Abnormal platelet Ca^{2+} handling accompanied by increased cytosolic free Mg^{2+} in essential hypertension

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Hiraga, Hiroyuki, Tetsuya Oshima, Mitsuisa Yoshimura, Hideo Matsuura, and Goro Kajiyama. Abnormal platelet Ca^{2+} handling accompanied by increased cytosolic free Mg^{2+} in essential hypertension. *Am. J. Physiol.* 275 (*Regulatory Integrative Comp. Physiol.* 44): R574–R579, 1998.—To test the hypothesis that abnormal platelet Ca^{2+} handling in essential hypertension results from cellular Mg^{2+} deficiency, cytosolic free Mg^{2+} concentration ($[Mg^{2+}]_i$) and Ca^{2+} metabolism were studied in mag-fura 2 and fura 2-loaded platelets from 30 essential hypertensive patients and 30 sex- and age-matched normotensive controls. Basal cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) and intracellular Ca^{2+} discharge capacity were higher in hypertensives than in normotensives (22 ± 5 vs. 18 ± 5 nM, $P < 0.05$; 743 ± 250 vs. 624 ± 144 nM, $P < 0.05$, respectively). The thrombin (0.03–1.0 U/ml)-evoked $[Ca^{2+}]_i$ response was also enhanced in platelets from hypertensives in both the absence and presence of extracellular Ca^{2+}. However, basal $[Mg^{2+}]_i$ was higher in hypertensives than in normotensives (437 ± 110 vs. 353 ± 85 μM, $P < 0.05$), whereas serum Mg^{2+} was similar in the two groups. These results oppose the Mg^{2+} deficiency hypothesis in platelets in essential hypertension.

platelets; mag-fura 2; fura 2

IN THE LAST SEVERAL DECADES, abnormal Ca^{2+} handling in many cell types from human subjects and animal models of primary hypertension has been reported and proposed as a factor in the pathogenesis of hypertension. Platelets are often used in the study of cellular cation metabolism in hypertension, because they are readily available for study and are thought to share a number of features with vascular smooth muscle cells (20). Most investigators have reported that basal levels of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) are higher in human subjects with essential hypertension than in normotensive subjects (5, 8, 11, 16, 30) and that there is a positive correlation between blood pressure and platelet $[Ca^{2+}]_i$ (5, 11). However, the reported values for hypertensives and normotensives cited in these studies vary widely, probably because of methodological differences. The mechanisms that contribute to evoked $[Ca^{2+}]_i$ under stimulated conditions differ from those that regulate basal $[Ca^{2+}]_i$, and it is unclear whether the small difference between cells from hypertensives and those from normotensives in basal $[Ca^{2+}]_i$ reflects a difference in the activated state associated with cell function. Therefore measurements of both basal $[Ca^{2+}]_i$ and $[Ca^{2+}]_i$ responses to agonists are important for any analysis of abnormalities in cellular Ca^{2+} handling.

Mg^{2+} has recently been reported to play an important role in the pathogenesis of essential hypertension. Altura et al. (1) reported that a deficiency in dietary Mg^{2+} can cause hypertension. Joffers et al. (15) showed an inverse relationship between dietary intake of Mg^{2+} and blood pressure. Because cellular Mg^{2+} is an essential cofactor in many cell functions, it is possible that abnormal Mg^{2+} handling at the cellular level may cause elevated blood pressure in hypertensive patients. Mg^{2+} deficiency has been reported to occur at both the serum and intraerythrocyte levels in hypertensives (28, 29), but serum or intraerythrocyte total magnesium may not accurately represent cellular magnesium activity (4). Because serum magnesium represents <1% of total body magnesium and protein-bound and anion complex magnesium is unavailable for biochemical processes, it is important to evaluate the levels of cytosolic free magnesium concentration ($[Mg^{2+}]_i$) exhibiting biological activity.

To test the hypothesis that a deficiency of $[Mg^{2+}]_i$ is involved in abnormal Ca^{2+} handling as the pathogenesis of essential hypertension, we compared platelet $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ between essential hypertensive patients and normotensive controls.

MATERIALS AND METHOD

Subjects. We studied 30 patients with essential hypertension (15 men, 15 women, mean age 51 ± 11 yr) and 30 sex- and age-matched normotensive controls (15 men, 15 women, mean age 50 ± 13 yr). Normotensive controls were recruited from healthy subjects who underwent annual physical examinations. Hypertension was defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg on each of three consecutive clinical visits. We measured blood pressure with a mercury sphygmomanometer in sitting subjects at least five times during each clinical visit and used the average value of these measurements. The blood pressure in normotensives was consistently <140/90 mmHg. None of the hypertensives or normotensives had received any medication for at least 4 wk before the study. Subjects with secondary forms of hypertension were excluded by careful clinical examination. Hypertensive patients and normotensive controls were maintained on a regular diet with an intake of 170 mmol/day NaCl to allow stabilization of the systemic Na^+ balance, and they ingested constant amounts of K^+ (2,000 mg/day), Ca^{2+} (500 mg/day), and calories (40 kcal/kg) for 7 days before the study. Venous blood was collected from fasting and resting subjects, slowly and steadily via a 19-gauge needle into a syringe containing 3.8% trisodium citrate (1:9 by vol, total 30 ml), using a two-syringe method (21) to separate platelets for measurement of $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$. Blood samples were centrifuged at room temperature for 5 min at 800 *g*, and $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ were measured by use of the resultant platelet-rich plasma. To minimize any time-dependent effects on platelet responsiveness and leakage of dyes, measurements of platelet $[Mg^{2+}]_i$ and $[Ca^{2+}]_i$ were performed separately by two independent investigators (*HH* measured $[Ca^{2+}]_i$; *MY* measured $[Mg^{2+}]_i$) in the same blood

