of human tissues have shown that about one-third of the cadmium normally present in the body is in the kidneys (3). Although fairly high amounts must be accumulated before renal damage occurs (4–6), the question arose as to whether the presence of cadmium might alter the action of drugs that exert their primary effect on the kidneys. Since the most important of these drugs are the diuretics, this investigation was initiated to determine if single or multiple doses of cadmium would alter the action of a commonly prescribed diuretic, hydrochlorothiazide, as measured by the excretion of sodium in the urine. Since diuretics increase potassium excretion as well, this ion was also studied.

Vander (7, 8), who infused cadmium into anesthetized dogs undergoing saline diuresis, found that during the first 2 hr cadmium caused a decrease in sodium excretion and, less consistently, a decrease in potassium excretion. However, his studies were conducted over relatively short time intervals. Also, cadmium was infused directly into the kidney in a mixture containing cysteine, which is known to bind cadmium. This paper reports studies of the effect of intraperitoneally administered cadmium on sodium excretion in the intact rat. The relationship between the dose of cadmium administered and the decrease in sodium excretion produced as well as the time during which a single dose of cadmium is effective in altering sodium excretion was investigated.

EXPERIMENTAL

Animals—Adult female rats[1], descendants of the Sprague–Dawley strain, were used. The rats weighed 190–210 g at the start of the experiments. They were housed individually in metal cages in temperature- and humidity-controlled rooms having a 12-hr day/night cycle, and they were allowed free access to food[2] and tap water except during urine collection. Prior to the experiments, the animals were acclimated for at least 1 week under these conditions.

Radionuclides—Both ^{24}Na and ^{42}K were obtained as high specific activity chloride salts in water. Radionuclidic purities were checked by comparison of the γ-spectra to reference spectra and by half-life determinations. The injection solution for ^{24}Na was prepared so that 0.2 ml contained about 20 μCi of ^{24}Na and 0.0018 mg of sodium chloride as carrier at the time of injection. The injection solution for ^{42}K was prepared so that 0.2 ml contained about 100 μCi of ^{42}K and 0.01 mg of potassium chloride as carrier. For both ^{24}Na and ^{42}K, the dose was 0.2 ml, which was given intraperitoneally.

Cadmium—The cadmium injection solutions were prepared by dissolving cadmium acetate in doubly distilled water. All doses were based on the cadmium ion, not the salt, and were given intraperitoneally.

Hydrochlorothiazide—The drug[3] was obtained as a white crystalline powder. It was given as a suspension in 0.5 ml of water. The dose chosen was 220 mg/kg/day given as divided doses every 8 hr.

Counting Equipment—Polyethylene bottles containing the urine samples were counted in a large well-type NaI(Tl) scintillation crystal housed in a 15.24-cm thick steel shield lined with lead and stainless steel. All samples were counted in the integral mode for a time sufficient to obtain a counting error less than 1%.

Kidney Analysis for Cadmium—Both kidneys from each animal were digested by heating with concentrated nitric acid. Cadmium concentration was determined by atomic absorption spectrophotometry.

Effect of Amount of Cadmium Given as a Single Dose on Sodium Excretion—Forty-two rats were injected with ^{24}Na and were placed immediately in individual metal metabolism cages where neither food nor water was provided. After 12 hr equilibration, the animals were divided randomly into seven groups of six animals. The dose of cadmium given to six of the groups was 8.0, 4.0, 2.0, 1.0, 0.5, or 0.1 mg/kg. The remaining group served as a control and received doubly distilled water. Urine was collected for 24 hr following cadmium administration. All animals were hydrated with 5 ml of doubly distilled water *per os* at the time of cadmium administration and at 8 and 16 hr. After completion of the experiment, the animals were sacrificed with chloroform and their kidneys were removed for cadmium analysis. The urine samples were counted as previously described.

Time during which a Single Dose of Cadmium Is Effective in Altering Sodium Excretion—Forty-two rats were randomly assigned to seven groups of six animals. Six of the groups were injected with 2.0 mg cadmium/kg at one of the following times prior to ^{24}Na administration: 240, 48, 24, 12, 2, and 0 hr. The remaining group served as a control and received doubly distilled water at the same time as the last cadmium injection. All animals were injected with ^{24}Na and were placed immediately in individual metal metabolism cages where neither food nor water was provided. Urine was collected for 24 hr following the ^{24}Na administra-

[1] Laboratory Supply Co., Indianapolis, Ind.
[2] Wayne Lab-Blox, Allied Mills Inc., Chicago, Ill.
[3] Merck Sharp & Dohme, West Point, Pa.

