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Effects of Lead on the Secretion and Disappearance of Renin in Rabbits[1]

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Effects of Lead on the Secretion and Disappearance of Renin in Rabbits. KEISER, J. A., VANDER, A. J., AND GERMAIN, C. L. (1983). *Toxicol. Appl. Pharmacol.* **69**, 117–126. The disappearance rate of renin from plasma was evaluated in both acutely and chronically lead-exposed rabbits. In addition, the effects of lead (Pb) on *in vitro* renin secretion were determined with rabbit renal cortical slices. Rabbits acutely exposed to Pb (0.3 to 2.0 mg/kg, iv) demonstrated no increase in plasma renin activity (PRA), but a markedly prolonged disappearance of renin following nephrectomy. Together, these observations suggest that renin secretion must have been inhibited; consistent with this hypothesis was the finding that rabbit renal cortical slices exposed to Pb (10^{-5} or 10^{-6} M) *in vitro* secreted significantly less renin than did controls. Thus, the effects of large acute doses of Pb in the rabbit are simultaneous inhibition of both renin secretion and clearance. Chronically Pb-exposed rabbits (500 or 1000 ppm in drinking water) had renin half-lives that were not different from controls (6 to 8 min). PRA was also not significantly different in the three groups. Renal slices from both groups of Pb-exposed rabbits secreted significantly more renin *in vitro* compared to controls, despite the fact that renal renin concentrations were similar in the three groups. However, the responsiveness to a beta adrenergic stimulus was significantly lower in the slices from rabbits treated with 1000 ppm Pb. Taken together these data suggest that PRA in the chronically Pb-exposed rabbit reflects a tendency for increased basal renin secretion, but a counteracting suppression of renin release secondary to adrenergically mediated stimuli; thus, PRA might be reduced, unchanged, or elevated depending upon experimental conditions. Clearance of renin does not seem to be altered in the chronically Pb-exposed rabbit.

Studies of the renin–angiotensin system in dogs after acute (iv) lead (Pb) exposure have demonstrated profound elevations in plasma renin activity (PRA) associated with a decreased hepatic removal of renin and no consistent changes in renin secretion (Goldman *et al.*, 1981). Significant elevations in PRA have also been reported in rats following both acute and chronic Pb exposure (Fleischer *et al.*, 1980; Mouw *et al.*, 1978; Victery *et al.*, 1982a). These observations have led to the hypothesis that the abnormal PRA found in the chronically Pb-exposed animal might be the result of a defect in the removal of renin rather than a change in renin secretion (Goldman *et al.*, 1981).

The present experiments were performed to evaluate this hypothesis by determining the effects of chronic lead exposure on the disappearance of endogenous renin following nephrectomy and on the secretion of renin from renal cortical slices *in vitro*. The rabbit, rather than rat or dog, was selected as the experimental animal because of its convenient size. The effects of Pb on the rabbit's

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renin–angiotensin system have not previously been examined; therefore, we first determined whether acute exposure to Pb inhibited renin clearance in a manner similar to that described for dog (Goldman *et al.*, 1981).

METHODS

All experiments were performed on male New Zealand white rabbits (*Oryctolagus cuniculus*), Spartan Labs, Lansing, Mich.) weighing 2.0 to 2.5 kg. Animals were housed in individual wire-floored cages in temperature-controlled rooms (24°C) on a fixed light schedule (0600–1800). Food (Teklad rabbit chow; Madison, Wisc.) and water were available *ad libitum*. Rabbits used for acute experiments were kept at least 1 week prior to use. Chronic animals were kept for 1 week before being allocated to specific treatment groups.