samples within 2 h after blood collection. Serum concentrations in electrolytes and lipids and mean platelet volume were measured by automated methods, and plasma renin activity and plasma aldosterone concentration were assayed by RIA in another blood sample.

Measurement of platelet $[Ca^{2+}]_i$. Platelet $[Ca^{2+}]_i$ was measured as described previously (13, 21, 22). In short, platelet-rich plasma prepared as described above was layered onto a Sepharose 2B-CL column (Pharmacia LKB Biotechnology, Uppsala, Sweden) that had been equilibrated with medium containing (in mM) 145 NaCl, 10 HEPES, 5 KCl, 5 glucose, and 1 $MgSO_4$ (pH 7.4) at room temperature. Washed platelets were eluted from this column with buffer and incubated at 37°C with 1 μM fura 2-AM (Molecular Probes, Eugene, OR) and 0.02% Pluronic F-127 (Molecular Probes) for 30 min at a platelet concentration of 10^8 cells/ml. After platelets had again been washed by gel filtration to remove any extracellular fura 2-AM, the platelet count was adjusted to 10^7 cells/ml, and $CaCl_2$ was added to the cell suspension at a final concentration of 1 mM. Incubation at 37°C for 7 min was performed to complete the hydrolysis of fura 2-AM, and platelet suspensions were then placed in cuvettes with stirrers at 37°C. Fluorescence was measured with a dual-excitation wavelength fluorometer (RF-5000, Shimadzu, Kyoto, Japan), using excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. After fluorescence in the basal state had been recorded, the $[Ca^{2+}]_i$ responses to thrombin (0.03, 0.1, 0.3, and 1.0 U/ml; Sigma Chemical, St. Louis, MO) were measured in the presence of 1 mM extracellular Ca^{2+} and in Ca^{2+}-free buffer prepared by the addition of 10 mM EGTA (Dojindo Laboratories, Kumamoto, Japan; Fig. 1). The discharge capacity of Ca^{2+} from intracellular storage sites was estimated by the $[Ca^{2+}]_i$ response to 5 μM ionomycin (Sigma) in the Ca^{2+}-free medium (13, 22). $[Ca^{2+}]_i$ was calculated using the following equation from Grynkiewicz et al. (7)

$$[Ca^{2+}]_i = K_d \cdot (R - R_{min}/R_{max} - R) \cdot Sf/Sb$$

where K_d represents the dissociation constant of fura 2 for Ca^{2+} (224 nM) and R_{max} and R_{min} are the ratios of fluorescence at 340 and 380 nm under Ca^{2+}-saturated and Ca^{2+}-free conditions, respectively. Sf and Sb are the fluorescence intensities at 380 nm for fura 2 with concentrations of zero and excess Ca^{2+}, respectively. R_{max} was determined with 50 μM digitonin in the presence of 1 mM Ca^{2+}. R_{min} was then determined by the addition of 10 mM EGTA after adjustment of pH to 8.3 with 30 mM Tris. Corrections were applied for extracellular fura 2 leaked from platelets because of EGTA usage and for autofluorescence by subtracting the fluorescence values of the unloaded platelets and test reagents (21, 22). Rapid initial drop in the fluorescence signal at 340 nm after EGTA addition was considered to reflect the contribution of the extracellular dye as extracellular Ca^{2+} was chelated. The ratio of the fluorescence change after EGTA at pH 7.4 in intact cell suspension to the change in the total dye in the tube (after cell lysis with digitonin) was regarded as the percentage of extracellular dye in the total dye (21). The calculated fluorescent signal of external dye was then subtracted from the original signal in the cell suspension. Cytosolic fura 2 concentration was estimated by comparing the fluorescence signal at 340 nm in the presence of 1 mM Ca^{2+} after cell lysis with that of a known concentration of fura 2.

