Parathyroid Hormone, Platelet Calcium, and Blood Pressure in Normotensive Subjects

Arnold Brickman, Michael Nyby, Kern von Hungen, Peter Eggena, and Michael Tuck

Relations between platelet cytosolic calcium, parathyroid hormone, and blood pressure were investigated in 91 normotensive subjects: 47 men and 44 women ranging in age from 24 to 70 years. The men had higher mean arterial blood pressure, serum creatinine, and body mass index than the women. Serum total calcium, plasma ionized calcium, and parathyroid hormone (measured as both intact hormone and mid-molecule fragment) were not different between men and women; however, serum phosphate was higher in women than in men. Basal platelet cytosolic calcium was higher in men than in women (113.7±1.9 versus 105.9±1.7, respectively; p<0.01), but there was no difference in the peak platelet cytosolic calcium responses to thrombin between the two groups. In the combined group of male and female subjects, platelet cytosolic calcium correlated with diastolic blood pressure and mean arterial pressure (r=0.17, p<0.001 and r=0.32, p<0.01, respectively). Intact parathyroid hormone correlated with systolic and mean arterial blood pressure (r=0.41, p<0.001 for both). Age correlated with both systolic blood pressure (r=0.40, p<0.001) and intact parathyroid hormone (r=0.51, p<0.001). When multiple regression analysis was performed using mean arterial pressure as the dependent variable, platelet cytosolic calcium and intact parathyroid hormone maintained significant correlations with mean arterial pressure. Platelet cytosolic calcium did not correlate with intact parathyroid hormone. These results suggest that both platelet cytosolic calcium and intact parathyroid hormone are associated with blood pressure regulation in normotensive subjects. However, the influences of these two factors on blood pressure are not interrelated. (Hypertension 1991;18:176-182)

Recently, several investigators have reported that platelet cytosolic calcium ([Ca$^{2+}$]) is elevated in subjects with essential hypertension.$^{1-6}$ Some of these studies demonstrated strong correlations between platelet [Ca$^{2+}$] and blood pressure when the results of both normotensive and hypertensive subjects were combined.$^{1,2}$ Most studies have focused on the differences in platelet [Ca$^{2+}$] between hypertensive and normotensive subjects and have not examined the relation between platelet [Ca$^{2+}$] and blood pressure in normotensive subjects. Only one study has reported a significant correlation between blood pressure and platelet [Ca$^{2+}$] in normotensive subjects.$^{1}$ The significance of the relation between platelet [Ca$^{2+}$] and blood pressure is thought to be indicative of a generalized increase in intracellular free calcium in individuals with higher blood pressure. The effect of increased intracellular free calcium in vascular smooth muscle cells would be increased vasoreactivity, vasoconstriction, and resultant elevation of blood pressure. The mechanism by which platelet [Ca$^{2+}$] is increased concomitant with increased blood pressure is presently not known.

A possible mediator for this elevation of platelet and vascular smooth muscle [Ca$^{2+}$] is parathyroid hormone (PTH). PTH has been shown to be elevated in essential hypertension,$^{8-11}$ and parathyroid function has been implicated as a necessary factor for the development of hypertension in spontaneously hypertensive rats and in deoxycorticosterone acetate–salt–treated rats.$^{12-14}$ The 1–34 fragment of PTH, PTH(1–34), at a concentration of 5 units/ml (equivalent to 800 ng/ml), has been shown to induce the influx of free calcium into isolated cardiac cells.$^{15}$ Likewise, PTH(1–34) at doses as low as 10$^{-9}$ M (4.1 ng/ml) has been shown to increase cytosolic calcium in osteoblastlike cells.$^{16}$ Thus, a role for PTH as a determinant of intracellular calcium and blood pressure can be suggested. In a previous study from this laboratory, we observed a significant correlation between PTH and platelet [Ca$^{2+}$] in platelets from hypertensive subjects.$^{17}$ However, this relation was
not observed in the small group of normotensive men included in that study.