Protocol 1. Effects of Acute Pb Exposure on the Disappearance of Renin

Three days prior to the renin-disappearance experiments each rabbit was anesthetized with a combination of xylazine (Rompun, Cutter Laboratories; 10 mg/kg, im) and ketamine hydrochloride (Vetalar, Parke Davis; 35 mg/kg, im). A midline incision (5 to 6 cm) was made on the ventral aspect of the neck, and the left external jugular vein was exposed and cannulated. The cannula was passed sc into the ear lobe and exteriorized. On the day of the experiment an unanesthetized rabbit was placed in a restrainer and given an infusion of Pb acetate (0.3 to 2.0 mg/kg; Pb treated) or an equimolar solution of sodium acetate (TC; time-control) via the jugular catheter. Solutions were made by dissolving either Pb or sodium acetate in sterile saline; the total volume infused was 2.0 ml/kg, and was given over approximately 50 min with a Harvard infusion pump. Two hours after completion of the infusion the rabbit was again anesthetized with xylazine and ketamine. An abdominal midline incision was made, and a catheter (PE-160) was inserted into the abdominal aorta proximal to its bifurcation. The right and left kidneys were isolated and cleared of connective tissue; loose ligatures were placed around the renal artery and vein on each side. A single blood sample (0.5 ml) was drawn from the aortic catheter and replaced with an equal volume of saline. Four to five minutes later the vessels to both kidneys were firmly tied and the kidneys removed from the abdomen. A simultaneous blood sample (0.5 ml), designated the zero sample, was withdrawn; additional samples were drawn at 2, 4, 6, 8, 10, 15, 20, 30, 40, and 50 min postnephrectomy. At completion of the protocol the animal was killed with an iv injection of sodium pentobarbital (120 mg/kg).

All blood samples were collected in tubes containing 0.05 ml of disodium ethylenediaminetetraacetate (0.2 M); samples for measurement of angiotensin converting enzyme (ACE) activity were collected in heparin (1000 μ/ml). Samples were chilled, centrifuged, and separated at 4°C, and the plasma was frozen for subsequent analysis.

Protocol 2: Effects of Chronic Pb Exposure on the Disappearance of Renin

Rabbits were randomly allocated to treatment groups of 0 (n = 5), 500 (n = 7), or 1000 (n = 5) ppm Pb (as Pb acetate) added to the drinking water. No attempt was made to determine any other Pb burden imposed by the air, food, or water available to the animals. During the seventh week of Pb treatment, rabbits were subjected to the same renin-disappearance experiments previously described (Protocol 1), except that the experiments were not preceded by acute Pb infusions.

Protocol 3: Effects of Chronic Pb Exposure on the Secretion of Renin in Vitro

In this series of experiments cortical slices were made from the kidneys of the rabbits described in Protocol 2 above. After nephrectomy the left kidney was placed in a beaker of ice-cold isotonic saline. The renal artery was cannulated and the kidney flushed with cold saline until the venous effluent became clear. Renal cortical slices (0.5-mm thick) were cut by hand with a Stadie–Riggs slicing apparatus. Slices were cut perpendicular to the surface of the kidney. The cortical slices were preincubated in a common flask containing 150 ml of a Krebs–Ringer bicarbonate buffer (124 mM NaCl, 5 mM KCl, 19 mM $NaHCO_3$, 2.6 mM $CaCl_2$, 1.2 mM NaH_2PO_4, and 0.2 g/dl glucose) aerated with 95% O_2 and 5% CO_2 at 38°C for 45 min. The medium was replaced 15 and 30 min into the preincubation with fresh prewarmed solution.

At the end of the preincubation, slices were randomly allocated to one of three treatment groups; 5 mM K^+, 35 mM K^+, or 35 mM K^+ with 10^{-6} M isoproterenol. Individual flasks containing 7.5 ml of buffer were incubated in a Dubnoff metabolic shaker bath at 38°C. A small aliquot (0.05 ml) of concentrated KCl was added to the flasks of the last two groups to achieve a final concentration of 35 mM K^+; in addition, the flasks of the final group received 0.075 ml of 10^{-4} M isoproterenol to achieve the final concentration of 10^{-6} M. Medium samples were collected into chilled tubes at 150 min. The samples were immediately centrifuged at 4°C to remove small tissue debris, and the supernatant fraction was stored at −30°C for later analysis of its renin concentration. At the end of the incubation period the slices were quickly removed, blotted, and placed in tared chilled tubes containing 0.5

ml of saline. The tubes were reweighed and stored at −30°C.