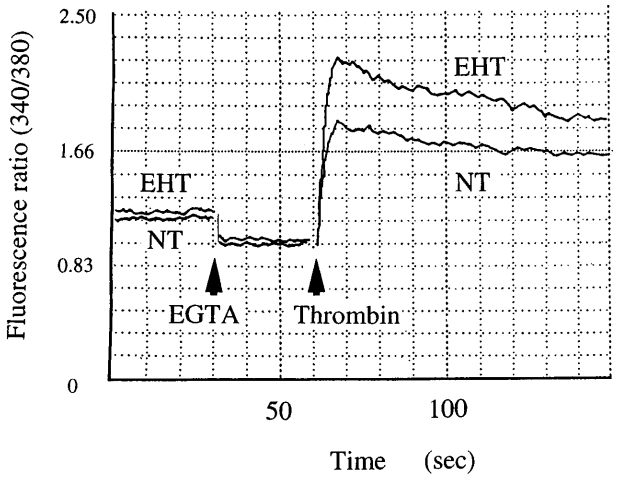

Fig. 1. Typical responses to thrombin (0.3 U/ml) in absence of extracellular Ca^{2+} in fura 2-loaded platelets from patients with essential hypertension (EHT) and normotensive controls (NT).

In the preliminary study, to determine whether citrate alone is sufficient to prevent cell activation during the preparation of platelets, we studied the effects of other anticoagulant agents on Ca^{2+} handling by gel-filtered platelets of essential hypertensives and normotensives. Platelet-rich plasma was divided into two batches. Apyrase (20 μg/ml), hirudin (0.05 U/ml), and PGI_2 (1 μM) were added to one batch, and no agent was added to the other batch. These conditions were maintained during fura 2 loading. After the cells were gel filtered, $[Ca^{2+}]_i$ was determined in the two batches. There was no effect of the anticoagulant cocktail on basal $[Ca^{2+}]_i$ or thrombin (0.1 U/ml)-stimulated $[Ca^{2+}]_i$ in seven subjects with essential hypertension (percentage of control: 101 ± 3 and 98 ± 5%, respectively) and eight normotensive controls (100 ± 4 and 99 ± 4%, respectively). We thus concluded that the use of citrate alone may be sufficient to inhibit $[Ca^{2+}]_i$ elevation induced by cell activation, when gel filtration is used to separate platelets.

Measurement of platelet $[Mg^{2+}]_i$. The washed platelets were incubated at 37°C with 2 μM mag-fura 2-AM (Molecular Probes) and 0.02% Pluronic F-127 for 30 min at a platelet concentration of ~5 × 10^7 cells/ml. Platelets were then washed again to remove extracellular mag-fura 2-AM, and $CaCl_2$ was added at a final concentration of 1 mM after resuspension of platelets in HEPES buffer at a platelet concentration of 10^7 cells/ml. Platelet suspension (3 ml) was incubated at 37°C for 7 min to complete the hydrolysis of mag-fura 2-AM, and samples were then placed in cuvettes and stirred magnetically at 37°C. Fluorescence was measured with a dual-excitation wavelength fluorometer (DM3000CM, SPEX, Edison, NJ), as described above. $[Mg^{2+}]_i$ was calculated using the equation from Raju et al. (24)

$$[Mg^{2+}]_i = K_d \cdot (R - R_{min})/R_{max} - R) \cdot Sf/Sb$$

where K_d is 1.5 mM. As in the preliminary study (31), we confirmed that R_{max} after saturation of the mag-fura 2 with 2 mM Ca^{2+} was similar to that with 50 mM Mg^{2+} (35.6 ± 2.2 vs. 35.4 ± 1.8), and we obtained R_{max} by saturating the mag-fura 2 with Ca^{2+}. R_{max} was therefore determined with 50 μM digitonin in the presence of 2 mM Ca^{2+} and 1 mM Mg^{2+}. R_{min} was then determined by the addition of 50 μM digitonin and 6 mM EDTA (Dojindo) after adjustment of the pH to 8.3 with 7 mM Tris in the Ca^{2+}-free medium. Sf and Sb are the fluorescence intensities at 380 nm for mag-fura 2 with concentrations of zero and excess Mg^{2+}, respectively. Corrections were applied for extracellular mag-fura 2 leakage from platelets by the use of 3 mM EDTA, which chelates extracellular Mg^{2+}, and for autofluorescence by the method described above. Cytosolic mag-fura 2 concentration was estimated by compar-

ing the fluorescence signal at 340 nm in the presence of 2 mM Ca^{2+} and 1 mM Mg^{2+} after cell lysis with that of a known concentration of mag-fura 2.

Statistics. Data are presented as means ± SD. Statistical comparisons were made using the Mann-Whitney *U* test. Curves were compared by using ANOVA for repeated measures. Statistical significance was defined as $P < 0.05$.

RESULTS

Basic information on the study subjects is shown in Table 1. There were no significant differences in serum total cholesterol, triglyceride, high-density lipoprotein-cholesterol, plasma renin activity, plasma aldosterone concentration, or mean platelet volume between the hypertensive and normotensive control groups. No differences were detected in the concentrations of platelet intracellular mag-fura 2 or fura 2 (hypertensives vs. normotensives: 402 ± 42 vs. 390 ± 45, 493 ± 81 vs. 512 ± 61 μM, respectively) or in extracellular leakage of mag-fura 2 or fura 2 (31.0 ± 4.4 vs. 30.0 ± 3.7, 8.6 ± 1.7 vs. 9.3 ± 1.7%), R_{max} of mag-fura 2 or fura 2 (31.9 ± 3.8 vs. 32.4 ± 3.5, 39.3 ± 10.0 vs. 35.7 ± 9.7), or R_{min} of mag-fura 2 or fura 2 (0.72 ± 0.02 vs. 0.73 ± 0.02, 0.83 ± 0.05 vs. 0.83 ± 0.05), indicating that platelets were loaded with the dyes to a similar extent in the two groups.

