Calcitropic Hormones, Platelet Calcium, and Blood Pressure in Essential Hypertension

Arnold S. Brickman, Michael D. Nyby, Kern von Hungen, Peter Eggenga, and Michael L. Tuck

Plasma ionized calcium, platelet cytosolic calcium (using the fura-2 method in gel-filtered platelets), parathyroid hormone (both the intact hormone and a midmolecule portion), calcitriol, and calcidiol were measured in 19 untreated male patients with essential hypertension and 19 age-matched normotensive male research subjects. Mean levels of platelet cytosolic calcium, parathyroid hormone, calcitriol, and calcidiol were all significantly higher, whereas plasma ionized calcium was significantly lower, in the hypertensive group compared with the normotensive group. Both platelet cytosolic calcium and intact parathyroid hormone were positively correlated with mean arterial pressure (r=0.58, p<0.001; r=0.54, p<0.001, respectively), whereas plasma ionized calcium was inversely correlated with mean arterial pressure (r=−0.60, p<0.001) in the combined group of all study subjects. All three of these correlations were significant in the hypertensive group alone but not in the normotensive group alone. When analyzed with plasma ionized calcium, body mass index, serum calcitriol, and calcidiol in a multivariable regression model, the significance of the partial regressions of platelet cytosolic calcium and parathyroid hormone with mean arterial pressure persisted. Intact parathyroid hormone was positively correlated to platelet cytosolic calcium (r=0.43, p<0.01) and plasma ionized calcium was inversely correlated to platelet cytosolic calcium (r=−0.44, p<0.01). These results confirm previous reports of disturbances of calcium metabolism in essential hypertension and suggest that the elevated platelet cytosolic calcium observed in essential hypertension may be linked to one or more of these alterations of calcium metabolism. (Hypertension 1990;16:515–522)

Several studies have described disturbances of calcium metabolism in subjects with essential hypertension. McCarron1 reported decreased plasma ionized calcium in patients with essential hypertension and also suggested that subjects with essential hypertension include less calcium in their diet.2 Further, McCarron and Morris3 also found that supplementing the dietary calcium intake of subjects with essential hypertension could decrease the blood pressure of some subjects. The blood pressure-lowering effect of dietary calcium has also been observed in rat models of hypertension.4,5 The major calcitropic hormones, parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D3 [calcitriol or 1,25(OH)2D3], have been shown to be increased in some forms of essential hypertension,6–9 which may reflect a compensatory increase of PTH secretion in response to a "renal calcium leak."

Besides the above mentioned alterations of calcium metabolism in essential hypertension, recent studies have indicated an elevation in levels of platelet cytosolic calcium concentration ([Ca2+]i) in individuals with essential hypertension.10–16 This elevation in platelet [Ca2+]i in essential hypertension may reflect intracellular calcium levels in other tissues, including vascular smooth muscle, where increased vascular smooth muscle reactivity would be expressed as increased vascular resistance and elevated blood pressure.17 The mechanism by which platelet [Ca2+]i is elevated in essential hypertension is yet to be identified.

The calcitropic hormones, which are elevated in essential hypertension, can influence intracellular calcium levels. PTH has been shown to stimulate the influx of free calcium into isolated cardiac cells18 and osteoblastlike cells.19 Also, 1,25(OH)2D3 reportedly increases the uptake of calcium in vascular smooth muscle cells20 and cardiac myocytes21 and increases cardiac contractility.22 Furthermore, 1,25(OH)2D3...
has been shown to increase calcium adenosine triphosphatase (Ca-ATPase) activity in a vascular smooth muscle cell line. Therefore, both elevated PTH and 1,25(OH)2D3 provide possible mechanisms whereby [Ca2+], is elevated in platelets in essential hypertension.

The present investigation was undertaken to examine the possible interrelations between blood pressure, plasma ionized calcium, platelet [Ca2+], PTH, and 1,25(OH)2D3 over a range of blood pressure as represented in carefully studied age-matched groups of individuals with and without essential hypertension.

