Abnormalities of calcium metabolism in essential hypertension

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(Received 7 July/16 December 1982; accepted 23 February 1983)

Summary
1. Calcium metabolism has been investigated in patients with essential hypertension and normal renal function to evaluate the renal calcium handling and the reported increase in renal calcium loss.
2. In 55 hypertensive and 55 sex- and age-matched healthy normotensive subjects creatinine clearance, serum total and ionized calcium, plasma parathyroid hormone and 24 h urinary excretion of calcium, sodium and cAMP were measured. In a subgroup of 20 hypertensive patients and 20 controls the fasting calcium excretion rate was also measured.
3. Both 24 h and fasting calcium excretion rates were higher in the hypertensive group; so also were plasma parathyroid hormone and urinary cAMP. Serum total and ionized calcium levels were not different in the two groups.
4. After intravenous calcium infusion (15 mg 3 h \(^{-1}\) kg \(^{-1}\)) in seven hypertensive patients and controls, the hypertensive patients excreted more calcium at all serum calcium concentrations.
5. These results support the hypothesis of primary renal calcium leak in essential hypertension. Enhanced urinary calcium excretion rate may cause compensatory parathyroid overactivity.

Key words: calcium, hypertension, parathyroid activity, urinary calcium excretion.

Introduction
Abnormalities of calcium metabolism have been described in patients with essential hypertension, including increased urinary calcium loss [1–3], decreased serum Ca\(^{2+}\) levels [4], higher incidence of primary hyperparathyroidism [5] and possibly of kidney stone disease [6]; some of these abnormalities have also been found in spontaneously hypertensive rats [7, 8].

These findings may be relevant to the pathophysiology of arterial hypertension, as calcium controls the contractile activity of vascular smooth muscle.

An increased urinary calcium excretion, accompanied by enhanced parathyroid gland activity, has been reported recently [1] in a small group of hypertensive subjects; a similar finding has been obtained from a preliminary study in our laboratory [2, 3].

The present study has been designed to establish the existence of an abnormal urinary calcium excretion in a larger series of hypertensive patients with normal renal function and to search for possible causes of enhanced renal calcium loss.

Subjects and methods
A group of 55 hypertensive patients, 28 males and 27 females, 42.7 ± 1.1 years, body mass index 27.4 ± 0.6, glomerular filtration rate (GFR) 63 ± 2 ml min\(^{-1}\) m\(^{-2}\) (mean ± SEM), seen consecutively in our Clinic, with a diastolic blood pressure (DBP) over 95 mmHg on two occasions, participated in the study. The diagnosis of uncomplicated primary hypertension with normal renal function was made by routine clinical investigations, including measurements of GFR by creatinine clearance, 24 h urinary aldosterone and catecholamine excretion, inspection of optic fundi, ECG, radioisotope renogram and rapid sequence intravenous pyelography.
The control group was made of 55 clinically healthy normotensive volunteers with comparable characteristics, 28 males and 27 females, 40.3 ± 0.6 years, GFR 65 ± 2 ml min⁻¹ m⁻².

At the time of the study all the participants were on an unrestricted diet and had no medications for at least 1 month.

A blood sample was obtained by venepuncture between 08.00 and 10.00 hours after a 12 h fast, at the end of a 24 h urine collection.

Total serum and urinary calcium was measured by atomic absorption spectrophotometry; urinary sodium by flame photometry; serum and urine creatinine by the picric acid colorimetric method. Serum Ca²⁺ was measured by a calcium-specific electrode (Orion SS 20). The immunoreactive parathyroid hormone (PTH) was determined by a double antibody radioimmunoassay (Sorin Biomedica), with bovine ¹²⁵I-labelled PTH and an antibody against the C-terminal part of the PTH molecule; urinary cAMP excretion was measured by a radioimmunoassay [9].

From a sample of 20 hypertensive patients and 20 sex- and age-matched controls, a timed urine collection was also obtained in the morning after a 12 h fast for determination of calcium excretion rate under fasting condition.

Finally in seven hypertensive and seven comparable normotensive men a more detailed investigation of calcium handling by the renal tubule was undertaken. An intravenous calcium load was given by constant rate infusion by the method of Mioni et al. [10]; 15 mg of Ca²⁺/kg body weight was infused over 3 h as 10% calcium chloride, further diluted in 100 ml of water.

Hourly urine specimens were collected for 2 h before, during the course of the infusion and at the end of a 1 h wash-out period, for the measurement of calcium, sodium and creatinine excretion.

Venous blood samples were obtained from the contralateral arm to the infusion at the mid-point of each urine collection, to determine total and ionized calcium and creatinine concentrations.

The statistical evaluation of results was carried out by one- or two-tailed Student's t-test for unpaired observations and Pearson linear regression analysis, as appropriate [11].

Results

The results of the whole series of 55 hypertensive patients and controls are shown in Table 1.

The 24 h urinary calcium excretion was significantly higher in essential hypertension: the average difference was similar in males and females (males: 4.75 ± 0.80 vs 3.77 ± 0.31, P<0.03; females 4.43 ± 0.34 vs 3.39 ± 0.27, P<0.02).

