Effect of calcium infusion on renal tubular reabsorption in the dog

BRIAN R. EDWARDS, ROGER A. L. SUTTON, AND JOHN H. DIRKS
Renal and Electrolyte Division, Department of Medicine, Royal Victoria Hospital, and
McGill University, Montreal 112, Quebec, Canada

Edwardes, Brian R., Roger A. L. Sutton, and John H. Dirks. Effect of calcium infusion on renal tubular reabsorption in the dog. Am. J. Physiol. 227(1): 13-18. 1974. Recollection micropuncture experiments were performed in hydropenic mongrel dogs to examine the effects of acute calcium infusion on renal tubular reabsorption. Three groups were studied: a) control group (n = 11), where plasma calcium concentration (P_Ca) remained unchanged, revealed the normal stability of renal tubular function. b) Group II (n = 23), P_Ca was raised from 4.9 to 7.9 meq/liter by intravenous infusion of 400 meq/liter CaCl₂ solution. c) Group III (n = 5), P_Ca was raised from 4.9 to 13.5 meq/liter by intravenous infusion of 800 meq/liter CaCl₂. In Group II, proximal fractional reabsorption of water, sodium, and calcium was reduced 7-10%. Despite differences in filtered loads of calcium and sodium, the ratio TF/UF_Ca; TF/P Na in the proximal tubule remained unchanged. This ratio was increased in the distal tubule implying a dissociation of fractional sodium and calcium reabsorption. This dissociation was more pronounced in the final urine. In Group III, GFR was greatly reduced and tended to mask the tubular effects of calcium infusion. However, fractional proximal reabsorption of sodium and calcium appeared to be dissociated.

Proximal tubule; Na reabsorption; Ca reabsorption; Ca-Na dissociation

Clearance studies in the mammalian kidney have demonstrated a close correlation between the clearance rates of calcium and sodium under a variety of experimental conditions (33, 34). Although suggestive of a common tubular transport system for these cations, the proportionality of calcium and sodium clearance rates can be disrupted by the administration of thiazide diuretics, parathyroid hormone, and mineralocorticoids (9, 10, 35). Micropuncture studies in the hydropenic rat and dog point to a close interrelation of sodium and calcium reabsorption by the individual renal tubule was also investigated before and following calcium infusion.

Materials and Methods

Experiments were performed on mongrel dogs (11-19 kg) of both sexes which had been allowed free access to standard laboratory chow and water. The surgical and experimental procedures for micropuncture in this laboratory have been previously described (11).

Inulin, which was used as a measure of glomerular filtration rate (GFR), was given as a priming dose followed by a constant intravenous infusion (0.97-1.36 ml/min) throughout the experiment. Late proximal and/or distal tubules were selected for puncture by periodic injection of 0.05 ml of 4% buffered FD & C green dye into the left renal artery. Following three 15-min control clearance periods during which micropuncture samples were also obtained, one of three procedures was followed: group I (n = 11), sustaining inulin infusion continued at the same rate. No CaCl₂ administered. Group II (n = 23), 400 meq/liter CaCl₂ given as a prime of 2.5 ml/kg body wt over 15 min followed by a constant infusion of 0.025 ml/kg per min. Group III (n = 5), 800 meq/liter CaCl₂ administered as for group II. For each group, at least 30 min were allowed to elapse before three further clearance periods were obtained together with recollection from as many of the previously punctured tubules as possible.

A venous blood sample was obtained at the midpoint of each urine collection. Hematocrit was determined and the plasma protein concentration was measured by refractometry. Femoral arterial blood pressure was monitored using a Statham transducer linked to a Sanborn recorder (Sanborn Co., Waltham, Mass.).

The analytical techniques for inulin and sodium in tubule fluid, urine, and plasma have been described elsewhere (11). Calcium in urine and plasma samples was analyzed by atomic absorption (model 303, Perkin-Elmer Corp., Norwalk, Conn.). The helium glow photometer (Montreal Polycrafters, Montreal, Quebec) was modified to read calcium in tubule fluid and plasma ultrafiltrates. The latter
were prepared using Amicon Centriflo ultrafiltration cones (Amicon Corp., Lexington, Mass.) as described by Raman (29). A series of 111 plasma ultrafiltrates from hydropenic dogs analyzed for calcium by the helium glow (HG) and by atomic absorption (AA) yielded a mean HG/AA ratio of 1.01 ± 0.01 (SE), which is not significantly different from unity. Addition of CaCl2 to plasma ultrafiltrates to increase the calcium concentration approximately 1.5 and 2.5 times yielded recoveries of 100.1 ± 1.5% (n = 20) and 100.9 ± 1.5% (n = 18), respectively.