**Methods**

Studies were carried out in 19 male patients who had been diagnosed with mild-to-moderate essential hypertension and 19 age-matched normotensive male research subjects. Informed consent was obtained from all subjects before the study. Seated blood pressure measurements were obtained by conventional sphygmomanometric methods between 8:00 and 10:00 AM by the same individual. Three determinations were performed on each subject with a 5-minute interval between each, and the average of these three determinations was used as the actual blood pressure. Subjects were considered normotensive if blood pressure was less than 140/90 mm Hg. All but two of the subjects with hypertension had blood pressure ranging from 130 to 190 mm Hg, and diastolic blood pressure ranged from 90 to 112 mm Hg. For purposes of subsequent data analysis, mean arterial blood pressure was used as the index of blood pressure and was calculated as the diastolic pressure plus one third the pulse pressure. Body weight and height from each subject were recorded and used in calculation of the body mass index (BMI) by the equation:

\[
\text{BMI} = \frac{\text{weight (kg)}}{\text{height (m)}^2}
\]

None of the participants in these studies were receiving medications known to affect platelet function. All subjects were on an unrestricted diet but were asked to fast for the 12 hours before the study. Individuals with chronic illnesses such as diabetes mellitus, obstructive lung disease, renal disease, or disorders known to affect calcium or calcitropic hormone homeostasis were excluded from selection.

For biochemical analyses, 35-ml venous blood samples were drawn; 20 ml blood was immediately added to 3.5 ml acid-citrate-dextrose solution (2.0 g sodium citrate, 1.5 g citric acid, 2.0 g dextrose in 100 ml water) and gently mixed. Samples for measurement of plasma ionized calcium were collected anaerobically in 3-ml tubes with heparin and chilled on ice until measurements were performed. Samples for plasma renin activity were collected in iced tubes containing ethylenedinitrilotetraacetic acid (EDTA). Serum samples were collected for measurement of levels of PTH, calcitriol, 25-hydroxyvitamin D3 [calcidiol or 25(OH)2D3], total calcium, magnesium, phosphorus, and creatinine. Twenty-four-hour urine samples were collected for determination of creatinine clearance and urinary excretion of calcium and sodium.

**Measurement of Platelet [Ca2+]i**

Platelet [Ca2+], was measured using the methods similar to those described by Erne et al.11 Briefly, the citrated blood was centrifuged at room temperature for 20 minutes at 140g. The resultant platelet-rich plasma fraction was then removed and layered onto a 2.5×10.0 cm Sepharose CL-2B (Pharmacia, Uppsala, Sweden) column that had been equilibrated with elution buffer containing 10 mM N-[2-hydroxyethyl]piperezine-N'-[2-ethanesulfonic acid] (HEPES) (Sigma Chemical Co., St. Louis, Mo.), 145 mM NaCl, 5 mM KCl, 1 mM MgSO4, 0.5 mM NaH2PO4, 6 mM glucose, pH 7.4 at 37°C. The platelet-rich plasma was then filtered through the gel column, and the fraction containing the platelets, recognized by its turbidity, was collected. The platelets were then incubated at 37°C with 3 μmol fura-2-acetoxyethyl ester (fura 2/AM, Calbiochem, La Jolla, Calif.) for 45 minutes, after which time the suspension was again gel-filtered on the Sepharose CL-2B column to remove free fura-2. Typically, this procedure yielded 7 ml of platelet suspension with a concentration between 1 and 2×109 platelets/ml. Three milliliters of platelet suspension was then placed in a quartz cuvette, and sufficient CaCl2 was added to establish a 1.0 mM extracellular calcium concentration. After 30 minutes of equilibration at 37°C, the cuvettes were placed in a Model 303 H & L Spectrofluorometer (H & L Instruments, Burlingame, Calif.). This instrument permits rapid changes of excitation wavelengths from 340 to 380 nm. Emission detection was limited using a Schott KV-470 filter (Schott Glass Technologies, Inc., Duryea, Pa.). Under temperature-controlled conditions, six measurements of basal platelet [Ca2+], were obtained at 1-minute intervals. To calibrate the sample for [Ca2+], determinations, the platelets were lysed using 3 μl Triton X-100 (Sigma), and the maximal 340/380 fluorescence ratio (range 20–30) was recorded. After adjusting the pH to 8.3, 2 mM ethylene glycol-bis(β-aminoethyl ether)-N,N',N''-tetraacetic acid (EGTA) (Sigma) was added to the cuvette contents. The minimal 340/380 ratio was then measured and was found to range from 1.2 to 1.4. Using these 340/380 ratios, [Ca2+], was calculated according to the equation:

\[
[\text{Ca}^{2+}] = 224 \frac{(R - R_{\text{min}})}{(R_{\text{max}} - R)} (S_f / S_b)
\]

where R is the 340/380 ratio in the intact platelet suspension, Rmin is the ratio after EGTA chelation, Rmax is the ratio after Triton X-100 platelet lysis, and
Sf2 and Sb2 are the emissions at 380 nm excitation of the free and bound forms, respectively. Measurement of platelet \([\text{Ca}^{2+}]\), was performed in duplicate platelet suspensions from each subject. In addition to measurement of basal \([\text{Ca}^{2+}]\), changes in platelet \([\text{Ca}^{2+}]\), were measured during incubation with human thrombin (Calbiochem) with cumulative doses of 0.01, 0.05, and 0.10 units/ml. These studies were performed in the second platelet suspension from each subject. In these studies, after the determination of baseline levels of \([\text{Ca}^{2+}]\), 0.01 units/ml human thrombin was added to the nonstirred platelet suspension (cuvette), mixed briefly with a pipette, and the emission ratios were monitored every 20 seconds for 6 minutes. Then the next dose of thrombin (0.05 units/ml) was added and the emission ratios again monitored for 6 minutes. Then 0.10 units/ml thrombin was added to the cuvette and the results monitored as before. Finally, the platelet sample was treated with Triton X-100 and EGTA as described above. The elapsed time between obtaining the blood samples from the study subjects and the measurement of platelet \([\text{Ca}^{2+}]\), ranged from 2 to 3 hours.

To examine the reproducibility of this method for measurement of platelet \([\text{Ca}^{2+}]\), studies were performed on two separate occasions in 21 subjects. The time interval between repeat measurements ranged from 1 to 12 months, with a mean interval period of 2.3 months. As shown in Figure 1, the correlation coefficient between repeat studies was 0.97 and the mean percent difference between studies was 3.1% with values ranging from 0.4% to 6.7%. To minimize leakage of fura-2 from platelets, the samples were maintained at room temperature until prepared for analysis. At the end of several experiments, while developing our methods, the platelets were examined and counted microscopically. We found that the platelets did not lyse and appeared normal after each day’s experiments were concluded. Occasional samples were discarded because lysis, as evidenced by a rapidly increasing baseline measurement, occurred during the measurements. In these cases, lysis was confirmed by microscopic examination.

**Blood Sample Analyses**

Samples for measurement of plasma ionized calcium were kept iced, and analyses were performed within 1 hour of venous sampling with a Nova 2 Ionized Calcium Analyzer (Nova Biomedical, Newton, Mass.). Serum samples for measurement of total calcium, phosphorus, magnesium, PTH, cholesterol, and plasma renin activity (PRA) were frozen and stored at \(-70\,^\circ\text{C}\) and subsequently analyzed in batch assays. Serum sodium, potassium, creatinine, chloride, as well as urine creatinine, calcium, and sodium were measured on an Astra 4 electrolyte analyzer (Beckman Instruments, Palo Alto, Calif.). Serum phosphorus was measured with a Technicon autoanalyzer (Technicon Instruments Corp., Tarrytown, N.Y.), and total serum calcium and magnesium were determined with a Perkin-Elmer Model 372 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.). PRA was measured by a previously reported method. Creatinine clearance was determined using the 24-hour urinary creatinine excretion in conjunction with serum creatinine and expressed as milligrams per milliliter per 1.73 meters squared of body surface area.

Two different commercial assays were used for measurement of PTH. Intact PTH [PTH(1–84)] was measured with an immunoradiometric assay kit obtained from Nichols Institute Diagnostics, San Juan Capistrano, Calif. This assay used a combination of two antisera that do not cross-react with the following PTH peptide fragments: 1–34, 39–68, 53–84, and 39–84. The second PTH assay we used is considered to be midmolecule specific (PTH-M) (Endocrine Metabolic Center, Oakland, Calif.). \(1,25(\text{OH})_2\text{D}_3\) and \(25(\text{OH})\text{D}_3\) were assayed with commercially available assay kits obtained from INCStar, Stillwater, Minn. These assays measure both \(\text{D}_3\) and \(\text{D}_2\) metabolites, and reported measurements express total levels of these respective sterols.