Table I—Effect of Amount of Cadmium Given as a Single Dose on Sodium Excretion

Dose, mg/kg	^{24}Na Excreted, cpm[a]	Cadmium in Kidneys, ppm[a,b]
0.0	1,328,360 ±460,640	Not detectable
0.1	981,220 ±279,570	1.8 ± 0.8
0.5	804,330 ±283,930	4.6 ± 0.7
1.0	446,560 ±233,590	6.2 ± 1.8
2.0	323,630 ± 71,320	9.6 ± 2.6
4.0	387,240 ± 71,250	14.3 ± 5.9
8.0	347,310 ±110,150	25.7 ± 11.8
Newman–Keuls Comparison[c]		
Dose, mg/kg	2.0 8.0 4.0 1.0	0.5 0.1 0.0

[a] Mean ± standard deviation. [b] Wet basis. [c] Ranked according to increasing sodium excretion. Doses underlined did not produce a significantly different effect ($p = 0.01$).

tion. All animals were hydrated with 5 ml of doubly distilled water *per os* at the time of ^{24}Na administration and at 8 and 16 hr. After completion of the experiment, the animals were sacrificed and their kidneys were removed for cadmium analysis. The urine samples were counted as previously described.

This experiment was repeated with 36 rats randomly assigned to six groups of six animals. Five of the groups were injected with 2.0 mg cadmium/kg at one of the following times prior to ^{24}Na administration: 15, 10, 7, 4, and 2 days. The remaining group served as a control and received doubly distilled water just prior to the ^{24}Na injection.

Effect of Single and Multiple Doses of Cadmium on Sodium and Potassium Excretion and on Action of Hydrochlorothiazide—The sodium study and the potassium study were carried out with 48 animals each during time intervals about 2 weeks apart. The 48 animals in each study were randomly divided into eight groups of six animals, with four groups used in the cadmium single-dose study and four in the multiple-dose study. In both cases, the four groups received the following treatments: control, cadmium, hydrochlorothiazide, and cadmium plus hydrochlorothiazide.

In the single-dose study, the animals used in the ^{24}Na experiments were injected with 10.0 mg cadmium/kg. The animals in the ^{42}K experiments received 5.0 mg cadmium/kg because of toxicity observed in the preceding ^{24}Na study. In the multiple-dose study, the animals in both the ^{24}Na and ^{42}K experiments were

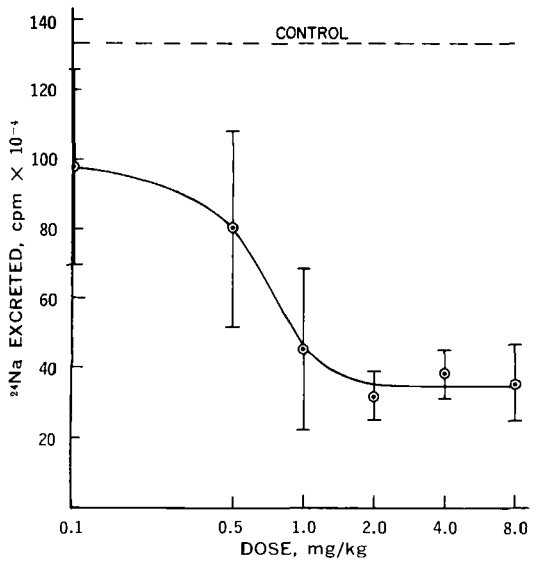

Figure 1—*Dose–response curve. Dose is plotted on a logarithmic scale. Curve was not mathematically fit.*

Table II—Time during which a Single Dose of Cadmium Is Effective in Altering Sodium Excretion