The present investigation was undertaken to study the relation of platelet \([Ca^{2+}]_i\), to blood pressure and PTH in a larger group of male and female normotensive subjects. Our purposes for this study were to substantiate the reported relation between platelet \([Ca^{2+}]_i\), and blood pressure in normotensive subjects and to investigate the possible relations between PTH and platelet \([Ca^{2+}]_i\), and between PTH and blood pressure in this group.

**Methods**

Ninety-one normal Caucasian subjects, 47 men and 44 women, were selected based on their age and general status of health. Subjects were considered normotensive if their blood pressure was lower than 140/90 mm Hg on three repeated measurements. None of the subjects were being treated with any medication for blood pressure control and had refrained from taking any other medications, including aspirin, for at least 2 weeks before the study. The subjects were asked to fast for 12 hours preceding the drawing of the blood samples for analyses. After informed consent was obtained from each subject, their seated blood pressure was measured three times (within 5 minutes) using conventional indirect methods. The diastolic pressure was taken as the pressure corresponding to Phase V Korotkoff sounds. Blood pressure measurements were repeated on all subjects at least 1 week later to confirm the accuracy of blood pressure measurements. The mean arterial pressure (MAP) was calculated as the diastolic pressure plus one third of the pulse pressure. After the blood pressure measurements were obtained, 40 ml venous blood was obtained from each subject's arm while they remained seated. Twenty milliliters of the blood was immediately added to 3.5 ml of acid-citrate-dextrose solution (2.5 g sodium citrate, 1.5 g citric acid, 2.0 g dextrose in 100 ml H2O) and was gently mixed. The remainder of the blood was distributed into separate evacuated containers as described below.

**Measurement of Platelet \([Ca^{2+}]_i\)**

Platelet \([Ca^{2+}]_i\) was measured using methods previously described in detail. Briefly, the citrated blood was centrifuged at room temperature for 15 minutes at 140g. The resultant platelet rich plasma was then layered onto a 2.5×10.0 cm Sephadex CL-2B (Pharmacia, Uppsala, Sweden) column that had been equilibrated with the elution buffer containing 10 mM N-2-hydroxyethyl]piperazine-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 platelets were eluted from the column with the buffer and a minimum of 7 ml platelet suspension was collected from this separation. The platelets were incubated at 37°C with 3 \(\mu\)M fura 2/AM (Calbiochem, La Jolla, Calif.) for 45 minutes; then they were again gel-filtered on the column to remove any free fura 2. Three milliliters of platelet suspension was then placed in a quartz cuvette, and sufficient calcium chloride was added to establish a 1.0 mM extracellular calcium concentration. The platelets were allowed to equilibrate with the calcium for 30 minutes at 37°C and then immediately were placed in the thermostatically controlled cuvette holder of a spectrofluorometer (model 303, H&L Instruments, Burlingame, Calif.). This instrument provides for rapid changes of excitation wavelength from 340 to 380 nm. Emission was controlled by using a 470 nm cutoff filter. Six basal \([Ca^{2+}]_i\), measurements of the unstirred platelet suspensions were then made at 60-second intervals. To calibrate the sample for calcium determinations, the platelets were lysed using 3 \(\mu\)l Triton X-100 (Sigma) and the maximal 340/380 fluorescence ratio recorded. Then 2 mM ethylene glycol-bis-(\(\beta\)-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) (Sigma) was added to the cuvette contents after adjusting the pH to 8.3 with NaOH. The minimal 340/380 ratio was then measured. Using these 340/380 ratios, \([Ca^{2+}]_i\), was calculated using the equation from Grynkiewicz et al:  