Standard formulas have been employed in the calculation on nonreabsorbed fractions of filtered load. Statistical analysis of the data incorporated the paired Student t test.

RESULTS

Clearance Data. For each dog, all clearance periods in each phase of the experiment have been averaged, and the mean values have been treated as individual data in the calculation of overall group means.

Table 1 summarizes the clearance data for group II. Data for the left (micropuncture) kidney are presented. The results for the right kidney are in no respect significantly different from the left. The elevation of ultrafilterable calcium (UFca) from 3.0 to 4.3 meq/liter produced a slight, but significant, fall in GFR. Mean urine flow for the whole group tended to increase, although this was not a consistent response. Fractional excretion of water was significantly increased, however. No consistent changes were noted in the absolute and fractional excretion of sodium or potassium. On the other hand, both absolute and fractional excretion of calcium were significantly increased, and hence the ratio FEca:FEK increased markedly. Other changes included a mean increase of mean arterial blood pressure of 12 mm Hg, no change in venous hematocrit, but a significant reduction of plasma protein concentration. The proportion of ultrafilterable calcium decreased from 60.4 ± 1.51 (SEM) to 55.5 ± 2.3% (P < 0.01). Plasma sodium concentration was reduced together with a consistent increase of plasma potassium from 3.66 ± 0.06 to 4.09 ± 0.08 meq/liter (P < 0.001).

Figure 1 depicts the individual clearance data obtained from five dogs with twice the calcium load of the previous group. The proportionally greater increase of total plasma calcium concentration over UFca was more marked in this group; percent UFca decreased from 60.8 ± 1.4 to 43.4 ± 4.0% (P < 0.01); GFR showed a mean decrease of 41% after calcium infusion, and although mean FEHo increased threefold, this was statistically not significant. FEK increased in these dogs but not to as great an extent as FEca. Again, mean arterial blood pressure increased (120 ± 9 to 136 ± 7 mmHg, P < 0.02), hematocrit did not change (41.7 ± 2.3 to 42.3 ± 2.7%), and plasma protein concentration fell (5.3 ± 0.2 to 4.9 ± 0.2 g/100 ml, P < 0.001). The increase of plasma potassium concentration in this group was from 3.8 ± 0.1 to 5.1 ± 0.3 meq/liter (P < 0.01).

<table>
<thead>
<tr>
<th>TABLE 1. Summary of clearance data of group II: calcium infusion 400 meq/liter</th>
<th>No. of Dogs</th>
<th>Hydropenia</th>
<th>Calcium Loaded</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSa , meq/liter</td>
<td>23</td>
<td>4.9 ± 0.05</td>
<td>7.9 ± 0.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>UFca , meq/liter</td>
<td>23</td>
<td>3.0 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PNa , meq/liter</td>
<td>23</td>
<td>133.2 ± 0.8</td>
<td>131.8 ± 1.0</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>GFR , ml/min</td>
<td>23</td>
<td>31.3 ± 1.5</td>
<td>28.7 ± 1.6</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>P3 , ml/min</td>
<td>23</td>
<td>0.39 ± 0.06</td>
<td>0.50 ± 0.09</td>
<td>NS</td>
</tr>
<tr>
<td>FEHo0 , %</td>
<td>23</td>
<td>1.27 ± 0.19</td>
<td>1.74 ± 0.27</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>U3, meq/min</td>
<td>23</td>
<td>50.6 ± 9.4</td>
<td>51.0 ± 12.0</td>
<td>NS</td>
</tr>
<tr>
<td>FEca0 , %</td>
<td>23</td>
<td>1.23 ± 0.19</td>
<td>1.16 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>UcaV, meq/min</td>
<td>23</td>
<td>1.18 ± 0.18</td>
<td>2.86 ± 0.50</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>FEca/FEca</td>
<td>23</td>
<td>1.28 ± 0.22</td>
<td>2.31 ± 0.36</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>21</td>
<td>117 ± 3</td>
<td>129 ± 3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hct , %</td>
<td>23</td>
<td>41.7 ± 1.0</td>
<td>41.4 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Protein, g/100 ml</td>
<td>18</td>
<td>5.5 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. PSa , PSa = plasma sodium and calcium concentration. UFca = plasma ultrafilterable calcium concentration. GFR = glomerular filtration rate. V = urine flow rate. FEHo0 , FEca0 = fractional excretion of water, sodium, and calcium. U3, V, UcaV = sodium and calcium excretion rate. MAP = mean arterial blood pressure. Hct = hematocrit. Protein = plasma protein concentration. NS = not significant.

