Effect of chronic mineralocorticoid administration on calcium excretion in the rat

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SUKI, WADI N., RICK S. SCHWETTMANN, FLOYD C. RECTOR, JR., AND DONALD W. SEDIN. Effect of chronic mineralocorticoid administration on calcium excretion in the rat. Am. J. Physiol. 215(1): 71-74. 1968.—The effect of prolonged 9-alpha-fluorohydrocortisone administration on sodium and calcium excretion was investigated in rats fed a low-calcium synthetic diet. In rats fed salt the administration of mineralocorticoid resulted in sodium retention lasting 4 days after which sodium excretion returned to control levels. The expansion of extracellular volume, occurring during the period of sodium retention, was associated with a two- to fivefold increase in calcium excretion that persisted even after sodium balance was re-established. Omitting salt from the diet prevented the effect of mineralocorticoids on calcium excretion. It was concluded that mineralocorticoids, by enhancing distal sodium reabsorption, expand extracellular fluid volume, thereby suppressing sodium as well as calcium reabsorption in the more proximal portions of the nephron.

9-alpha-fluorohydrocortisone; sodium excretion; volume expansion; proximal tubule

Urineary excretion of calcium under a variety of experimental conditions has been reported by many investigators to vary directly with sodium excretion (8, 9, 11, 15). This observation led to the suggestion that calcium reabsorption in the kidney is linked to sodium reabsorption (15). The excretion of calcium, however, can be dissociated from that of sodium: prolonged administration of thiazide diuretics reduces the urinary excretion of calcium despite an augmentation of sodium excretion (7).

These seemingly conflicting observations could be reconciled by the hypothesis that the relation between calcium and sodium excretion was the passive consequence of changes in effective extracellular fluid volume (ECF). It has been demonstrated that shrinkage of effective ECF augments (4), whereas expansion of effective ECF suppresses, the reabsorption of sodium in the proximal tubule (3, 12). It is conceivable that if sodium diuresis is associated with a shrinkage of ECF (administration of thiazide diuretics) the reabsorption not merely of sodium but also of calcium in the proximal tubule would be augmented. Calcium excretion would, therefore, fall. On the other hand, if sodium diuresis is associated with expansion of ECF (saline infusion) the reabsorption of calcium as well as of sodium in the proximal tubule will be suppressed. Calcium excretion would, therefore, rise.

To test this hypothesis, 9-alpha-fluorohydrocortisone was administered to rats with access to dietary salt to stimulate the reabsorption of sodium in the distal tubule. The resulting expansion of ECF would be expected to cause a suppression of the proximal tubular reabsorption of filtrate. In the steady state, therefore, when ECF is overexpanded calcium excretion should be increased and sodium excretion should be normal. If 9-alpha-fluorohydrocortisone is given without dietary salt no expansion in ECF would occur and, therefore, no augmentation in calcium excretion should be observed.

MATERIALS AND METHODS

Studies were performed on female Sprague-Dawley rats weighing 180-220 g. Two groups of rats were placed in metabolic cages and fed 30 ml daily of a synthetic semiliquid diet. Calcium was omitted from the diet in order to eliminate contamination of the urine by fecal calcium. The diet given rats in group I contained 20 g NaCl and 15 g KCl in each liter of diet; group II received only 15 g KCl/liter of diet. After at least 1 week on the experimental diet, 24-hr urine collections under oil were begun. On the 5th day, 0.5 mg of 9-alpha-fluorohydrocortisone dissolved in alcohol and water was added daily to the diet of each rat. Group I received 9-alpha-fluorohydrocortisone for a total of 10 days following which the drug was omitted and urine collections continued for another 9 days. Rats in group II were treated in exactly the same way during the control period, but urine collections were stopped after 5 days on 9-alpha-fluorohydrocortisone since preliminary experiments indicated that the peak effect was attained by this time.

Urine volume was measured daily and recorded and examined for calcium and sodium content. Total calcium and sodium excretion was calculated as the product of the daily urine volume and the concentration of calcium and sodium in the urine.