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

where \(R\) is the 340/380 ratio in the intact platelet suspension, \(R_{min}\) is the ratio after EGTA chelation, \(R_{max}\) is the ratio after Triton X-100 lysis, and \(S_f^2\) and \(S_b^2\) are the emissions at 380 nm excitation of the free and bound forms, respectively. A second sample of each subject's platelets was used for duplicate measurement of basal \([Ca^{2+}]_i\), as well as determination of \([Ca^{2+}]_i\), responses to thrombin stimulation. In these samples, following the determination of basal \([Ca^{2+}]_i\), as described above, 0.01 units/ml thrombin (Calbiochem) was added and quickly mixed with a 1.0 ml pipet. The resulting changes in fura-2 fluorescence were monitored every 15 seconds for 5 minutes with no additional stirring of the platelet suspension. Then 0.05 units/ml thrombin was added, and the resulting change in fluorescence was monitored as before. Five minutes after the final thrombin dose had been administered, the platelets were lysed and calibrated as described above.

The preincubations of the platelet suspensions with 1.0 mM calcium at 37°C were staggered 10 minutes apart so that all samples would be exposed to the external calcium at this temperature for the same length of time.

**Other Assays**

Samples for plasma ionized calcium were anaerobically drawn in 3-ml heparinized evacuated tubes and immediately chilled in ice. Ionized calcium was measured within 1 hour using a Nova 2 Ionized Calcium Analyzer (Nova Biomedical, Newton, Mass.). Blood for serum electrolytes was collected in a 10 ml serum separation tube and centrifuged at 4°C. The resultant serum was frozen at −70°C and assayed within 3 months. Serum sodium, potassium,
creatinine, and chloride were measured on an automated chemistry analyzer (Astra 4, Beckman Instruments, Palo Alto, Calif.). Total serum calcium and magnesium were determined using atomic absorption spectrophotometry (model 372, Perkin-Elmer Corp., Norwalk, Conn.). Serum cholesterol and serum uric acid were measured using automated methods.

Two separate assays were used for measuring PTH. One assay, obtained in kit form from Nichols Institute Diagnostics, San Juan Capistrano, Calif., measures intact PTH (PTH(1-84)); the other assay, also a kit, obtained from Dade (Baxter Travenol Diagnostics, Inc., Cambridge, Mass.), measures mid-molecule PTH (PTH-M). Both assays were performed according to kit instructions. According to the manufacturer, the combination antisera used in the PTH(1-84) assay do not cross-react with human PTH(l-84). Plasma renin activity was assayed using previously published methods.19

Data Analysis

Data was compiled and analyzed using computer-assisted techniques. Data was analyzed by both single variable regression analysis and multiple regression analysis using NWA STATPAK software (Northwest Analytical, Portland, Ore.) on a personal computer. Student's t test was used to compare means of grouped data. On all analyses, a value of p<0.05 was considered to indicate a significant difference or correlation. All values reported in the tables are the mean±SEM.

Results

Basic demographic information of the study subjects is shown in Table 1. For the sake of comparing the male subjects with the female subjects, this table shows the results divided by gender. As could be expected, the male subjects have higher body mass index and blood pressure than the female subjects. The male subgroup also had a higher serum creatinine than females (0.94±0.02 and 0.73±0.01 mg/dl, respectively, p<0.001). There were no differences between genders in serum sodium, potassium, chloride, magnesium, cholesterol, or plasma renin activity.

Table 2 lists calcium-related parameters, again divided by gender. The values for platelets [Ca2+]i obtained in the present study are in the range reported by other investigators for normotensive subjects, using either fura-2 or quin-2 methods.1-6 Basal platelet [Ca2+]i was found to be higher in the men, whereas serum phosphate was lower in this group. There was no difference between men and women in serum ionized calcium or in PTH by either assay. To confirm that gender itself was not a determinant of the higher platelet [Ca2+]i in men, we performed a multivariate regression test on the entire study group using platelet [Ca2+]i as the dependent variable and those factors we found to be different between men and women as independent variables. To include sex as a factor, women were given the numerical representation of 0 and men were given 1. As seen in Table 3, sex itself does not appear to be an important factor in determining platelet [Ca2+]i. The use of partial correlation coefficients allowed us to determine the correlation of mean arterial pressure to platelet [Ca2+]i, with the influence of the other variables listed in Table 3 removed. The observed differences in platelet [Ca2+]i between men and women seems to be mainly associated with the differences in blood pressure between these groups.