Protocol 4: Effects of in Vitro Pb Exposure on Renin Secretion by Cortical Slices

In this series of experiments renal cortical slices were exposed to Pb *in vitro* during the incubation period. Kidneys were obtained from rabbits which had undergone no previous treatment. Slices were prepared as described in Protocol 3. Slices were preincubated and incubated in a solution having the same composition as that of protocol 3, except the KCl concentration was 25 mM (this value was used to provide somewhat higher renin-secretion values than seen at 35 mM so that it would be possible to see any potential Pb-induced inhibition of secretion). Following the preincubation, slices were randomly allocated to one of three treatment groups; control, 10^{-5} M Pb, or 10^{-6} M Pb. A 10^{-3} M Pb acetate stock solution was made in deionized distilled water, and the pH was adjusted to 5.5 to prevent precipitation. Aliquots (0.075 ml) of this stock or of a 10-fold dilution were added to incubation flasks of the lead-treated groups to achieve final concentrations of 10^{-5} and 10^{-6} M Pb, respectively. Medium samples were collected after 60, 90, 120, and 150 min of incubation and handled as described in Protocol 3.

Analytical Methods

Hematocrit was measured on a microhematocrit reader after centrifugation in a microcapillary centrifuge for 5 min. Plasma sodium and potassium concentrations were determined by flame photometry. Blood lead was measured by graphite furnace atomic absorption (Varian Instruments, Model 375 CRA 90) by methods of addition. ACE activity was measured in plasma and lung tissue from chronic animals only. A commercially available kit for plasma ACE microdeterminations was obtained from Ventrex Corporation. The supernatant fraction of homogenized lung tissue was spectrophotometrically assayed for ACE activity by the method of Wallace *et al.* (1978) with the major modification that the phosphate buffer was replaced with a Tris buffer. This assay measures the rate of generation of hippuric acid from hippuryl-L-histidyl-L-leucine by lung homogenates. Protein in the pulmonary supernatant fraction was assayed by the method of Lowry *et al.* (1951).

Methods for the measurement of PRA, plasma renin substrate (angiotensinogen, PRS), and renal renin concentration (RRC) have been described previously (Mouw *et al.*, 1978); the maleate buffer used in assaying rabbit samples was pH 6.5. Cortical slice media were assayed for renin activity after the addition of substrate to the incubation mixture. The renin substrate used was plasma from rabbits nephrectomized 48 hr previously; the substrate concentration of the final incubation solution was equivalent to 800 ng angiotensin I/ml. Maleate buffer (50 ml, 0.2 M, pH 6.5), dimercaprol (1.7 g%, 5 ml), 8-hydroxyquinolone (6.6 g%, 5 ml), and EDTA (0.2 M, 2 ml) were added to 100 ml of the substrate. Aliquots (0.162 ml) of this solution were pipetted into individual tubes and stored at −30°C for later use. Medium samples (0.05 ml) were added to these substrate tubes and incubated as previously described for the renin assay (Mouw *et al.*, 1978).

Statistical Analysis

Data were analyzed with analysis of variance (ANOVA) by a one-way classification (Steel and Torrie, 1960). Differences between treatment groups within the ANOVA were determined with Duncan's New Multiple Range test (DNMR) (Steel and Torrie, 1960). A p value less than 0.05 was accepted as significant. All group values are given as means ± 1 SE.

RESULTS

Protocol 1: Effects of Acute Pb Exposure on the Disappearance of Renin

Zero-time values for PRA were 10.4 ± 3.9 ng AI/ml/hr in the TC rabbits; the mean value in Pb-treated rabbits was 5.6 ± 0.7. Figure 1 depicts mean curves for the fall in PRA (expressed as a percentage of the PRA at time zero) in the acutely Pb-exposed and TC rabbits; data are plotted on semilog scale. It is apparent that although PRA fell in both groups during the first 10 min, the slope of the line for the TC group was much steeper. The mean half-life in the controls was 8.2 ± 2.4 min. In four of the five Pb-treated rabbits, PRA never reached 50% of its initial value during the 50-min sampling period. The single exception had a half-life of 4 min, a value similar to those observed in the TC rabbits. No statistical comparison of half-lives for the two groups could be made since four of the five Pb rabbits never reached this endpoint. PRS was measured in two animals from each group at 10, 30, and 50 min postnephrectomy; values were similar in all four animals and did not fall in any of the rabbits during the course of the experiment.