Platelet basal $[Ca^{2+}]_i$ was significantly higher in the hypertensive group than in the normotensive group (22.3 ± 5.3 vs. 17.8 ± 5.3 nM; Fig. 2), although there was a considerable overlap in distribution between groups. Thrombin (0.03, 0.1, 0.3, and 1.0 U/ml)-evoked $[Ca^{2+}]_i$ responses were significantly enhanced in the hypertensive group in the presence or absence of extracellular Ca^{2+} (Fig. 3, *A* and *B*). Differences in $[Ca^{2+}]_i$ increase between the presence and absence of extracellular Ca^{2+}, representing thrombin-evoked external Ca^{2+} influx, were also enhanced in the hypertensive group (Fig. 3*C*); i.e., external Ca^{2+} influx and discharge of Ca^{2+} from intracellular stores were both enhanced in thrombin-stimulated platelets from the hypertensive group. The discharge capacity of Ca^{2+} from intracellular storage sites, which was assessed by the $[Ca^{2+}]_i$ response to the addition of 5 μM ionomycin in a Ca^{2+}-free medium, was greater in the hypertensive than the normotensive group (743.0 ± 250.4 vs. 624.2 ± 144.2 nM). However, basal $[Mg^{2+}]_i$ was significantly higher in hypertensives than in normotensives (436.6 ± 109.9 vs. 353.0 ± 85.3 μM, Fig. 4), whereas serum total Mg^{2+} was similar in the two groups (Table 1).

Table 1. *Characteristics of patients with essential hypertension and normotensive controls*

	EHT	NT
Age, yr	51.2 ± 11.4	49.9 ± 12.5
Systolic blood pressure, mmHg	158.7 ± 14.4*	114.3 ± 14.9
Diastolic blood pressure, mmHg	99.6 ± 10.5*	71.1 ± 11.0
Mean blood pressure, mmHg	119.2 ± 11.2*	85.5 ± 11.7
Body mass index, kg/m^2	25.2 ± 3.5	23.9 ± 2.8
Total cholesterol, mM	5.32 ± 1.22	5.17 ± 0.96
Triglyceride, mM	1.21 ± 0.50	1.23 ± 0.50
HDL cholesterol, mM	1.26 ± 0.37	1.31 ± 0.30
Mean platelet volume, μm^3	8.6 ± 0.6	8.9 ± 0.7
PRA, $ng \cdot l^{-1} \cdot s$	0.31 ± 0.33	0.36 ± 0.31
PAC, pg/ml	79.3 ± 7.5	75.2 ± 6.4
Serum Na^+, mM	142 ± 2.2	142 ± 1.9
Serum K^+, mM	4.0 ± 0.3	4.0 ± 0.3
Serum total Ca^{2+}, mM	2.23 ± 0.11	2.30 ± 0.09
Serum total Mg^{2+}, mM	0.87 ± 0.08	0.89 ± 0.06
Urinary Na^+, mmol/day	152.2 ± 13.4	157.5 ± 12.3

Values are means ± SD. EHT, patients with essential hypertension; NT, normotensive controls; HDL, high-density lipoprotein; PRA, plasma renin activity; PAC, plasma aldosterone concentration. *$P <$ 0.05 vs. NT.

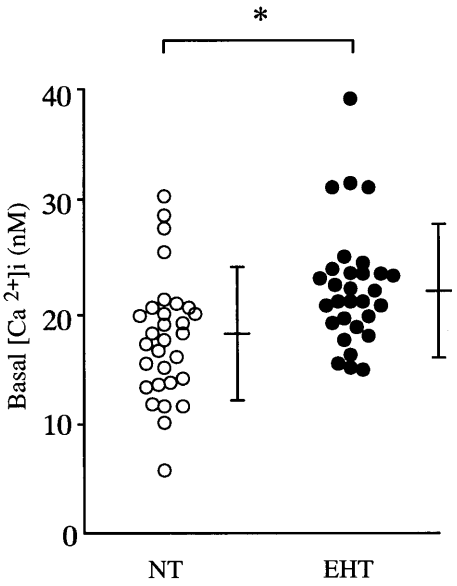

Fig. 2. Resting cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in platelets from EHT and NT subjects. Resting $[Ca^{2+}]_i$ was significantly higher in EHT. Results are means ± SD. *$P < 0.05$.

DISCUSSION

We (19) and other investigators (5, 9) have reported an increase in the intracellular concentration of Na^+ and in basal $[Ca^{2+}]_i$ in blood cells, such as platelets and lymphocytes, of subjects with essential hypertension. In the present study, the basal $[Ca^{2+}]_i$ in platelets was elevated in hypertensive subjects, confirming most previous results (5, 8, 11, 16, 30). However, there was a considerable overlap in distribution between hypertensives and normotensives. This overlap may be due to the heterogeneity of essential hypertension, which should not be regarded as a single disease entity. Essential hypertensive patients have heterogeneity in several factors, such as renin status (19), blood pressure level (5), age (2), and salt intake (20), each of which may influence intracellular cation characteristics and are difficult to control precisely.