Despite the observed difference in basal platelet [Ca2+]i between men and women, there was no difference in the platelet [Ca2+]i response to thrombin stimulation. In men, the response to 0.05 units/ml thrombin was 193.7±11.6 nmol/l. In women, the response was 183.8±11.9 nmol/l, respectively. In both groups

<p>| TABLE 1. Descriptive Data of Male and Female Subgroups |
|---------|---------|---------|---------|---------|---------|</p>
<table>
<thead>
<tr>
<th>Sex</th>
<th>n</th>
<th>Age</th>
<th>SBP (mm Hg)</th>
<th>DBP (mm Hg)</th>
<th>MAP (mm Hg)</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>47</td>
<td>47.2±2.0</td>
<td>120.5±1.6</td>
<td>78.5±0.9</td>
<td>92.5±0.9</td>
<td>25.0±0.4</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>45.4±2.1</td>
<td>115.2±1.6*</td>
<td>74.1±1.1*</td>
<td>87.8±1.2*</td>
<td>22.6±0.4</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; BMI, body mass index.

*p<0.05 between groups.

\*p<0.001 between groups.

<p>| TABLE 2. Calcium Related Parameters in Male and Female Subgroups |
|---------|-------------|----------|----------|----------|----------|----------|</p>
<table>
<thead>
<tr>
<th>Sex</th>
<th>Serum total Ca (mg/dl)</th>
<th>Plasma ionized Ca (mg/dl)</th>
<th>Serum phosphate (mg/dl)</th>
<th>PTH(1-84) (pg/ml)</th>
<th>PTH-M (pg/ml)</th>
<th>Platelet [Ca2+]i (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>9.65±0.04</td>
<td>4.95±0.02</td>
<td>3.37±0.07</td>
<td>37.2±1.8</td>
<td>207±9</td>
<td>113.7±1.9</td>
</tr>
<tr>
<td>F</td>
<td>9.59±0.04</td>
<td>4.94±0.02</td>
<td>3.70±0.06f</td>
<td>35.2±1.9</td>
<td>202±10</td>
<td>105.9±1.7f</td>
</tr>
</tbody>
</table>

M, male; F, female; PTH(1-84), intact parathyroid hormone; PTH-M, mid-molecule fragment of parathyroid hormone; [Ca2+]i, cytosolic calcium concentration.

*p<0.01 between groups.

f<p<0.001 between groups.
platelet \([Ca^{2+}]\), reached a peak between 30 and 60 seconds after the addition of thrombin to the un-stirred platelet suspensions. The magnitude of the peak response to thrombin at either dose did not correlate with age or blood pressure.

Because the platelet \([Ca^{2+}]\), difference between the male and female subjects was apparently not due to gender itself, we combined the male and female groups for subsequent data analysis. Using single variable linear regression analysis, we observed significant linear correlations between platelet \([Ca^{2+}]\) and both diastolic and mean arterial pressure as shown in Figure 1. There was no significant correlation between platelet \([Ca^{2+}]\) and systolic blood pressure. We also found significant correlations between PTH(1–84) and both systolic blood pressure and mean arterial pressure (Figure 2) but not between PTH(1–84) and diastolic blood pressure. Both PTH(1–84) and systolic blood pressure were correlated with age as shown in Figure 3. Similar correlations were present in the male and female groups when analyzed alone. There was no correlation evident between age and platelet \([Ca^{2+}]\) or between PTH(1–84) and platelet \([Ca^{2+}]\) (Figure 4) by this analysis.