**Statistical Methods**

Statistical analyses were carried out with NWA Statpak (Northwest Analytical, Portland, Ore.). Data were analyzed by both single variable (linear, log, exponential, and power curve fitting) and multivariate regression analyses. Student's t test for unpaired data was used to compare means of grouped data. On all analyses, a value of \(p<0.05\) was considered to indicate a probable significant difference or correlation. Data reported on tables and figures is expressed as the mean±SEM.

**Results**

Table 1 summarizes age, BMI, and blood pressure in the normotensive and hypertensive research subjects. Mean and range of ages in the two groups were similar, 55.6±3.1 (range 26–73 years) and 57.0±3.1 (range 25–79 years), respectively, in normotensive and hypertensive groups. 24 Metabolic studies were performed on two separate occasions in 21 subjects. The time interval between repeat measurements ranged from 1 to 12 months, with a mean interval period of 2.3 months. As shown in Figure 1, the correlation coefficient between repeat studies was 0.97 and the mean percent difference between studies was 3.1% with values ranging from 0.4% to 6.7%. To minimize leakage of fura-2 from platelets, the samples were maintained at room temperature until prepared for analysis. At the end of several experiments, while developing our methods, the platelets were examined and counted microscopically. We found that the platelets did not lyse and appeared normal after each day’s experiments were concluded. Occasional samples were discarded because lysis, as evidenced by a rapidly increasing baseline measurement, occurred during the measurements. In these cases, lysis was confirmed by microscopic examination.
and hypertensive subjects. In the normotensive group, mean arterial pressure ranged from 79 to 102 mm Hg, and from 103 to 125 mm Hg in hypertensive subjects. BMI was greater in the hypertensive group than in the normotensive group.

Table 2 summarizes the biochemical parameters measured in the two groups of study subjects. Of these parameters, mean plasma ionized calcium was significantly lower and levels of PTH, measured both as intact hormone [PTH(1–84)] and PTH-M, were significantly higher in the hypertensive group than in the normotensive group. Both 1,25(OH)2D3 and 25(OH)D3 were also significantly higher in the hypertensive group alone (r=0.533, p<0.01) but not within the normotensive group alone. The values for platelet [Ca2+]i in the present study are in agreement with those reported in hypertensive and normotensive subjects by Erne et al10 (168 and 108 nmol/l, respectively) and Cooper et al,11 as well as those of the present study, are somewhat elevated due to this leakage. However, the results of the present study are qualitatively similar to those reported by groups who did correct for dye leakage. One of these groups, Pritchard et al,16 reported a mean platelet [Ca2+]i of 98 nmol/l in a combined group of 18 normotensive and hypertensive subjects and found a significant correlation between platelet [Ca2+]i and mean arterial blood pressure (r=0.534, p<0.02). Another group who corrected for dye leakage, Hvarfner et al,15 reported a platelet [Ca2+]i of 122.0 in 29 essential hypertensive subjects and 99.4 nmol/l in 22 normotensive subjects and also found a significant correlation between platelet [Ca2+]i and mean arterial pressure (r=0.30, p=0.02). Thus, it appears that the lack of correction for dye leakage in the present study did result in somewhat higher absolute values for platelet [Ca2+]i in the two groups, but the differences between the groups and the relation between platelet [Ca2+]i and blood pressure are not appreciably different from those obtained in studies where correction for leakage was made.

### Table 1. Characteristics of Study Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normotensive subjects (n=19)</th>
<th>Hypertensive subjects (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>55.6±3.14</td>
<td>57.0±3.1</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>123±2.8</td>
<td>150±3.9*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>78±1.3</td>
<td>97±0.9*</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>98±1.5</td>
<td>115±1.6*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26±0.6</td>
<td>31±1.2*</td>
</tr>
</tbody>
</table>