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### TABLE 1. Comparison of data from normotensive (n = 55) and hypertensive (n = 55) subjects

<table>
<thead>
<tr>
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<th>Normotensive</th>
<th>Hypertensive</th>
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<tr>
<td>Urinary Ca (mmol/24 h)</td>
<td>3.58 ± 0.20</td>
<td>4.60 ± 0.26**</td>
</tr>
<tr>
<td>Urinary Na (mmol/24 h)</td>
<td>160 ± 9</td>
<td>184 ± 9</td>
</tr>
<tr>
<td>100 × Urinary Ca/Na ratio</td>
<td>2.28 ± 0.11</td>
<td>2.69 ± 0.18*</td>
</tr>
<tr>
<td>Serum total Ca (mmol/l)</td>
<td>2.54 ± 0.03</td>
<td>2.42 ± 0.04</td>
</tr>
<tr>
<td>Serum ionized Ca (mmol/l)</td>
<td>1.10 ± 0.01</td>
<td>1.09 ± 0.01</td>
</tr>
<tr>
<td>Plasma PTH (m.i.u./ml)</td>
<td>2.20 ± 0.08</td>
<td>2.78 ± 0.19*</td>
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<tr>
<td>Urinary cAMP (nmol/100 ml of GF)</td>
<td>2.69 ± 0.12</td>
<td>3.57 ± 0.17***</td>
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The 24 h sodium was not significantly higher in hypertensive patients but was directly related to calcium excretion, both in patients (r = 0.48, P < 0.001) and controls (r = 0.55, P < 0.001). To correct for the different sodium excretion, the average urinary calcium/sodium ratio was then calculated: this index was significantly increased in the hypertensive group.

The serum total and ionized calcium concentrations were comparable in patients and controls. The mean plasma PTH concentration was found to be higher in the hypertensive group, with borderline statistical significance. Urinary cAMP excretion was significantly increased. A weak but significant direct correlation was found between urinary cAMP and calcium excretion (r = 0.50, P < 0.001) in hypertensive subjects.

Fig. 1 shows the urinary calcium excretion rates in comparable groups of normotensive and hypertensive subjects under fasting condition. The average calcium excretion was significantly higher in the hypertensive group and the distribution of the values showed little overlap with the control group. The values from controls were distributed within a relatively small range, whereas the variability within the hypertensive group appeared to be larger. No significant difference was found in either serum ionized (1.07 ± 0.01 vs 1.09 ± 0.01 mmol/l) or total (2.47 ± 0.05 vs 2.50 ± 0.05 mmol/l) calcium concentration between patients and controls.

The data obtained after the intravenous calcium load are summarized in Fig. 2. The urinary calcium excretion rate measured in hourly urine fractions was plotted against the serum Ca²⁺ concentration found at the mid-point of the urine collection. Separate regression analysis was done for each subject: the slopes of the single regression lines from the hypertensive and control groups were
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*FIG. 1. Urinary calcium excretion rates in fasting normotensive (n = 20; 10 male, 10 female) and hypertensive (n = 20; 10 male, 10 female) subjects (means ± SEM).

*FIG. 2. Comparison of urinary calcium excretion rates in normotensive (•: n = 7 male) and hypertensive (○: n = 7 male) subjects during intravenous calcium infusion. A regression equation of urinary calcium excretion on serum ionized calcium concentration was set up for each subject. The individual a and b coefficients were averaged to obtain the regression lines of the normotensive (y = -49.81 + 46.51x) and the hypertensive (y = -77.45 + 75.58x) group respectively.

different with minimum overlap (75.58 ± 6.30 vs 46.51 ± 5.00, P < 0.01). It was apparent that the hypertensive patients excreted on average more calcium at any level of serum calcium concentration and of filtered calcium load.

The theoretical threshold for calcium excretion, given by the value of the intercept of the regression line on the x axis, appears to be slightly, but not significantly, lower in the hypertensive group.

Discussion

The present data confirm the results of a preliminary study, recently reported by our laboratory [2, 3], and provide further evidence in favour of a defect in renal tubular calcium handling in essential hypertension.

The average increase in 24 h urinary calcium output of hypertensive patients was 28%, with no appreciable difference between the sexes. We found two male and three female patients with 24 h urinary calcium output above the accepted cut-off points for hypercalciuria (300 mg for males and 250 mg for females) [12].

Since differences in the dietary intake of sodium chloride are known to affect calcium transport by the renal tubule [13, 14], we evaluated the calcium/sodium ratio in 24 h urine to control for the influence of this potentially confounding variable. The hypertensive patients in this study had slightly, but not significantly, higher 24 h sodium excretion than controls, in agreement with previous observations [15–17]. Nevertheless the urinary calcium/sodium ratio was still significantly enhanced in the hypertensive group, indicating an average 20% increase in calcium excretion at any given level of urinary sodium.