Because age, body mass index, and sex can be considered as variables that could influence blood pressure, we performed a multivariate regression analysis including these three factors as well as platelet \([Ca^{2+}]\), and PTH(1–84) as independent variables with mean arterial pressure as the dependent variable. These regression results, shown in Table 4, indicated that both platelet \([Ca^{2+}]\), and PTH(1–84) maintained significant partial correlations with blood pressure even with these other factors included, and thus controlled for, in the regression.

**TABLE 3. Multivariate Analysis: Partial Correlations of Gender-Related Differences With Platelet \([Ca^{2+}]\), as the Dependent Variable**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Partial correlation coefficient with platelet ([Ca^{2+}])</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure</td>
<td>0.256</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum phosphate</td>
<td>0.148</td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>0.206</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Body mass index</td>
<td>-0.134</td>
<td>NS</td>
</tr>
<tr>
<td>Sex</td>
<td>0.121</td>
<td>NS</td>
</tr>
</tbody>
</table>

\([Ca^{2+}]\), cytosolic calcium concentration; NS, not a significant correlation. Multiple regression correlation coefficient=0.47, \(p<0.01\).
The results of this investigation demonstrate that serum PTH is associated with blood pressure in normotensive subjects. We found that PTH(1–84) was strongly correlated with systolic blood pressure and that this relation was not eliminated when age was controlled for in the regression. A similar relation between PTH and blood pressure has been demonstrated by Zacariah et al. in hypertensive subjects but only when serum calcium was used in a ratio with PTH. The greater sample size in our study allowed this relation between PTH and blood pressure to be apparent without including serum calcium as a factor. To our knowledge, no other report has been made regarding an association between PTH and blood pressure within the blood pressure range of the normotensive population. The significance of this observation is uncertain but may reflect physiological changes not unlike those that occur in essential hypertension.

Unlike the situation with normotensive subjects, the role of PTH in blood pressure regulation in essential hypertension has been repeatedly studied. PTH has been shown to be elevated in essential hypertension. Resnick et al. reported that PTH was elevated in hypertensive subjects, particularly in those hypertensive subjects with "low renin" hyper-

tension. Grobbee et al. reported elevated intact PTH in young hypertensive subjects when compared with young normotensive subjects. McCarron et al. proposed the existence of a renal calcium leak in essential hypertension that would thereby result in compensatory elevation of PTH in these cases. In earlier studies from our laboratory, we observed that elevated PTH in essential hypertension was associated with decreased plasma ionized calcium. However, in the present study of normotensive subjects we observed no decrease of plasma ionized calcium concomitant with the higher levels of PTH. This suggests that higher levels of PTH(1–84) observed in the normotensive group were the result of some mechanism other than compensation to decreased plasma ionized calcium.

Despite the documented occurrence of elevated PTH in essential hypertension, the role of PTH, if any, in regulating blood pressure is not known, and available information is somewhat contradictory. Parathyroid function has been shown to be necessary for the development of hypertension in hypertensive rats and parathyroid glands from hypertensive rats demonstrate hypertrophy. Yet Pang et al. were unable to detect elevations of any serum PTH fragments in spontaneously hypertensive rats during the development of hypertension. PTH has been shown to increase calcium entry into cardiac cells and osteoblastlike cells, an action that, if it occurred in vascular smooth muscle cells, could possibly lead to increased vascular constriction and elevated blood pressure. However, PTH(1–84) in concentrations ranging from 82 to 287 ng/ml has been shown to antagonize calcium entry into vascular tissue. Furthermore, available evidence suggests that PTH has a dilator effect on vascular tissue and lowers blood pressure when injected at doses of 160 ng to 16 μg/kg body weight into a variety of animals. Therefore, it appears most likely that PTH affects blood pressure by a mechanism other than its direct effects on vascular tissue. Because measurement of PTH(1–84) can be regarded as an index of parathyroid gland secretion, the possibility exists that some other secretory product of the parathyroid gland may be a factor more important to blood pressure regulation. Such a substance has recently been identified in the blood of subjects with essential hypertension by Lewanzuk et al.