MAP, mean arterial blood pressure; BMI, body mass index. p<0.01

### Table 2. Biochemical Parameters in Study Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive subjects (n=19)</th>
<th>Hypertensive subjects (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet [Ca2+]i (nmol/l)</td>
<td>112±2.4</td>
<td>127±3.0*</td>
</tr>
<tr>
<td>Total serum calcium (mg/dl)</td>
<td>9.6±0.06</td>
<td>9.5±0.09</td>
</tr>
<tr>
<td>Plasma ionized calcium (mg/dl)</td>
<td>4.94±0.04</td>
<td>4.74±0.04*</td>
</tr>
<tr>
<td>Serum phosphate (mg/dl)</td>
<td>3.3±0.10</td>
<td>3.1±0.09</td>
</tr>
<tr>
<td>PTH(1-84) (pg/ml)</td>
<td>39±2.9</td>
<td>57±6.0*</td>
</tr>
<tr>
<td>PTH-M (pg/ml)</td>
<td>198±10.7</td>
<td>270±33.5*</td>
</tr>
<tr>
<td>1,25(OH)2D3 (pg/ml)</td>
<td>25.7±1.2</td>
<td>31.9±2.6*</td>
</tr>
<tr>
<td>25(OH)D3 (ng/ml)</td>
<td>23.1±1.3</td>
<td>29.2±1.5*</td>
</tr>
<tr>
<td>CREATININE CLEARANCE (mg/ml/1.73 m²)</td>
<td>114.5±6.9</td>
<td>99.3±6.9</td>
</tr>
</tbody>
</table>

[Ca2+]i: cytosolic calcium concentration; PTH(1-84), intact parathyroid hormone; PTH-M, midmolecule fragment of parathyroid hormone; 1,25(OH)2D3, calcitriol or 1,25-dihydroxyvitamin D3; 25(OH)D3, calcidiol or 25-hydroxyvitamin D3; PRA, plasma renin activity.

*p<0.05.

tp<0.01.
Levels of PTH measured either as PTH(1–84) or PTH-M correlated strongly with mean arterial pressure when the groups were combined. The strongest correlation was observed between mean arterial pressure and PTH(1–84) (Figure 3). This relation was also evident in the hypertensive subset (r=0.467, p<0.025) but not in the normotensive group. The correlation coefficient for the relation between mean arterial pressure and PTH-M in the combined group was 0.333, p<0.025.

Plasma ionized calcium correlated inversely with mean arterial pressure (r=-0.60, p<0.001), platelet [Ca²⁺]ᵢ, PTH(1–84), and PTH-M (r=-0.28, p<0.05) in the combined group. Although none of these correlations was significant in the normotensive group alone, the inverse correlation between plasma ionized calcium and platelet [Ca²⁺]ᵢ was significant in the hypertensive group alone (r=-0.46, p<0.025).

Creatinine clearance correlated inversely with PTH-M (r=-0.45, p<0.01) and with PTH(1–84) (r=-0.34, p<0.03). There were no significant correlations evident between creatinine clearance and blood pressure, serum ionized calcium, platelet [Ca²⁺]ᵢ, or 1,25(OH)₂D₃.

Other parameters that differed between the two study groups could influence the relations among blood pressure, platelet [Ca²⁺]ᵢ, and PTH. Age matching and restriction of the study population to men excluded two of these variables. Possible additional relations with plasma ionized calcium, 1,25(OH)₂D₃, 25(OH)D₃, and BMI were examined by application of multivariate regression analysis. These results are shown in Table 3. With this analysis, platelet [Ca²⁺]ᵢ, PTH(1–84), and plasma ionized calcium maintained significant correlations with mean arterial pressure.

In the combined group, a significant correlation also was observed between platelet [Ca²⁺]ᵢ, and PTH-M. Age matching and restriction of the study population to men excluded two of these variables. Possible additional relations with plasma ionized calcium, 1,25(OH)₂D₃, 25(OH)D₃, and BMI were examined by application of multivariate regression analysis. These results are shown in Table 3. With this analysis, platelet [Ca²⁺]ᵢ, PTH(1–84), and plasma ionized calcium maintained significant correlations with mean arterial pressure.