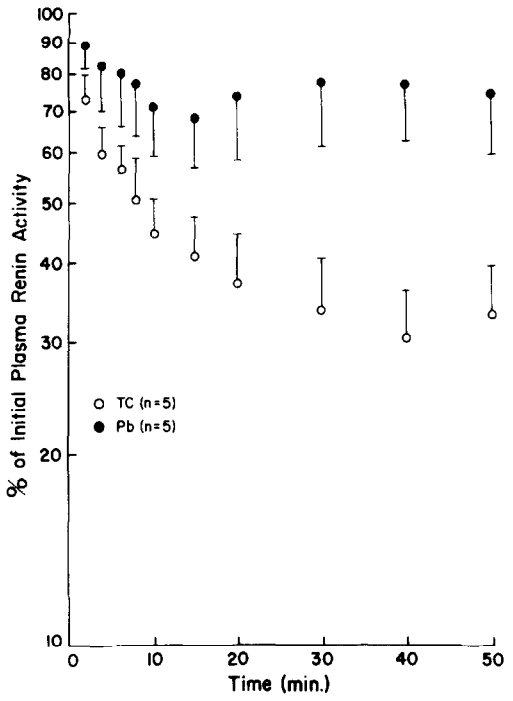

FIG. 1. Postnephrectomy fall in plasma renin activity (PRA) in rabbits acutely given sodium acetate (time-controls, TC) or Pb acetate (Pb) iv. See text for description of Pb exposure.

Protocol 2. Effects of Chronic Pb Exposure on the Disappearance of Renin

Zero time values for PRA were not different between the three groups: TC = 13.0 ± 5.0 ng AI/ml/hr, 500 ppm = 14 ± 7.1, 1000 ppm = 18.2 ± 5.2. There were no significant differences in the half-life of renin in the three groups of chronic rabbits. The postnephrectomy fall in PRA (normalized to percentage of the initial value) in the three groups is depicted in Fig. 2. All three groups showed a rapid drop within the first 15 min and a slower fall during the remainder of the experiment. The mean half-lives for renin in the three groups are depicted in Fig. 3: TC = 8.2 ± 2.4 min, 500 ppm = 7.0 ± 1.2, and 1000 ppm = 6.4 ± 2.2.

Blood Pb concentrations were elevated in the 500 and 1000 ppm groups to 66 ± 10 and 109 ± 15 μg/dl, respectively (Table 1). Body weights and kidney weights, plasma [Na] and [K], and both lung and plasma ACE activities were similar in the three groups. Hematocrit was depressed by approximately 10% in the 1000-ppm rabbits compared to the other two groups, consistent with a modest Pb-induced anemia. Mean arterial blood pressures at the time of nephrectomy were 71 ± 4 (TC), 71 ± 5 (500 ppm), and 67 ± 8 (1000 ppm); these differences were not statistically significant.

Protocol 3: Effects of Chronic Pb Exposure on the in Vitro Secretion of Renin

Previously performed pilot studies had demonstrated that renin secretion from cortical slices was linear from 90 to 150 min of incubation and so, for ease of analysis, only the 150-min samples were used. These pilot experiments also confirmed the findings by others (Churchill and Churchill, 1979, 1980) that elevation of potassium concentration in the medium reduced renin secretion from the