As shown in the present investigation, PTH(1–84) increases significantly with age as does systolic blood pressure. Age-related increases of PTH have been described by Marcus et al. As explained by these authors, their use of a mid-region-specific antisera still allowed the possible interpretation that the age-related increase in PTH was due to reduced removal of carboxy fragments of the PTH molecule by the kidney. Their data also demonstrated that renal function decreased concomitantly with the increased PTH. In the present investigation, the use of an intact-molecule PTH assay suggests that the increase of PTH with age is not due to decreased renal function but actually is due to increased parathyroid
gland secretion. Further, the lack of correlation between age and mid-molecule PTH in the present study suggests that there was no age-related change in renal clearance of PTH fragments in these subjects. Chapuy et al. \(^{26}\) suggested that age-related decreases in intestinal absorption of calcium results in increased secretion of PTH and that the decreased absorption is due to reduced synthesis of 1,25-dihydroxyvitamin D in older people as reported by Gallagher et al. \(^{27}\) We could not confirm this hypothesis with our results since we observed no decrease of serum calcium with age.

The results of the present study show that platelet [Ca\(^{2+}\)], is correlated with blood pressure within the normal blood pressure range. Erne et al. \(^{1}\) also found that platelet [Ca\(^{2+}\)], correlated significantly with blood pressure within this blood pressure range. The platelet has some biochemical features in common with vascular smooth muscle cells, such as calcium-linked contractile mechanisms and adenylate-cyclase-dependent \(\alpha\)-adrenergic receptors. \(^{28}\) Because of these common features, platelets are used as a model for the study of some aspects of vascular smooth muscle cell physiology. Vascular smooth muscle contraction is a calcium-regulated process, \(^{29}\) therefore an increase in free cytosolic calcium in these tissues is assumed to stimulate vasoconstruction, which increases vascular resistance in that particular blood vessel. Many factors regulate the concentration of [Ca\(^{2+}\)], in platelets, \(^{30}\) and one or more of these factors could be altered in both platelets and vascular smooth muscle. Because the relation between platelet [Ca\(^{2+}\)], and blood pressure exists within the range of "normal" blood pressures, this suggests further that the alteration is not limited to the entity of essential hypertension but is continuously modified throughout the range of blood pressures in the human population.

Although both PTH(1–84) and platelet [Ca\(^{2+}\)], were correlated with blood pressure in the present study, there was no demonstrable interrelation between these two factors. Hvarfner et al. \(^{6}\) reported an inverse relation (\(r = -0.26, p = 0.05\)) between PTH and platelet intracellular calcium in a combined group of normotensive and hypertensive subjects. Several differences between their methods and the methods used in the present investigation make comparison difficult. In our previous studies in men with essential hypertension, we were able to demonstrate a significant correlation between PTH(1–84) and platelet [Ca\(^{2+}\)]. \(^{17}\) This result, combined with our inability to demonstrate a correlation in the normotensive population, suggests that PTH, or a substance associated with PTH, may have little influence on platelet [Ca\(^{2+}\)], in normal subjects but may be more of an influence on platelet [Ca\(^{2+}\)], in subjects with essential hypertension.

From our results, we conclude that both PTH and platelet [Ca\(^{2+}\)], are associated with blood pressure in normotensive subjects but through independent mechanisms. The results obtained imply that an intrinsic mechanism alters platelet [Ca\(^{2+}\)], in proportion to blood pressure not only in hypertensive subjects as previously reported but in normotensive subjects as well. We also conclude that PTH, or an associated product of parathyroid gland activity, may be a participant in blood pressure regulation in normotensive men and women but has no influence on platelet [Ca\(^{2+}\)], in this population.

References


**KEY WORDS** • calcium • parathyroid hormone • blood pressure
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