Although basal platelet [Ca²⁺]ᵢ was higher in the hypertensive group, no differences were observed in the thrombin-stimulated [Ca²⁺]ᵢ response between
the two groups (Figure 6). Thrombin at all doses raised platelet [Ca\(^{2+}\)], to a peak between 40 and 100 seconds followed by a slow return toward baseline. Because these were cumulative doses of thrombin in each sample, there was noticeable desensitization to thrombin after the dose of 0.05 units/ml, resulting in a lower response to 0.1 units/ml. Systolic blood pressure correlated with the response to 0.01 units/ml thrombin in the hypertensive group (r=0.64, p<0.01).

**Discussion**

The results of the present study confirm previous reports of altered calcium metabolism in essential hypertension by other investigators.6-9 In the present study, the patients with essential hypertension had significantly higher levels of platelet intracellular calcium, PTH(1-84), PTH-M, 1,25(OH)\(_2\)D\(_3\), and 25(OH)D\(_3\) as well as decreased plasma ionized calcium. We found no difference in serum phosphate between the groups. This result differs from results of studies by McCarron et al\(^{10}\) and Kjeldsen et al\(^{26}\) in which decreased serum phosphate in subjects with essential hypertension was observed. However, additional studies other than our own by Gennari et al\(^{27}\) and Folsom et al\(^{28}\) in which decreased serum phosphate in subjects with essential hypertension was observed. However, additional studies other than our own by Gennari et al\(^{27}\) and Folsom et al\(^{28}\) have also failed to demonstrate a compensatory response to a renal calcium leak. Increased urinary excretion of calcium concomitant with elevated serum PTH in 102 patients with essential hypertension has been reported by Gennari et al.\(^{27}\) Resnick et al\(^{7}\) reported that PTH was elevated in individuals with "low renin" hypertension. Grobbee et al\(^{27}\) also found elevated intact PTH and decreased serum calcium in young hypertensive subjects and reported a positive correlation between intact PTH and blood pressure. We were unable to demonstrate increased urinary calcium excretion in the hypertensive group in the present study, but we did observe an inverse correlation between creatinine clearance and PTH. These results suggest that impaired renal function has some relation to the elevated PTH observed in essential hypertension.

Two distinctive and contrasting actions of PTH on blood pressure regulation have been described. Acute administration of PTH to several animal species has a hypotensive and vasodepressor effect.\(^{29,30}\) PTH infusions also antagonize the pressor responses to both norepinephrine and angiotensin II in dogs.\(^{30}\) Additionally, PTH has been viewed as a functional calcium antagonist, reducing calcium uptake in vascular smooth muscle.\(^{31}\)

The second and contrasting action of PTH appears to be one of facilitating development of hypertension. Studies of the spontaneously hypertensive rat (SHR) model of hypertension suggest that PTH is a factor in the development of hypertension.\(^{32,33}\) In this regard, parathyroidectomy can ameliorate the degree of blood pressure rise in SHR.\(^{34}\) Other studies in the rat have indicated that PTH exerts a permissive role in the pressor effect of calcium infusion.\(^{35}\) The mechanism of this putative permissive action of PTH in blood pressure regulation is unknown. Pang et al\(^{36}\) were unable to detect an increase in any serum PTH fragments during the development of hypertension in SHR, yet others have shown definite evidence of increased parathyroid gland activity in this same model.\(^{37}\) In normal humans, chronic administration of PTH increases blood pressure, although this pressor effect could be due to the resultant increase of serum calcium.\(^{38}\)
PTH has been shown to increase calcium entry into osteoblastlike cells and cardiac myocytes. Recent preliminary data indicate that PTH increases intracellular calcium concentration in isolated vascular smooth muscle cells. The mechanism of this ionophoric action of PTH on calcium transport has not yet been described.

Our results suggest an association between PTH(1-84) and platelet cytosolic calcium; the positive correlation obtained in the hypertensive group between these two factors demonstrates that changes in each occur proportionally. The lack of correlation between PTH(1-84) and platelet \([Ca^{2+}]_i\) in the normotensive group further suggests that PTH may not be an influence on platelet \([Ca^{2+}]_i\) in the normal population. Hvarfner et al. observed a negative correlation between PTH and platelet \([Ca^{2+}]_i\) in their combined group of normotensive and hypertensive subjects. However, the PTH assay that they used was sensitive not only to intact PTH, as is the case with the PTH(1-84) assay that we used, but also to fragments in the midmolecule and C-terminal portions of the molecule. Using this assay, Hvarfner et al. did not observe elevated PTH in their hypertensive subjects, despite decreased plasma ionized calcium. In our use of a PTH-M assay, we found no correlation between PTH-M and platelet \([Ca^{2+}]_i\) in the hypertensive group. Therefore, our demonstration of a correlation with intact PTH and a lack of correlation with the PTH-M assay suggests that platelet \([Ca^{2+}]_i\) may be associated uniquely to intact PTH.