Time Interval[a] Hours	Time Interval[a] Days	^{24}Na Excreted, cpm[b]	Cadmium in Kidneys, ppm[b,c]
0		332,120 ±144,530	5.9 ± 0.8
2		354,850 ±216,070	5.6 ± 1.1
12		135,200 ±174,030	10.1 ± 1.8
24		1,005,560 ±489,840	11.6 ± 3.2
48		2,246,080 ±823,250	14.5 ± 3.1
240		982,160 ±301,550	20.0 ± 4.4
Control		2,140,495 ±997,450	Not detectable
	2	863,120 ±407,280	12.4 ± 1.5
	4	715,500 ±387,060	15.1 ± 1.8
	7	905,210 ±259,560	15.2 ± 6.8
	10	1,151,140 ±748,000	19.8 ± 5.6
	15	1,072,360 ±609,520	17.4 ± 3.6
	Control	1,654,410 ±653,240	Not detectable
Newman–Keuls Comparison for 240-hr Study[d]			
Time interval, hr	12 0 2	24 240	48

[a] Time interval between cadmium and ^{24}Na administrations. [b] Mean ± standard deviation. ^{24}Na excretion values for the two studies are not comparable because the ^{24}Na solution injected was different. [c] Wet basis. [d] Ranked according to increasing sodium excretion. Times underlined did not produce a significantly different effect ($p = 0.01$).

injected with 0.5 mg cadmium/kg every other day for 20 times. Hydrochlorothiazide was administered *per os* every 8 hr for 24 hr.

In the single-dose study, the cadmium and the first hydrochlorothiazide dose were administered 12 hr after the ^{24}Na or ^{42}K injections. In the multiple-dose study, the ^{24}Na or ^{42}K was administered 48 hr after the last dose of cadmium; the first dose of hydrochlorothiazide was given 12 hr later to allow for equilibration of ^{24}Na or ^{42}K.

Following the nuclide administration, the animals were placed in individual metal metabolism cages where neither food nor water was provided. Urine was collected for 24 hr following the first administration of hydrochlorothiazide. At 0.5 hr prior to each diuretic administration, all animals were hydrated with 5 ml of doubly distilled water *per os*. Immediately after completion of each study, all animals of the ^{42}K study and the single-dose animals of the ^{24}Na study were sacrificed and their kidneys were removed for cadmium analysis. The urine samples were counted as previously described.

RESULTS AND DISCUSSION

A preliminary study showed that the only applicable parameter for assessing the treatment effects was the ^{24}Na activity in the urine. Urine weights did not correlate with ^{24}Na activity, since cadmium had no significant effect on the weight of urine excreted while it did significantly decrease the excretion of ^{24}Na. Fasting the animals also had no significant effect on the ^{24}Na excreted by the rats receiving cadmium. However, since experiments of this kind are conventionally performed with fasted animals and since the amount of food eaten might introduce an added source of variability, it was decided to fast the animals during urine collection.

In each experiment, the net counts per minute of ^{24}Na or ^{42}K was corrected for decay to the time of nuclide administration. The data were tested for homogeneity of variance, and square root transformations were performed to achieve homogeneity.

Effect of Amount of Cadmium Given as a Single Dose on Sodium Excretion—The ^{24}Na activities of the urine samples and

Table III—Effect of Single and Multiple Doses of Cadmium on Sodium and Potassium Excretion and on Action of Hydrochlorothiazide

Single Dose				Multiple Dose			
No Diuretic		Diuretic		No Diuretic		Diuretic	
Control	Cadmium	Control	Cadmium	Control	Cadmium	Control	Cadmium
^{24}Na in Urine, cpm							
1,713,460 ±325,500	453,960 ±517,000	3,077,660 ±455,760	1,440,680 ±524,080	1,597,520 ±315,790	1,607,380 ±415,140	3,104,820 ±443,010	3,289,900 ±1,008,310
^{42}K in Urine, cpm							
1,869,750 ±623,270	1,922,970 ±641,450	1,904,140 ±360,510	2,275,140 ±364,120	1,682,910 ±120,820	1,817,060 ±358,880	2,041,400 ±445,200	2,574,700 ±306,410

the cadmium concentrations in the kidneys are shown in Table I. A one-way analysis of variance on the ^{24}Na activities showed a significant (p = 0.01) dose effect. A Newman–Keuls comparison (Table I) showed two distinct groups, with no significant differences (p = 0.01) within each group. The two lowest doses of 0.1 and 0.5 mg/kg fell in the group with the controls, indicating no cadmium effect. The other four doses fell in the second group, indicating the same degree of sodium retention for doses of 1.0 mg/kg or greater. A dose–response curve (Fig. 1) shows a sharp fall corresponding to the break between the two groups and a flat portion corresponding to the leveling off of the effect at high doses.