Urine volume was measured daily and recorded and
then an aliquot removed for analysis of sodium, potassium, and calcium. Sodium, potassium, and calcium in the urine were analyzed in a Technicon AutoAnalyzer, sodium and potassium by internal standard flame photometry and calcium by the colorimetric method of Gitelman (personal communication). In group I the mean excretion of calcium in the 4 control days was compared to that in the last 3 days at the peak of 9-alpha-fluorohydrocortisone effect. In group II the control excretion was compared to that in the last 3 days of the experimental period. Potassium excretion in the control period of group I rats was compared to that in the first 5 days following 9-alpha-fluorohydrocortisone administration. The significance of the differences between the means was tested by the Student t test (6).

RESULTS

An example of the effect of 9-alpha-fluorohydrocortisone on a rat fed a diet containing both potassium and sodium chloride is shown in Fig. 1. During the 4 control days the rat excreted 1 mg/day of calcium and 6 mEq/day of sodium. The administration of 9-alpha-fluorohydrocortisone resulted in a progressive fall in the excretion of sodium, reaching a trough on the 3rd experimental day and then returning to the control level by the 5th day. Concomitant with these changes in sodium excretion calcium excretion rose on the 2nd experimental day, reaching a peak excretion on the 4th day, then leveling off at a rate of excretion amounting to more than threefold the control value. The withdrawal of 9-alpha-fluorohydrocortisone was followed by a rise in sodium excretion. This increase in sodium excretion was attended by a gradual fall of calcium excretion to the control level.

The mean calcium excretions from each of 8 rats in group I are shown in Table 1. The control daily rate of calcium excretion in each rat ranged from 0.27 to 1.81 mg/day; the mean daily excretion in the control period ranged from 0.35 to 1.72 mg/day. The administration of 9-alpha-fluorohydrocortisone resulted in a two- to fivefold increase in calcium excretion in every rat, the daily excretion of calcium in the experimental period ranging from 1.73 to 4.93 mg/day and the mean daily excretion in the experimental period from 1.79 to 4.67 mg/day. This change was highly significant in every instance.

In contrast to the rats in group I were the rats in group II from whose diet salt was omitted. One typical experiment on a rat in group II is shown in Fig. 2. During the control period calcium excretion was stable around 0.75 mg/day. The urine, however, was virtually free of sodium. With the administration of 9-alpha-fluorohydrocortisone sodium excretion in the urine remained very low. The excretion of calcium fluctuated somewhat but the excretion rate was not significantly different in the experimental period from that in the control period.

The results from all seven rats in group II are shown in Table 2. The control daily rate of calcium excretion ranged from 0.38 to 1.21 mg/day; the mean daily excretion in the control period varied from 0.45 to 0.97 mg/day. Calcium excretion during the administration of 9-alpha-fluorohydrocortisone in this group appeared to fluctuate in either direction but in no instance was
there a statistically significant change. The daily excretion of calcium in the experimental period ranged from 0.35 to 1.67 mg/day; the mean daily excretion in the experimental period ranged from 0.41 to 1.18 mg/day.

The difference in the effect of 9-alpha-fluorohydrocortisone on calcium excretion in group I and II rats is not due to potassium depletion in the former group. The rate of potassium excretion in the first 5 days following 9-alpha-fluorohydrocortisone was not significantly different from that in the control period in any of the eight rats studied.

**Discussion**

The results of these experiments demonstrate that in rats fed salt the administration of a potent mineralocorticoid, 9-alpha-fluorohydrocortisone, results in a transient retention of sodium lasting about 5 days, at the end of which time the excretion returns to control levels (Fig. 1). This return of sodium excretion to normal despite the continued administration of a mineralocorticoid is similar to that described in man following the administration of deoxycorticosterone (13), aldosterone (1), and 9-alpha-fluorohydrocortisone (14). Concomitant with these changes in urinary sodium excretion, the excretion of calcium in the urine rose progressively to a peak rate of excretion on the 4th to 5th day and then leveled off at this enhanced rate of excretion thereafter. When 9-alpha-fluorohydrocortisone was discontinued, urine sodium excretion rose and calcium excretion gradually fell to control levels.