1 In general these dogs reacted adversely to the high calcium load. Renal function temporarily ceased in many instances, although this response varied in intensity and duration. Only 5 of 10 dogs attempted were suitable for the second phase of micropuncture. Because of the small number of animals and the variability between animals, individual clearance data are presented.
CALCIUM INFUSION AND TUBULAR REABSORPTION

TF/UF_{Ca} 0.29 ± 0.02 to 0.30 ± 0.02. Again, these changes were not significant. The interrelationship of calcium and sodium reabsorption is depicted by the ratio TF/UF_{Na}. In the proximal and in the distal tubule, this ratio remained stable in these control dogs. Mean values ± SE for the two phases were: for the late proximal tubule, 1.11 ± 0.02 to 1.12 ± 0.03, and for the distal tubule, 1.04 ± 0.07 to 1.10 ± 0.10.

In group II, a total of 44 proximal tubules were punctured in 14 dogs and 19 distal tubules in 9 dogs. Figure 2 illustrates the proximal TF/P_{In} data expressed as means per animal. All but three of the points fall below the line of identity. Mean values are shown in Table 2 together with the TF/P_{Na} and TF/UF_{Ca} ratios. While transtubular electrolyte concentration ratios were unchanged, proximal fluid reabsorption, as reflected by TF/P_{In} ratio, was significantly reduced following calcium infusion.

In the distal tubule, five of the nine dogs examined showed a pronounced increase of TF/P_{In} ratios (Fig. 3). As shown in Table 2, mean TF/P_{In} increased significantly from 3.50 to 5.07. There was a tendency for distal TF/P_{Na} to fall and TF/UF_{Ca} to rise after calcium infusion, but neither change was significant. However, a significant dissociation of calcium and sodium reabsorption, as expressed by the ratio TF/UF_{Ca}:TF/P_{Na}, occurred during hypercalcemia.

In group III, where twice the previous calcium load was infused, 18 proximal tubules were punctured in five dogs. Because of the marked reduction of GFR and intratubular flow, recollection micropuncture in the distal tubule proved technically impossible. Individual proximal TF/P_{In} ratios before and after calcium infusion are illustrated in Fig. 4. A major increase of fractional fluid reabsorption occurred in the proximal tubules of this group. The massive reduction of GFR undoubtedly contributed to this phenomenon and was further reflected by the marked increase of proximal transit time from 18.1 ± 1.1 to 35.7 ± 5.8 s (P < 0.02). While proximal TF/P_{Na} showed a slight, though significant increase, TF/UF_{Ca} rose to a greater extent yielding a significant increase of the ratio TF/UF_{Ca}:TF/P_{Na}. Thus, although fractional calcium reabsorption was essentially unchanged during marked hypercalcemia, fractional sodium and water reabsorption were significantly enhanced (Table 3).

DISCUSSION

GFR has been reported to remain unchanged in rats and man (7, 24) and to decrease in dogs (5, 31) following calcium infusion. Elevation of plasma calcium concentration produces a vasoconstriction in the renal vascular bed.
fractional fluid reabsorption was reduced 7% in the face of marked hypercalcemia, the influence of the massive reduction of percent UF_{Ca} with increasing P_{Ca} in the present study. Ultrafilterability at the glomerulus would presumably be affected. Preliminary studies on the Munich-Wistar strain of rats, which have glomeruli accessible for micropuncture, reveal a similar pattern. The formation of ultrafilterable calcium and phosphate complexes also has been postulated (5). Assuming that only ionic calcium can be reabsorbed by the renal tubule, any filtered calcium complex may be considered physiologically “inactive.” Speculating further, complex formation may explain the lack of any appreciable increase of absolute calcium reabsorption in the proximal tubule in the face of an increased filtered load of calcium. Additional formation of nonreabsorbable calcium complexes in group III may account for the elevation of TF/UF_{Ca} ratio.