The concentration of cadmium in the kidneys steadily increased with the administered dose. A correlation test performed to relate the kidney concentration of cadmium to the amount of sodium excreted did not show a significant relationship even at p = 0.10. Although these kidney concentrations of cadmium were determined after the completion of the sodium excretion study and are not the concentrations that existed during the study, it is assumed that the relationship between dose level and kidney concentration was essentially the same both during and after the study.

Time during which a Single Dose of Cadmium Is Effective in Altering Sodium Excretion—The results of the two time studies are shown in Table II and Fig. 2. The data for each study were analyzed separately by a one-way analysis of variance. There was a significant time effect (p = 0.01) for the 240-hr study but no time effect (p = 0.10) for the 15-day study.

For the 240-hr study, a Newman–Keuls comparison (Table II) showed the greatest sodium retention with animals injected 0, 2, and 12 hr prior to receiving ^{24}Na and the least retention with the 48-hr animals (p = 0.01). The animals injected at 24 and 240 hr had intermediate values of sodium retention. A two-tailed t test to compare the ^{24}Na excretion of the control group with the excretions of the 24-, 48-, and 240-hr groups showed significant differences (p = 0.05) for the 24- and 240-hr groups but not for the 48-hr group. For the 15-day study, the values for the 2-, 4-, and 7-day groups differed significantly from the control, whereas the values for the 10- and 15-day groups did not.

A correlation test to relate the kidney concentration of cadmium (Table II) to the excreted sodium was run separately for both studies. No significant (p = 0.10) relationship was found for either study. Again, these kidney concentrations of cadmium were determined after the completion of the sodium excretion study. However, it was previously found (9) that the levels of cadmium in the kidney do not change appreciably between 8 and 240 hr.

Although the results of the statistical analyses are somewhat inconsistent for the two studies, it is clear that cadmium greatly affects sodium excretion within the first 12 hr after administration (Fig. 2). It appears that beyond 12 hr and up to 7 days it is still exerting an appreciable effect. Beyond 7 days, a significant effect could not be shown statistically.

Effect of Single and Multiple Doses of Cadmium on Sodium and Potassium Excretion and on Action of Hydrochlorothiazide—The ^{24}Na and ^{42}K activities of the urine samples are shown in Table III. Since the sum of the multiple doses of cadmium equaled the single dose, the ^{24}Na excretion data were analyzed by an analysis of variance for a three-factor factorial design. The three factors were: *(a)* type of dosing, single or multiple; *(b)* diuretic, presence or absence; and *(c)* cadmium, presence or absence. A Newman–Keuls comparison was made where necessary. The single dose of cadmium significantly increased (p = 0.01) the retention of sodium, whereas the multiple doses did not. This effect was obtained in spite of the fact that the levels of cadmium in the kidneys of the single-dose animals were about 30 ppm, wet weight, while those of the multiple-dose animals were about 150 ppm. The increase in sodium excretion produced by the diuretic was expected because of the known properties of the diuretic. Most important was the finding that the cadmium treatment and diuretic treatment each exerted its effect independently of the presence of the other. Therefore, the presence of cadmium in the kidney, even at the high concentrations following multiple dosing, did not alter (p = 0.01) the action of hydrochlorothiazide.

The ^{42}K excretion data were analyzed separately for the single- and multiple-dose studies by an analysis of variance for a two-factor factorial design since the sum of the multiple doses did not equal the single dose. The two factors were cadmium and diuretic each at two levels, absence or presence. For the single-dose study, neither cadmium nor diuretic had any effect on potassium excretion at any significance level. For the multiple-dose study, both cadmium (p = 0.05) and the diuretic (p = 0.01) caused increased potassium excretion.

Hydrochlorothiazide would be expected to exert at least a minimal effect on potassium excretion under the experimental conditions. The discrepancy in its action in the two studies cannot be explained.

For the multiple-dose study, the F value for the interaction between the cadmium and diuretic treatments approached significance at the 0.10 level and was significant at the 0.25 level. For this reason, a Newman-Keuls comparison of the means was performed. Only the combination of cadmium and diuretic had a significant effect (p = 0.05).