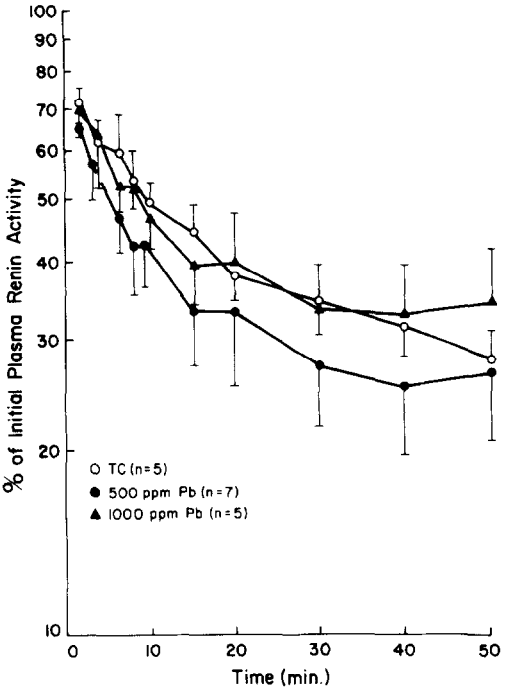

FIG. 2. Effects of chronic Pb-exposure on the postnephrectomy fall in plasma renin activity (PRA) in rabbits. See text for description of treatment.

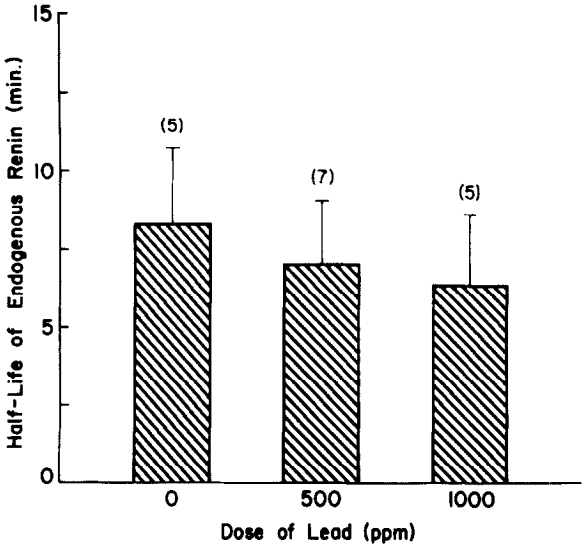

FIG. 3. The mean half-life of endogenous renin in the three groups of chronic rabbits depicted in Fig. 2.

slices to values similar to those occurring *in vivo*. Therefore, for evaluation of "basal" renin secretion by the slices, we chose a potassium concentration (35 mM) which reduced renin secretion to approximately 33% of the value observed with 5 mM K^+. Accordingly, two different methods for stimulating renin secretion above "basal" were then used: isoproterenol and medium K^+ concentration of 5 mM.

Table 2 presents data on the secretion of renin *in vitro* from the three groups of chronically Pb-exposed rabbits used in Protocol 2. Basal renin secretion (35 mM K^+) was approximately twofold higher in the flasks containing slices from chronically Pb-treated rab-

TABLE 1

BLOOD LEAD CONCENTRATIONS, ORGAN WEIGHTS, AND OTHER PARAMETERS IN LEAD-EXPOSED RABBITS

Indices	Dose of lead[a] added to drinking water		
	0 ppm (control, n = 5)	500 ppm (n = 7)	1000 ppm (n = 5)
Blood [Pb] (μg/dl)	7 ± 3	66 ± 10	109 ± 15
Body weight (kg)	3.24 ± 0.12	3.42 ± 0.13	3.31 ± 0.13
Kidney weight (g)	8.67 ± 0.22	8.43 ± 0.50	8.70 ± 0.44
Hematocrit (×100)	38.9 ± 1.4	38.2 ± 1.4	34.2 ± 2.1
Plasma [Na] (mM)	140.5 ± 3.0	141.2 ± 1.3	141.2 ± 2.3
Plasma [K] (mM)	3.89 ± 0.23	4.25 ± 0.22	4.33 ± 0.21
Lung converting enzyme activity (mmol/min/mg protein)	31.8 ± 1.8	29.3 ± 1.5	30.9 ± 1.6
Plasma converting enzyme activity (nmol/min/ml plasma)	503.5 ± 20.0	474.9 ± 17.6	476.3 ± 61.2

Note. Data are group means ± 1 SE.

[a] As lead acetate.