Modest hypercalcemia is associated with reduced fractional reabsorption of sodium and water in the proximal tubule. These studies provide no evidence for a competitive inhibition of sodium reabsorption by calcium. With more marked hypercalcemia, the influence of the massive reduction of GFR does not permit direct analysis of the primary tubular effect of the higher calcium load. However, in contrast to group II, a dissociation of fractional reabsorption of sodium and calcium was evident as reflected by the increase in systemic blood pressure after calcium infusion if transmitted to the peritubular capillary may be involved in the reduction in Na reabsorption. However, similar changes in these parameters were accompanied by opposite changes in fractional sodium reabsorption in groups II and III as serum calcium was progressively increased.

Another factor which may be of importance is the complexing of calcium as a “colloidal calcium-phosphate-protein complex.” This has been suggested as the cause of the decreased ultrafilterability of calcium sometimes reported in hypercalcemic states (3, 16, 18, 30, 32) and could account for the progressive reduction of percent UF_{Ca} with increasing P_{Ca} in the present study. Ultrafilterability at the glomerulus would presumably be affected. Preliminary studies on the Munich-Wistar strain of rats, which have glomeruli accessible for micropuncture, reveal a similar pattern. The formation of ultrafilterable calcium and phosphate complexes also has been postulated (5). Assuming that only ionic calcium can be reabsorbed by the renal tubule, any filtered calcium complex may be considered physiologically “inactive.” Speculating further, complex formation may explain the lack of any appreciable increase of absolute calcium reabsorption in the proximal tubule in the face of an increased filtered load of calcium. Additional formation of nonreabsorbable calcium complexes in group III may account for the elevation of TF/UF_{Ca} ratio.

Modest hypercalcemia is associated with reduced fractional reabsorption of sodium and water in the proximal tubule. These studies provide no evidence for a competitive inhibition of sodium reabsorption by calcium. With more marked hypercalcemia, the influence of the massive reduction of GFR does not permit direct analysis of the primary tubular effect of the higher calcium load. However, in contrast to group II, a dissociation of fractional reabsorption of sodium and calcium was evident as reflected by the increase in systemic blood pressure after calcium infusion if transmitted to the peritubular capillary may be involved in the reduction in Na reabsorption. However, similar changes in these parameters were accompanied by opposite changes in fractional sodium reabsorption in groups II and III as serum calcium was progressively increased.

Another factor which may be of importance is the complexing of calcium as a “colloidal calcium-phosphate-protein complex.” This has been suggested as the cause of the decreased ultrafilterability of calcium sometimes reported in hypercalcemic states (3, 16, 18, 30, 32) and could account for the progressive reduction of percent UF_{Ca} with increasing P_{Ca} in the present study. Ultrafilterability at the glomerulus would presumably be affected. Preliminary studies on the Munich-Wistar strain of rats, which have glomeruli accessible for micropuncture, reveal a similar pattern. The formation of ultrafilterable calcium and phosphate complexes also has been postulated (5). Assuming that only ionic calcium can be reabsorbed by the renal tubule, any filtered calcium complex may be considered physiologically “inactive.” Speculating further, complex formation may explain the lack of any appreciable increase of absolute calcium reabsorption in the proximal tubule in the face of an increased filtered load of calcium. Additional formation of nonreabsorbable calcium complexes in group III may account for the elevation of TF/UF_{Ca} ratio.

Modest hypercalcemia is associated with reduced fractional reabsorption of sodium and water in the proximal tubule. These studies provide no evidence for a competitive inhibition of sodium reabsorption by calcium. With more marked hypercalcemia, the influence of the massive reduction of GFR does not permit direct analysis of the primary tubular effect of the higher calcium load. However, in contrast to group II, a dissociation of fractional reabsorption of sodium and calcium was evident as reflected by the increase in systemic blood pressure after calcium infusion if transmitted to the peritubular capillary may be involved in the reduction in Na reabsorption. However, similar changes in these parameters were accompanied by opposite changes in fractional sodium reabsorption in groups II and III as serum calcium was progressively increased.

Another factor which may be of importance is the complexing of calcium as a “colloidal calcium-phosphate-protein complex.” This has been suggested as the cause of the decreased ultrafilterability of calcium sometimes reported in hypercalcemic states (3, 16, 18, 30, 32) and could account for the progressive reduction of percent UF_{Ca} with increasing P_{Ca} in the present study. Ultrafilterability at the glomerulus would presumably be affected. Preliminary studies on the Munich-Wistar strain of rats, which have glomeruli accessible for micropuncture, reveal a similar pattern. The formation of ultrafilterable calcium and phosphate complexes also has been postulated (5). Assuming that only ionic calcium can be reabsorbed by the renal tubule, any filtered calcium complex may be considered physiologically “inactive.” Speculating further, complex formation may explain the lack of any appreciable increase of absolute calcium reabsorption in the proximal tubule in the face of an increased filtered load of calcium. Additional formation of nonreabsorbable calcium complexes in group III may account for the elevation of TF/UF_{Ca} ratio.