The mechanisms that contribute to evoked $[Ca^{2+}]_i$ under stimulated conditions are different from those regulating basal $[Ca^{2+}]_i$. Most previous reports have been limited to the measurement of basal $[Ca^{2+}]_i$. Even in a few previous studies with stimulated platelets, the status of the $[Ca^{2+}]_i$ response was controversial: an enhanced $[Ca^{2+}]_i$ response to thrombin was reported by Lechi et al. (16) and to ANG II by Touyz and Schiffrin (30) in platelets from hypertensive patients, whereas

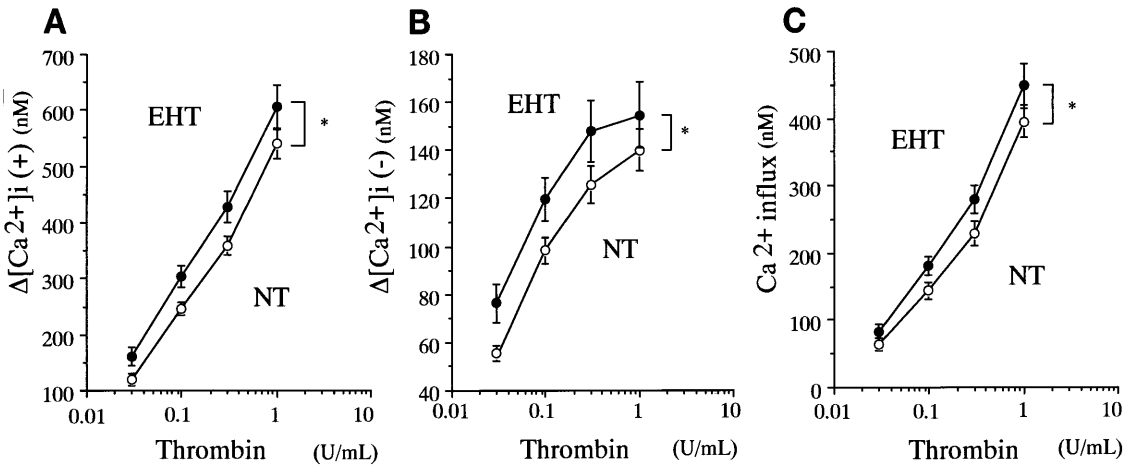

Fig. 3. Rise in platelet $[Ca^{2+}]_i$ ($\Delta[Ca^{2+}]_i$) in response to thrombin in presence (+; *A*) or absence (−; *B*) of extracellular Ca^{2+} and difference between $\Delta[Ca^{2+}]_i(+)$ and $\Delta[Ca^{2+}]_i(-)$ (*C*), representing external Ca^{2+} influx, in EHT and NT subjects. Rise in $[Ca^{2+}]_i$ was significantly greater in EHT than in NT using ANOVA for repeated measures. $*P < 0.05$.

Haller et al. (8) showed that the change in $[Ca^{2+}]_i$ in response to thrombin was similar in platelets from hypertensives and those from normotensives. In the present study, the evoked $[Ca^{2+}]_i$ responses to thrombin were enhanced in hypertensives, both in the absence and presence of extracellular Ca^{2+}. Platelets from hypertensive subjects exhibited not only an increase in basal $[Ca^{2+}]_i$ but also an enhanced Ca^{2+} discharge from intracellular stores, an increase in Ca^{2+} influx under thrombin-stimulated conditions, and an increase in intracellular Ca^{2+} discharge capacity.

We have repeatedly emphasized that methodological issues are important in the assessment of $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ in fluorescent dye-loaded platelets (7, 13, 14, 18, 21). Accordingly, the present study was carried out so as to minimize platelet activation during blood collection by using a 19-gauge needle and a two-syringe method.

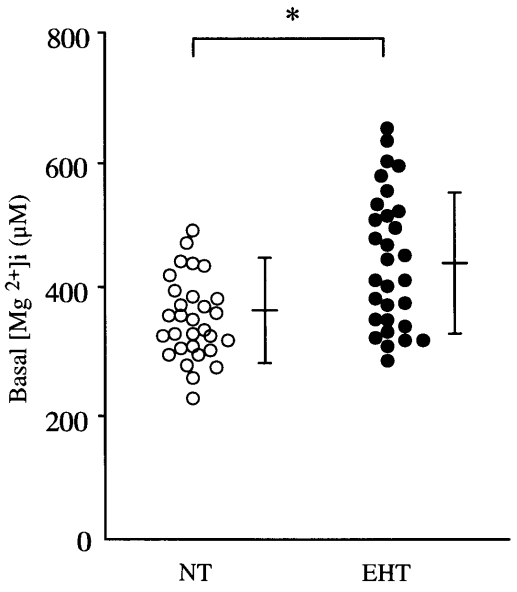

Fig. 4. Resting cytosolic free Mg^{2+} concentration ($[Mg^{2+}]_i$) in platelets from EHT and NT subjects. Resting $[Mg^{2+}]_i$ was significantly higher in EHT. Results are means ± SD. $*P < 0.05$.