TABLE 2

In Vitro Renin Secretion During 150-min Incubation with Slices from Chronically Lead-Exposed Rabbits

Renin secreted (ng angiotensin I/mg kidney)	Dose of lead[a] added to drinking water		
	0 ppm (control, $n = 6$)	500 ppm ($n = 6$)	1000 ppm ($n = 6$)
A. 35 mM K^+	0.84 ± 0.19	1.61 ± 0.31[b]	1.63 ± 0.30[b]
B. 35 mM K^+ with 10^{-6} M Isoproterenol	1.63 ± 0.30	2.78 ± 0.35[b]	2.01 ± 0.29
C. 5 mM K^+	2.34 ± 0.50	3.63 ± 0.34	3.97 ± 0.72
Ratio			
B/A	2.09 ± 0.31	1.87 ± 0.20	1.32 ± 0.20
C/A	3.32 ± 0.94	2.59 ± 0.46	2.60 ± 0.45

Note. Data are group means ± 1 SE.

[a] As lead acetate.

[b] Significantly different from control, $p < 0.05$.

bits compared to time controls ($p < .05$ for both Pb-treated groups). Stimulation of renin secretion with isoproterenol (10^{-6} M) increased the renin secretion in all three treatment groups above that in 35 mM K^+ alone. The absolute value for secretion in 35 mM K^+ with isoproterenol was greatest in the 500 ppm group and was significantly different from the other two groups ($p < .05$); the value for the 1000-ppm group did not differ significantly from control. Stimulation of renin secretion with 5 mM K^+ increased renin secretion approximately threefold in all three groups; there were no significant differences between the absolute values for the three groups.

To isolate the effects of isoproterenol and those of low K^+ from those of high K^+ (i.e., to take into account the fact that basal secretion rates were different in the three groups), the data for these two other media were evaluated as the ratios to secretion rates in 35 mM K^+ and are presented in Table 2. The percentage stimulation by isoproterenol was significantly less in the 1000-ppm group then in the control group.

Despite the elevated basal renin secretion exhibited by the Pb-treated groups, renal renin concentrations in the three groups of rabbits were similar: TC = 331 ± 47 ng AI/mg kidney, 500 ppm = 308 ± 37, and 1000 ppm = 382 ± 49.

Protocol 4: Effects of in Vitro Pb Exposure on Renin Secretion by Cortical Slices

Renin secretion rates in the presence of 0, 10^{-5}, and 10^{-6} M Pb (added to the incubation media) are shown in Fig. 4. These cortical slices were prepared from rabbits that had not undergone previous Pb treatment. At each sampling the mean absolute values of secreted renin were lower for the Pb-exposed slices than for controls, but these differences were not significant. However, since basal secretion varied widely from animal to animal, we normalized data between animals, i.e., compared the values for Pb-exposed slices to those for the same animals' control slices. Slices incubated in 10^{-5} M Pb secreted renin at 89 ± 11, 86 ± 9, 86 ± 10, and 79 ± 4% of the values for control flasks at 60, 90, 120, and 150 min, respectively. Mean secretion from slices incubated in 10^{-6} M Pb was 72 ± 19, 82 ± 9, 83 ± 12, and 89 ± 12% during these same intervals. Because the two groups had similar responses, the data were pooled for analysis. The percentage inhibition of 10^{-5} or 10^{-6} M

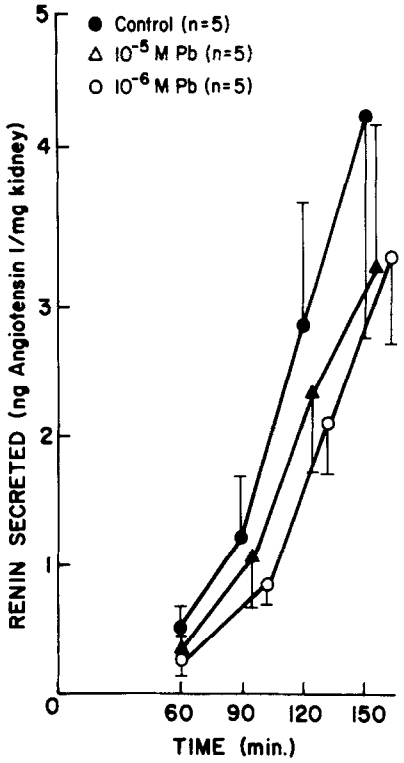

FIG. 4. Renin secretion from kidney cortical slices exposed to Pb (as Pb acetate) *in vitro* during 150-min incubation.

lead compared to normalized control secretion at 150 min was significantly different ($p < .05$) by a paired t statistic.