Modest hypercalcemia is associated with reduced fractional reabsorption of sodium and water in the proximal tubule. These studies provide no evidence for a competitive inhibition of sodium reabsorption by calcium. With more marked hypercalcemia, the influence of the massive reduction of GFR does not permit direct analysis of the primary tubular effect of the higher calcium load. However, in contrast to group II, a dissociation of fractional reabsorption of sodium and calcium was evident as reflected by the increase in systemic blood pressure after calcium infusion if transmitted to the peritubular capillary may be involved in the reduction in Na reabsorption. However, similar changes in these parameters were accompanied by opposite changes in fractional sodium reabsorption in groups II and III as serum calcium was progressively increased.

Another factor which may be of importance is the complexing of calcium as a “colloidal calcium-phosphate-protein complex.” This has been suggested as the cause of the decreased ultrafilterability of calcium sometimes reported in hypercalcemic states (3, 16, 18, 30, 32) and could account for the progressive reduction of percent UF_{Ca} with increasing P_{Ca} in the present study. Ultrafilterability at the glomerulus would presumably be affected. Preliminary studies on the Munich-Wistar strain of rats, which have glomeruli accessible for micropuncture, reveal a similar pattern. The formation of ultrafilterable calcium and phosphate complexes also has been postulated (5). Assuming that only ionic calcium can be reabsorbed by the renal tubule, any filtered calcium complex may be considered physiologically “inactive.” Speculating further, complex formation may explain the lack of any appreciable increase of absolute calcium reabsorption in the proximal tubule in the face of an increased filtered load of calcium. Additional formation of nonreabsorbable calcium complexes in group III may account for the elevation of TF/UF_{Ca} ratio.
increased TF/UF, TF/PNa ratio. This finding may be explicable in terms of the formation of ultrafilterable, non-reabsorbable calcium complexes.

The effects of hypercalcemia on loop of Henle and distal tubular function remain controversial. Although studies on the isolated toad bladder indicate an inhibitory effect of high calcium concentrations on ADH activity (1, 27), an altered osmotic permeability of the distal nephron is not considered an important determinant of diminished concentrating ability (2, 31). However, intratubular injection of calcium in the rat has been shown to reduce distal osmotic permeability during stopped flow (22). Sodium reabsorption by the ascending limb of Henle's loop has been reported to be both decreased (31) and unaffected (2, 19) in hypercalcemia. The unchanged distal TF/PNa in the present study supports the latter proposal. The reason for the elevated distal TF/PN is unclear, although the decrease of GFR and prolongation of distal transit time may be important factors. Reduced GFR in the rat consequent to renal arterial constriction is associated with elevated parathyroid hormone. The unchanged distal TF/PN in values (21). Only a slight dissociation of fractional calcium and sodium reabsorption was evident at the distal puncture site, indicating the ability of the loop to compensate for more proximal impairment. Further dissociation of fractional calcium and sodium reabsorption occurred beyond the distal puncture site as indicated by the Ca/Na ratios. This is in line with other studies in this laboratory where chlorothiazide and parathyroid hormone both cause disproportionate changes in the reabsorption of these cations beyond the distal puncture site (9, 10). The likely suppression of parathyroid hormone with hypercalcemia may account for at least part of the distal dissociation in Ca/Na transport observed in this study.

However, in clearance studies calcium infusion increased the Ca/Na ratio independently of parathyroid hormone, and direct effects of Ca may account for the distal dissociation of Ca from Na reabsorption (26).

The authors are indebted to Miss Valerie Cripps, Miss Evelyn Crystal, and Mrs. Antoinette Oilbrich for their expert technical assistance; to Mr. James Pettit for modifying the helium glow photometer; and to Drs. Carol A. Harris and Philip G. Baer for additional help in some experiments.

Dr. Edwards and Sutton were Fellows of the Canadian Medical Research Council.

This work was funded by Grant MT-1915 of the Canadian Medical Research Council and by the Banting Research Foundation.

This study was presented in part at the meeting of the Canadian Society for Clinical Investigation, Toronto, January 26th, 1972.

Received for publication 99 June 1973.

REFERENCES