Attention must be paid to the possible activation of platelets and the coagulation system under the conditions of blood collection. Second, corrections were applied for extracellular leakage of dye, which leads to overestimations of $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ in the presence of extracellular Ca^{2+} and Mg^{2+} when a cell suspension system is used. Corrections for extracellular fura 2 should be made by using EGTA in agonist-stimulated conditions. Because Mn^{2+} enters platelets via the Ca^{2+} channel, $MnCl_2$ may be unsuitable for correction for dye leakage in estimating agonist responses. Third, in any comparison of Mg^{2+} or Ca^{2+} handling between hypertensives and normotensives, all aspects of intracellular dye metabolism, such as cytosolic fluorescent dye concentration and the degree of hydrolysis of fluorescence, should be similar in the two groups. The extent of dye ester hydrolysis affects fluorescence dynamics. However, many investigators have failed to clearly define the method of blood collection, correction for extracellular dye leakage, and the comparison of fluorescent dye metabolism. In the many reports concerning basal $[Ca^{2+}]_i$ in human platelets, the findings have been variable, ranging from 20 to 200 nM (5, 8, 11, 13, 16, 30). Our basal $[Ca^{2+}]_i$ level probably reflects improved methods with minimal activation of platelets and correction for dye leakage from platelets. Recent data from careful investigations of methods have shown basal $[Ca^{2+}]_i$ in human platelets as low as the level determined in our study (6, 11, 17, 27).

We have previously reported differences in abnormal Ca^{2+} handling by fura 2-loaded platelets from several types of hypertensive rats (12, 21, 23). Basal $[Ca^{2+}]_i$ is increased in spontaneously hypertensive rats (21, 23) but decreased in Dahl salt-sensitive and DOCA-salt hypertensive rats (12) and similar in stroke-prone spontaneously hypertensive rats (17) compared with those from normotensive control rats. The evoked $[Ca^{2+}]_i$ responses to thrombin in the absence of extracellular Ca^{2+} were enhanced in these three strains of hypertensive rats, whereas in the presence of extracellular Ca^{2+},

the $[Ca^{2+}]_i$ increase was enhanced in spontaneously hypertensive rats (21, 23), decreased in Dahl salt-sensitive rats (12), and similar in DOCA-salt rats compared with values in normotensive control rats. Thus differences in platelet intracellular Ca^{2+} handling exist between strains of hypertensive rats, and platelets from models of hypertension do not always show an elevation of $[Ca^{2+}]_i$. However, platelets from essential hypertensive subjects may be comparable to those from spontaneously hypertensive rats with respect to basal $[Ca^{2+}]_i$ and $[Ca^{2+}]_i$ responses to thrombin.

Mg^{2+} is an important constituent of cells and an essential cofactor in many cell functions, including the regulation of receptor systems, transmembrane flux of cation, and activation of cellular enzyme, since certain enzyme activity, including Ca^{2+}-ATPase and Na^+-K^+-ATPase, depends entirely on Mg^{2+} (3) and Na^+ transport and cellular Ca^{2+} handling, which may be affected by $[Mg^{2+}]_i$ (25). Rat studies have shown that a deficiency of dietary Mg^{2+} is associated with the development of hypertension (1). Epidemiological studies suggest an inverse relationship between the dietary intake of Mg^{2+} and blood pressure (15). One could therefore hypothesize that an Mg^{2+} deficiency is associated with a decrease in Ca^{2+}-ATPase and Na^+-K^+-ATPase activity, an elevated cytosolic Ca^{2+} level, and hence an increase in vascular resistance in hypertensive patients. To test this hypothesis, $[Mg^{2+}]_i$ and Ca^{2+} metabolism were studied in platelets from hypertensive patients and normotensive controls matched for age and gender. Unexpectedly, $[Mg^{2+}]_i$ was elevated significantly in platelets from patients with essential hypertension; furthermore, serum total magnesium was similar in the hypertensive and normotensive groups. Thus we could not support the hypothesis that hypertension results from a cellular deficiency of Mg^{2+}.

Resnick et al. (26, 27) previously described a decrease in intraerythrocyte concentration of free Mg^{2+} in essential hypertension based on studies employing nuclear magnetic resonance spectroscopy. Results contrary to ours may be due to differences in the cells used for $[Mg^{2+}]_i$ measurement. Furthermore, a few previous reports (11, 30) using mag-fura 2 have shown the decrease in $[Mg^{2+}]_i$ in platelets from subjects with essential hypertension. This discrepancy may result from differences in the methods used, such as isolation of platelets or correction for extracellular dye. In other reports (28, 29), intracellular Mg^{2+} measurement by atomic absorption spectroscopy represents intracellular total magnesium concentration, and this may not accurately reflect cellular Mg^{2+} activity, since protein-bound and anion complex magnesium are unavailable for biochemical processes, whereas free Mg^{2+} does have biological activity. Similarly, we could not find a significant difference between hypertensives and normotensives in serum total magnesium, which may not represent intracellular Mg^{2+} metabolism.