Two possible explanations can be advanced to account for these changes. It is possible that the observed increase in urinary calcium excretion is the result of a direct physiologic or pharmacologic effect of 9-alpha-fluorohydrocortisone on the intestinal absorption or renal excretion of calcium. The fact that the urinary excretion of calcium increased in rats fed a diet from which calcium was omitted argues against this effect being due to increased gastrointestinal absorption of calcium. Furthermore, when salt was also omitted from the diet the effect of mineralocorticoid administration was completely obliterated (Fig. 2, Table 2). These observations militate strongly against a direct effect on gastrointestinal absorption or renal excretion of calcium.

An alternative explanation is that the observed increase in calcium excretion is secondary to sodium repletion. The failure of mineralocorticoids to augment calcium excretion when salt is eliminated from the diet is strong evidence in favor of this hypothesis.

It has been demonstrated by a number of workers that sodium retention resulting from excessive amounts of exogenous or endogenous mineralocorticoids leads to an expansion of ECF volume. Expansion of ECF volume has been shown to inhibit sodium reabsorption in the proximal tubule of both dogs and rats (3, 12). It is conceivable, therefore, that as distal sodium reabsorption is enhanced, under the influence of mineralocorticoids, ECF volume expands and fractional reabsorption of filtrate in the proximal tubule is reduced. Preliminary evidence supports this hypothesis; jugular venous blood from dogs chronically treated with Doca contains a factor that inhibits the intrinsic reabsorptive capacity of the proximal tubule (F. C. Rector, Jr., unpublished results). From micropuncture studies it is now evident that about 80% of filtered calcium is reabsorbed in the proximal tubule (10). If the concentration of calcium in the proximal tubule following volume expansion remains unaltered (F. C. Rector, Jr., unpublished results) or drops slightly (5), the reduction in fractional reabsorption of filtrate in the proximal tubule can then lead to an increased excretion of calcium in the urine. It may be argued that the expansion of ECF volume of the rat results in a rise in the glomerular filtration rate and the rise in the filtered load of calcium leads to the observed increase in calcium excretion. The observation of Blythe, Gitelman, and Welt that expansion of ECF volume remains unaltered (F. C. Rector, Jr., unpublished results) or drops slightly (5), the reduction in fractional reabsorption of filtrate in the proximal tubule can then lead to an increased excretion of calcium in the urine. It may be argued that the expansion of ECF volume of the rat leads to a rise in the glomerular filtration rate and the rise in the filtered load of calcium leads to the observed increase in calcium excretion. The observation of Blythe, Gitelman, and Welt that expansion of ECF volume increases calcium excretion despite a reduction in the filtration rate induced by clamping of the aorta (2) argues against this being the explanation for our findings.

On the basis of alterations in ECF volume, an explanation for previous observations on the relationship of sodium to calcium excretion may be offered. Saline infusion, by expanding ECF volume, inhibits fractional reabsorption in the proximal tubule and, therefore, in-

<table>
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<th>Rat No.</th>
<th>Calcium Excretion, mg/day</th>
<th>Potassium Excretion, mEq/day</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>After 9-alpha-FH*</td>
<td>Control</td>
<td>After 9-alpha-FH*</td>
</tr>
<tr>
<td>1</td>
<td>0.59±0.16</td>
<td>0.56±0.16</td>
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<td>3</td>
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<td>1.12±0.20</td>
<td>&gt;0.3</td>
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<td>4</td>
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<tr>
<td>7</td>
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</tr>
<tr>
<td>9</td>
<td>0.76±0.28</td>
<td>0.4±0.02</td>
<td>&gt;0.1</td>
</tr>
</tbody>
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* 9-Alpa-fluorohydrocortisone
creases the excretion of both sodium and calcium. Salt deprivation or thiazide diuretics shrink ECF volume and stimulate proximal reabsorption. The increased fractional reabsorption in the proximal tubule results in a diminution in the excretion of calcium.

To account for the above observations it is not necessary to invoke an actual link between the reabsorption of sodium and that of calcium in the proximal tubule. If alterations in ECF volume exert an effect on the fractional reabsorption in the proximal tubule of bulk filtrate and not of sodium alone, the same changes in calcium excretion would be observed.

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REFERENCES