For the single-dose study, it was observed that the difference in means for the group receiving cadmium plus diuretic and for the group receiving only diuretic was about the same as the difference for the respective means in the multiple-dose study. The conclu-

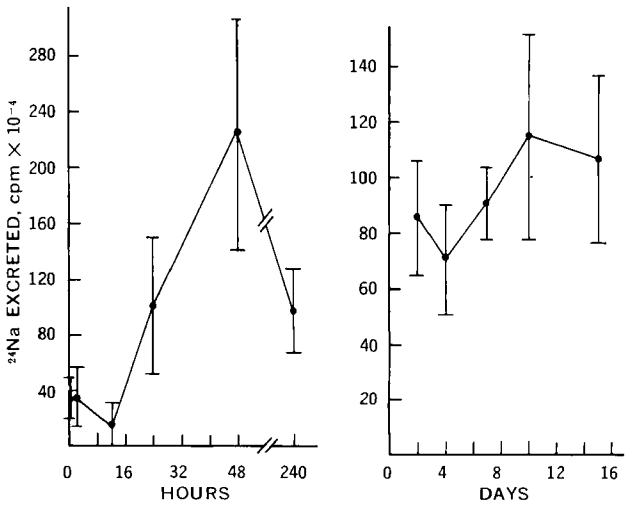

Figure 2—*Time–response curves. ^{24}Na excretion values for the two studies are not comparable because the ^{24}Na solution injected was different.*

sion drawn from these results is that both cadmium and the diuretic affect potassium excretion to a slight degree and, when both are given together, they exert an additive effect for which it may or may not be possible to show statistical significance.

The cadmium concentrations in the kidneys of the single-dose animals in the ^{42}K study were about 15 ppm, wet basis. These were about half the concentrations in the single-dose ^{24}Na study where the animals received a dose of cadmium twice as high. The concentrations for the multiple-dose animals were about 150 ppm, as previously stated.

CONCLUSIONS

The antinatriuretic effect found in the animals that received cadmium as a single dose of 10.0 mg/kg may have been due to the same mechanism, a direct effect on the proximal tubule, as that postulated by Vander (8) to explain his observations following infusion of 0.025 mg/kg into anesthetized dogs. More recent evidence would suggest, however, that cadmium may affect sodium excretion indirectly through stimulation of the renin–aldosterone system. Renin production is known to be stimulated by a fall in blood pressure, and there is evidence that cadmium may decrease blood pressure. Youkilis *et al.* (10) found that 3 mg/kg produced significant *in vivo* dilatation of venules and arterioles of innervated bat wings, and Scott and Haddy (11) found decreased peripheral resistance in canine limbs during infusion of $3\text{–}24 \times 10^{-4}$ *M* cadmium ion. The increased renin production following the fall in blood pressure would cause an increase in aldosterone production. Sodium retention would thus be increased after cadmium treatment by the action of the higher levels of aldosterone on the distal tubule.

The lack of any effect of 20 multiple cadmium doses of 0.5 mg/kg on sodium excretion is not surprising in view of the protective action of pretreatment with small doses of cadmium against the toxicity of a single large dose (12, 13). This detoxification has been attributed to the formation of a complex between cadmium ions and a low molecular weight protein known as metallothionein (14). The discovery of increased amounts of metallothionein in the livers (14) and blood (15) of animals exposed to cadmium may explain why a single dose of cadmium affects sodium excretion whereas multiple smaller doses do not, since the basal level of metallothionein is not sufficient to handle all of the ions introduced in a single large dose. Thus, even though the cadmium concentration was much higher in the kidneys of animals receiving the multiple doses, the cadmium was not physiologically free to exert its effect.

The increased excretion of potassium which was apparent but not statistically significant in the animals that received cadmium as a single dose of 5.0 mg/kg can be explained as the direct consequence of the increased reabsorption of sodium since aldosterone is known to cause exchange of sodium, which is reabsorbed, for potassium, which is excreted. The apparent increase in excretion of potassium in the animals that received multiple doses of cadmium cannot be similarly explained since no effect on sodium excretion was found in these animals.

If the proposed mechanism for the effect of cadmium on electrolyte excretion is correct, cadmium would not be expected to alter the action of any diuretic, regardless of its site of action in the nephron. However, further studies with other diuretics are necessary before such a conclusion can be drawn.

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