DISCUSSION

These experiments demonstrate that the disappearance of renin from plasma following nephrectomy in acutely Pb-exposed rabbits is markedly inhibited, whereas chronically Pb-exposed rabbits are unaffected. Quantitation of the data depends upon the manner in which the disappearance curves are evaluated. The disappearance curves for renin clearly do not fit a single exponential. There is a rapid fall in PRA during the first 10 to 15 min post-nephrectomy followed by a much slower decline, a pattern previously reported by other investigators (Michelakis and Mizukoshi, 1971; Schaechtelin *et al.*, 1964; Schneider *et al.*, 1968).[4] There is disagreement concerning the mechanisms contributing to this shape of the disappearance curve. Schneider and colleages (1968) have suggested that the initial rapid fall in renin is due to a redistribution of renin into a second compartment (for example, the extravascular space) rather than to actual elimination by the liver, and that the slower component of the renin disappearance curve actually reflects the true clearance of renin. Using this criterion, Schneider estimated a renin half-life of 45 min in the anesthetized dog (Schneider *et al.*, 1968). Using a similar approach to the data, Michelakis and Mizukoshi (1971) predicted a slow-component renin half-life of 93 ± 17 (SD) min in dogs and 280 ± 94 (SD) min in humans.

Implicit in the logic behind the redistribution concept is the assumption that renin secretion must have been stimulated during anesthesia or nephrectomy and that equilibration between plasma and interstitial fluid had not yet been reached at the moment of nephrectomy. In our experiments there was no consistent rise or fall in PRA between the first two samples obtained in the present experiment (pre and zero), which suggests that no large burst of renin secretion had occurred. However, we have no direct proof of equilibration between plasma and interstitial fluid.

If one chooses not to separate the disappearance curves into multiple components, but simply uses the raw data, a much shorter half-life results. For example, Oates (1974) measured an endogenous renin half-life of 10 min in the anesthetized rat, a value similar to our present value for the rabbit. These lower values are more consistent with directly measured values for hepatic renin extraction in the literature, which average approximately 30% for all species studies (Goldman *et al.*, 1981; Horky *et al.*, 1970; Mitch *et al.*, 1979;

[4] A possible problem in the present experiments could be an increase in PRS following nephrectomy, since such an increase would elevate PRA. However, no change in substrate was observed during the 50 min postnephrectomy; therefore, PRA was a valid indicator of plasma renin concentration.

Tapia *et al.*, 1973). Even assuming a total extracellular fluid volume of distribution for renin in the steady state, this extraction ratio coupled with the very high rates of hepatic blood flow leads to calculated half-lives much shorter than 45 to 90 min.

Since the physiological significance of multiple components in the renin disappearance curves is unclear, we chose to estimate a single value for half-life. Renin half-life was approximately 8 min in acute TC rabbits. In four of the five acutely Pb-exposed animals, the half-life was markedly prolonged (>50 min). This finding is consistent with the report by Goldman *et al.* (1981) that directly measured hepatic renin extraction was virtually eliminated in dogs following acute Pb exposure. The renin half-life of the fifth Pb-exposed rabbit in this study was comparable to controls; this rabbit received the lowest dose of Pb (0.3 mg/kg), suggestive of a possible threshold effect.

The mean value for PRA tended to be lower at the start of the experiment in the acutely Pb-exposed rabbits (5.6 ± 0.7 ng AI/ml/hr) compared to TC animals (10.4 ± 4.0). One would have predicted an elevated PRA if a decrease in the clearance of renin were the only Pb-induced effect. One must postulate, therefore, that renin secretion was also virtually eliminated by acute Pb-exposure. Consistent with this hypothesis was the finding that the acute exposure of renal slices to Pb *in vitro* resulted in inhibition of renin secretion. The ability of Pb to inhibit renin secretion has been previously suggested by data from people (Bertel *et al.*, 1978) and rats whose exposure was begun *in utero* (Victery *et al.*, (1982b)).