In summary, abnormal Ca^{2+} handling, including higher basal $[Ca^{2+}]_i$, enhanced thrombin-evoked $[Ca^{2+}]_i$ responses in the presence or absence of extracellular Ca^{2+}, and a greater Ca^{2+} discharge capacity was observed in platelets from hypertensive patients. Platelet $[Mg^{2+}]_i$ was higher in hypertensives than in normotensives. Our data appear to negate the hypothesis that abnormal Ca^{2+} handling in platelets from hypertensive subjects results from a cellular Mg^{2+} deficiency.

Perspectives

Mg^{2+} deficiency has been recognized to be associated with the pathogenesis of several cardiovascular diseases, such as arrhythmia and coronary heart disease. Hypertension is an established risk factor for these cardiovascular diseases. In the present study, we have clarified the increased $[Mg^{2+}]_i$ in essential hypertension. Thus further studies are necessary to clarify the relation between systemic Mg^{2+} balance and cellular Mg^{2+} metabolism. The Mg^{2+} balance study might solve this problem. Furthermore, the reason for increased $[Mg^{2+}]_i$ in essential hypertension is not clear, since the precise mechanisms that regulate $[Mg^{2+}]_i$ are not fully understood. Intracellular ATP concentration and Mg^{2+}/Na^+ exchanger are reported to regulate $[Mg^{2+}]_i$. These factors should be studied in the cardiovascular diseases and their risk factors.

We thank Y. Omura for secretarial assistance.

This work was supported by Grants-in-Aid for Scientific Research 07407065 and 08457639 from the Ministry of Education, Science and Culture of Japan, and by grants from the Kurozumi Medical Foundation of Japan and the Clinical Pathology Research Foundation of Japan.

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Received 27 November 1996; accepted in final form 1 April 1998.

REFERENCES

1. **Altura, B. M., B. T. Altura, A. Gebrewold, H. Ising, and T. Gunther.** Magnesium deficiency and hypertension: correlation between magnesium-deficient diets and microcirculatory change in situ. *Science* 223: 1315–1317, 1984.
2. **Bruschi, G., M. E. Bruschi, M. Caroppo, G. Orlandini, M. Spaggiari, and A. Cavatorta.** Cytoplasmic free $[Ca^{2+}]$ is increased in the platelets of spontaneously hypertensive rats and essential hypertensive patients. *Clin. Sci. Lond.* 68: 179–184, 1985.
3. **Elin, R. J.** Assessment of magnesium status. *Clin. Chem.* 33: 1965–1970, 1987.
4. **Elin, R. J.** Magnesium metabolism in health and disease. *Dis. Mon.* 34: 161–219, 1988.
5. **Erne, P., P. Bolli, E. Burgisser, and F. R. Buhler.** Correlation of platelet calcium with blood pressure: effect of antihypertensive therapy. *N. Engl. J. Med.* 310: 1084–1088, 1984.
6. **Gardner, J. P., J. H. Cho, J. H. Skurnick, G. Awad, M. Gutkin, L. H. Byrd, N. Lasker, and A. Aviv.** Blood pressure inversely correlates with thrombin-evoked calcium rise in platelets. *Hypertension* 23: 703–709, 1994.
7. **Grynkiewicz, G., M. Poenie, and R. Y. Tsien.** A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260: 3440–3450, 1985.
8. **Haller, H., C. Lindschau, P. Quass, and A. Distler.** Protein phosphorylation and intracellular free calcium in platelets of patients with essential hypertension. *Am. J. Hypertens.* 5: 117–124, 1992.
9. **Hilton, P. J.** Cellular sodium transport in hypertension. *N. Engl. J. Med.* 314: 222–229, 1986.
10. **Hinssen, R., J. D'Haese, J. V. Small, and A. Sobieszek.** Mode of filament assembly of myosins from muscle and nonmuscle cells. *J. Ultrastruct. Res.* 64: 282–302, 1978.