After McCarron’s report [1] of a possible urinary calcium leak in essential hypertension, this is the first demonstration of an absolute increase in calcium excretion in human hypertension. In that study [1] the authors actually compared calcium excretion in 24 h urine collections from hypertensive patients and in timed fast urine specimens from controls.

An increase in 24 h calcium excretion may be associated with a number of metabolic disorders [18], including increased rate of bone reabsorption (as in thyrotoxicosis, Paget’s disease, skeletal metastasis, immobilization), increased net intestinal calcium absorption (as in most cases of so-called idiopathic hypercalciuria) and a primary renal defect in calcium handling.

In our patients there was no clinical or laboratory evidence to support the hypothesis of resorptive hypercalciuria, a condition associated with depressed PTH levels and enhanced serum calcium
tial hypertension is not dependent on an increased concentration of calcium filtered load, but is likely to be accounted for by a primary renal defect. The latter finding is also contrary to the hypothesis of an absorptive hypercalciuria [18]. Nevertheless, we evaluated this possibility further by measuring the urinary calcium excretion rate in the fasting state, which is independent of circadian variations in serum calcium concentration related to meals and is particularly suited to differentiate between absorptive hypercalciuria and a primary renal calcium leak [21]. The fasting calcium excretion rates in our patients were twice those in controls with little overlap, despite the evidence of an increased parathyroid gland activity. As the serum total and ionized calcium levels were comparable in the two groups, this result indicates that the enhanced urinary calcium output in essential hypertension is not dependent on an increased calcium filtered load, but is likely to be accounted for by a primary renal defect.

Further evidence in favour of a renal tubular defect in essential hypertension, with compensatory increase in PTH secretion, comes from the evaluation of urinary calcium titration curves, obtained by gradually increasing the filtered load of calcium by means of an intravenous calcium infusion. The regression line for the hypertensive group lay to the left of that of controls, indicating that calcium excretion was higher at any level of filtered calcium. This finding is especially relevant as parathyroid overactivity is known to shift the calcium titration curve to the right [22]. The slope of the regression line for control subjects was close to previously reported values for normal subjects [10, 22, 23], whereas that of hypertensive patients was markedly increased in six out of seven cases. According to Mioni et al. [10], this result can be interpreted as the expression of a defect in calcium reabsorption in the proximal tubule. It is then reasonable to assume that parathyroid overactivity compensates to some degree for the proximal calcium leak by increasing calcium reabsorption in the distal part of the nephron [23, 24].

The process of calcium reabsorption is dependent on the passive entry of calcium from the tubular lumen into the cell interior down its electrochemical gradient and on its active extrusion at the contraluminal surface [23]. A reduced rate of calcium efflux has been found in erythrocytes of spontaneously hypertensive rats [25]: a similar defect in the renal tubular cell could possibly explain the increase in urinary calcium excretion observed in essential hypertension. The hypothesis can be proposed that this defect is the expression of a widespread abnormality of transmembrane calcium transport in hypertension, in accordance with findings of several authors [25-27].

Whether the increased urinary calcium loss and the subsequent derangement in calcium metabolism can contribute to blood pressure elevation, is difficult to assess at the present time. It has been proposed that a reduction in serum Ca\(^{2+}\) concentration, secondary to the renal calcium leak, could increase the excitability of vascular smooth muscle [4]. A reduction in serum Ca\(^{2+}\) has not been found in our patients, although patients selection could explain this failure as more severely hypertensive patients were included in the McCarron [4] series. In addition it is unlikely that a decrease in extracellular calcium concentration would act solely on vascular smooth muscle and not on other excitable systems (myocardium, skeletal muscle) [18].

Kesteloot & Geboers [28] have reported a weak direct association between total calcium and blood pressure in a cross-sectional study of a Belgian population. However, no data were given for subjects with arterial hypertension as defined in our study. Thus a comparison with our findings is impossible.

A possible contributory role of the increased PTH levels to hypertension may be suspected, as there is circumstantial evidence for a permissive role of PTH on blood pressure. In parathyroidec-tomized rats, genetic or DOCA-induced hypertension develops to a milder degree than in intact controls [29, 30]. Calcium turnover, and probably the intracellular concentration of calcium, are increased by PTH in different cell types, including myocytes of the arterial wall [31, 32]. This in turn would increase the tone of the vascular bed or enhance the contractile response to adrenergic stimuli [27].

Finally, what are the possible clinical implications of the defect in renal calcium handling? Rosenthal & Roy [5] have reported from a population survey a higher incidence of parathyroid adenomas in asymptomatic subjects with essential hypertension. It is conceivable that these cases might be a consequence of chronic stimulation of parathyroid glands secondary to renal calcium loss (so-called tertiary hyperparathyroidism) [33].

In addition, our finding of five cases of hypercalciuria out of 55 hypertensive patients raises a possible predisposition to kidney stone formation in patients with arterial hypertension [6, 8]. Further studies are indicated to evaluate these clinical problems and the metabolic effects of antihypertensive drugs known to interfere with renal
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calcium handling (thiazides) or in general with transmembrane calcium transport (calcium antagonists).

References