In contrast to the acute experiments, there was no difference in the renin disappearance curves in chronically Pb-exposed rabbits, indicating that any observed changes in PRA are not due to altered renin clearance but to altered renin secretion. This hypothesis can be tested directly in the intact animal only by obtaining measurements of renal plasma flow and renin concentrations in arterial and renal venous plasma. This procedure is extremely difficult to achieve in unanesthetized, relatively small experimental animals and so we chose to use renal cortical slices instead, since it is generally accepted that release of renin from slices *in vitro* parallels renin secretion *in vivo* (Park *et al.*, 1978). Moreover, measuring renin secretion *in vitro* also eliminates many of the feedback loops that alter renin secretion in the intact animal, and permits the testing of direct effects of Pb (added *in vitro*).

These experiments demonstrate that basal renin secretion by rabbit cortical slices is indeed increased following chronic Pb exposure. It has been demonstrated that basal renin secretion both *in vitro* and *in vivo* is highly correlated with kidney renin stores (Park *et al.*, 1978). The increased secretory rates seen in these experiments would be readily explained if renal renin content were elevated in the Pb-exposed rabbit, as is the case for rats exposed to a similar dietary dose (Fleischer *et al.*, 1980). However, there were no significant differences in renal renin content among the three groups of rabbits. It is possible that the total renin content measured may not reflect the pool from which renin secretion actually occurs, and that Pb may alter distribution between different pools.

The fact that chronic Pb exposure stimulated renin secretion from cortical slices is consistent with the hypothesis that Pb competes with calcium for influx into juxtaglomerular cells. If the two cations vied for entry into the justaglomerular cells, increasing concentrations of Pb would decrease calcium influx and thereby stimulate renin secretion (Churchill and Churchill, 1980; Fray, 1980; Park and Malvin, 1978). Kapoor and van Rossum (1977) have reported that 2×10^{-4} M Pb inhibited net movement of calcium into rat kidney slices.

The lesser increment in renin release following a β-adrenergic stimulus from the chronic Pb-exposed slices is consistent with previous reports on both Pb-exposed rats and people (Bertel *et al.*, 1978; Fleischer *et al.*, 1980), and adds further support to the use of

slices as an analogue for *in vivo* renin secretion. A number of investigators have proposed that β-adrenergic stimuli promote renin secretion by increasing the sequestration or extrusion of calcium from intracellular pools; our results are consistent with this hypothesis since low concentrations of Pb have been shown to inhibit the calcium-accumulating ability of mitochondria (Parr and Harris, 1976). Although all our results from chronic lead-exposed rabbits can be interpreted in terms of lead interfering with calcium fluxes, many other interpretations are possible. For example, the inhibition of response to isoproterenol could reflect a direct interference with β receptors.

The combination of chronically increased basal renin secretion and unchanged renin clearance should lead to increased PRA in the intact animal. The failure to observe such an increase in the samples taken just prior to nephrectomy was a surprising finding, (particularly since an increase in PRA does occur in rat [Fleischer *et al.*, 1980; Victery *et al.*, 1982a]) and could call into question whether the acute disappearance curves and *in vitro* slice data are adequate indicators of *in vivo* steady-state processes. However, it must be emphasized that the samples for PRA were taken following anesthesia and surgery, whereas in the previous studies with rats, samples were obtained by decapitation of carefully conditioned animals. Accordingly, there should be a considerable increase in sympathetic activity in the rabbits, acting as a stimulus for renin secretion. The fact that Pb-exposure blunts the renin response to β-adrenergic stimulation could account for a smaller rise in PRA, i.e., no difference in PRA between time-control and Pb-exposed groups would be expected when renin secretion is under strong sympathetic stimulation.

In summary, responses of the rabbit to acute high-dose and chronic low-dose Pb-exposure are quite different. For the first case, the data indicate inhibition of both the secretion and clearance of renin. For the second case, the data indicate that clearance is unchanged, basal secretion is enhanced, and sympathetically mediated secretion is blunted.

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