11. **Hsieh, S. T., K. Saito, H. Sano, and M. Yokoyama.** Decreased platelet free magnesium with increased free calcium in mild essential hypertension. *Hypertens. Res.* 15: 105–110, 1992.
12. **Ishida, T., M. Ishida, H. Matsuura, R. Ozono, G. Kajiyama, and T. Oshima.** Lack of increase in cellular Ca^{2+} in Dahl salt-sensitive and deoxycorticosterone acetate-salt hypertensive rats (Abstract). *Hypertension* 24: 405, 1994.
13. **Ishida, T., H. Matsuura, M. Ishida-Kainouchi, R. Ozono, M. Watanabe, G. Kajiyama, and T. Oshima.** Na^+-Ca^{2+} exchange modulates Ca^{2+} handling of human platelets by altering intracellular Ca^{2+} store size. *J. Hypertens.* 11: 1089–1095, 1993.
14. **Ishida-Kainouchi, M., H. Matsuura, T. Ishida, G. Kajiyama, and T. Oshima.** Platelet calcium handling in spontaneously hypertensive rats and in three strains of normotensive rats. *J. Hypertens.* 11: 509–514, 1993.
15. **Joffers, M. R., D. M. Reed, and K. Yano.** Relationship of magnesium intake and other dietary factors to blood pressure: the Honolulu Heart Study. *Am. J. Clin. Nutr.* 45: 469–475, 1987.
16. **Lechi, A., C. Lechi, G. Bonadonna, D. Sinigaglia, P. Carradini, R. Polignano, E. Arosio, G. Covi, and P. de Togni.** Increased basal and thrombin-induced free calcium in platelets of essential hypertensive patients. *Hypertension* 9: 230–235, 1987.
17. **Ono, N., T. Oshima, M. Ishida, T. Ishida, H. Matsuura, M. Kambe, and G. Kajiyama.** Platelet Ca^{2+} is not increased in stroke-prone spontaneously hypertensive rats. Comparative study with spontaneously hypertensive rats. *Hypertension* 27: 1312–1317, 1996.
18. **Oshima, T., T. Ishida, H. Matsuura, M. Ishida, K. Ishibashi, R. Ozono, M. Watanabe, G. Kajiyama, and M. Kanbe.** Lack of effect of ouabain on calcium homeostasis in rat platelets: comparative study with human platelets. *Am. J. Physiol.* 266 (*Regulatory Integrative Comp. Physiol.* 35): R651–R657, 1994.
19. **Oshima, T., H. Matsuura, K. Kido, K. Matsumoto, H. Fujii, S. Masaoka, M. Okamoto, Y. Tsuchioka, G. Kajiyama, and T. Tsubokura.** Intralymphocytic sodium and free calcium and plasma renin in essential hypertension. *Hypertension* 12: 26–31, 1988.
20. **Oshima, T., H. Matsuura, K. Matsumoto, K. Kido, and G. Kajiyama.** Role of cellular calcium in salt sensitivity of patients with essential hypertension. *Hypertension* 11: 703–707, 1988.
21. **Oshima, T., E. W. Young, R. D. Bukoski, and D. A. McCarron.** Abnormal calcium handling by platelets of spontaneously hypertensive rats. *Hypertension* 15: 606–611, 1990.
22. **Oshima, T., E. W. Young, R. D. Bukoski, and D. A. McCarron.** Rise and fall of agonist-evoked platelet Ca^{2+} in hypertensive rats. *Hypertension* 18: 758–762, 1991.
23. **Oshima, T., E. W. Young, and D. A. McCarron.** Abnormal platelet and lymphocyte calcium handling in prehypertensive rats. *Hypertension* 18: 111–115, 1991.
24. **Raju, B., E. Murphy, L. A. Levy, R. D. Hall, and R. E. London.** A fluorescent indicator for measuring cytosolic free magnesium. *Am. J. Physiol.* 256 (*Cell Physiol.* 25): C540–C548, 1989.
25. **Reinhart, R. A.** Clinical correlates of the molecular and cellular actions of magnesium on the cardiovascular system. *Am. Heart J.* 121: 1513–1521, 1991.
26. **Resnick, L. M., R. K. Gupta, K. K. Bhargava, H. Gruenspan, M. H. Alderman, and J. H. Laragh.** Cellular ions in hypertension, diabetes, and obesity: a nuclear magnetic resonance spectroscopic study. *Hypertension* 17: 951–957, 1991.
27. **Resnick, L. M., R. K. Gupta, B. DiFabio, M. Barbagallo, S. Mann, R. Marion, and J. H. Laragh.** Intracellular ionic consequences of dietary salt loading in essential hypertension. *J. Clin. Invest.* 94: 1269–1276, 1994.
28. **Resnick, L. M., R. K. Gupta, H. Gruenspan, M. H. Alderman, and J. H. Laragh.** Hypertension and peripheral insulin resistance: possible mediating role of intracellular free magnesium. *Am. J. Hypertens.* 3: 373–379, 1990.
29. **Touyz, R. M., F. J. Milne, and S. G. Reinach.** Platelet and erythrocyte Mg^{2+}, Ca^{2+}, Na^+, K^+ and cell membrane adenosine triphosphatase activity in essential hypertension in blacks. *J. Hypertens.* 10: 571–578, 1992.
30. **Touyz, R. M., and E. L. Schiffrin.** The effect of angiotensin II on platelet intracellular free magnesium and calcium ionic concentrations in essential hypertension. *J. Hypertens.* 11: 551–558, 1993.
31. **Yoshimura, M., T. Oshima, H. Matsuura, M. Watanabe, Y. Higashi, N. Ono, H. Hiraga, M. Kambe, and G. Kajiyama.** Effect of the transmembrane gradient of magnesium, and sodium on the regulation of cytosolic free magnesium concentration in human platelets. *Clin Sci. Lond.* 89: 293–298, 1995.