The differences between the two PTH assays used by us may yield some further insight into the relation between hypertension and parathyroid gland activity. Intact PTH, which accounts for only about 10% of the total immunoreactive PTH-related peptides present in serum, is considered to be an index of parathyroid gland secretion. Serum concentrations of PTH fragments, which are not detected by the PTH(1-84) assay, are not only the result of parathyroid gland secretion but also the result of circulatory cleavage of the intact molecule and renal clearance of these fragments. In support of this, we found a stronger correlation between PTH-M (a measure of PTH fragments) and creatinine clearance than between PTH(1-84) and creatinine clearance. Therefore, the fact that intact PTH is elevated in essential hypertension suggests increased parathyroid activity and secretion in this condition. PTH itself may not be the only secretory product that results from increased parathyroid activity in hypertension; perhaps some other yet to be identified substance secreted by the parathyroid glands has a role in blood pressure regulation. The existence of such a substance, parathyroid hypertensive factor, has been proposed by Lewanczuk et al. This parathyroid gland product is found not only in human subjects with essential hypertension but also in SHR and, when infused into normotensive rats, produces hypertension and increased vascular reactivity to a number of vasoconstrictors.

The relation between vitamin D, either 1,25(OH)\(_2\)D\(_3\) or 25(OH)D\(_3\), and blood pressure regulation has been studied less than that of PTH and blood pressure. A recent publication by Sowers et al. reported that 1,25(OH)\(_2\)D\(_3\) was positively correlated with blood pressure in a group of 373 women. Resnick et al. also observed elevated 1,25(OH)\(_2\)D\(_3\) in essential hypertensive patients with low renin and low ionized calcium. Our group of essential hypertensive participants seems to fit into this category. However, others have reported decreased levels of 1,25(OH)\(_2\)D\(_3\) in hypertensive humans and SHRs. The reason for these discrepancies in hypertensive humans is unclear but may relate to dietary or age differences of the subject groups between studies. Our results support the observations of elevated 1,25(OH)\(_2\)D\(_3\) in some older populations of essential hypertensive men. Furthermore, the elevated levels of 1,25(OH)\(_2\)D\(_3\) observed in our studies are in agreement with the associated elevations of PTH, which stimulates 1-hydroxylation of 25(OH)D\(_3\) in the kidney. However, a mechanism that would also include elevation of 25(OH)D\(_3\) is not so apparent. A study of 25(OH)D\(_3\) in subjects with essential hypertension by Kokot et al. suggested that 25(OH)D\(_3\) concentrations were actually lower in hypertensive subjects. However, these authors speculated that some of the reduction of 25(OH)D\(_3\) in the present study may have been due to treatment with hydrochlorothiazide. The reasons for the observed increase of 25(OH)D\(_3\) in the hypertensive group in the present study are not clear.

Overall, our results confirm earlier reports of a dramatic alteration of calcium metabolism in essential hypertension. This change may result from a renal calcium leak that decreases plasma ionized calcium, which in turn stimulates PTH secretion and subsequently 1,25(OH)\(_2\)D\(_3\) production. Our results also suggest that small decreases in renal function may have a role in altering calcitropic hormone metabolism in essential hypertension. Additionally, our results demonstrate that decreased plasma ionized calcium coexists with increased platelet \([Ca^{2+}]_i\) in essential hypertension. This divergence between extracellular calcium and intracellular calcium suggests the existence of a factor that alters compartmentalization of ionized calcium in essential hypertension, a factor that allows intracellular calcium to be elevated beyond the normal proportion of intracellular to extracellular calcium. Our results also suggest that PTH, or a substance associated with PTH secretion in essential hypertension, may account for elevation of platelet intracellular calcium in subjects with essential hypertension.

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KEY WORDS • calcium • parathyroid hormone • vitamin D • essential hypertension
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