

# Hypertension Does Not Affect Intracellular Calcium Uptake in Human Platelets

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The relationship between the  $\text{Ca}^{2+}$  transport of platelet endoplasmic reticulum and hypertension was analyzed in 17 untreated patients exhibiting various degrees of hypertension. Each patient underwent a 24-h recording of ambulatory blood pressure. Platelets from patients were permeabilized with saponin and the rate of ATP-driven thapsigargin-sensitive  $\text{Ca}^{2+}$  uptake determined using the fluorescent  $\text{Ca}^{2+}$  indicator fluo-3. No relationship between blood pressure (systolic, diastolic, day, night) and the rate of  $\text{Ca}^{2+}$  uptake into the

sarcoplasmic reticulum of platelets was found. A weak but insignificant correlation between  $\text{Ca}^{2+}$  uptake and the heart rate was noted. Therefore, the increase in cytosolic  $\text{Ca}^{2+}$  of platelets in hypertension may not be due to changes of the activity of  $\text{Ca}^{2+}$  uptake into the sarcoplasmic reticulum. *Am J Hypertens* 1996;9:136-142

KEY WORDS: Platelets, calcium transport, hypertension.

Despite extensive research, the relationship of calcium and arterial hypertension remains unclear. Intracellular  $\text{Ca}^{2+}$  is a major determinant of basal myogenic tone of vascular muscle. Therefore, an increase of cytosolic  $\text{Ca}^{2+}$  in arterial smooth muscle leads to an increase in peripheral resistance characteristic for hypertension. The systemic hormonal signals associated with hypertension influence endothelial cell, vascular muscle cells and blood cells. Erne et al<sup>1</sup> have documented an elevation of platelet cytosolic free  $\text{Ca}^{2+}$  in hypertension and a close correlation of the  $\text{Ca}^{2+}$  levels in platelets and blood pressure in untreated hypertensive patients. Furthermore, both platelet  $\text{Ca}^{2+}$  and vasoconstriction were normalized by antihypertensive treatment,<sup>1,2</sup> indicating a close relationship between plate-

let  $\text{Ca}^{2+}$  dynamics and circulating hormonal factors. Cytosolic  $\text{Ca}^{2+}$  overload has been described in a variety of cells in both hypertension<sup>3-5</sup> and pressure-induced cardiac dysfunction.<sup>6</sup>

Whereas the relationship of platelet cytosolic  $\text{Ca}^{2+}$  and vasoconstriction has been clearly established, the molecular basis of elevated  $\text{Ca}^{2+}$  levels remains unclear. The rapid rise in cytosolic  $\text{Ca}^{2+}$  observed upon exposure of platelets to humoral factors from hypertensives<sup>7,8</sup> indicates that systemic hormonal factors influence platelet cytosolic  $\text{Ca}^{2+}$  and that the changes in platelet cytosolic  $\text{Ca}^{2+}$  are effected by a rapid mechanism, ie.  $\text{Ca}^{2+}$  influx across the plasma membrane or intracellular  $\text{Ca}^{2+}$  mobilization. The reversal of  $\text{Ca}^{2+}$  levels by means of  $\text{Ca}^{2+}$  antagonists has led to the hypothesis that  $\text{Ca}^{2+}$  influx is responsible for elevated platelet  $\text{Ca}^{2+}$ .<sup>1,9</sup> However, the enhanced hormone-induced elevation of platelet cytosolic  $\text{Ca}^{2+}$  is not corrected by antihypertensive therapy,<sup>10</sup> demonstrating that the fundamental defect in  $\text{Ca}^{2+}$  dynamics has not been restored. Moreover, voltage-operated  $\text{Ca}^{2+}$  channels do not mediate agonist-evoked  $\text{Ca}^{2+}$  entry in platelets.<sup>11</sup> Besides voltage-operated  $\text{Ca}^{2+}$  channels, a variety of other mechanisms contribute to the  $\text{Ca}^{2+}$  dynamics of platelets<sup>11,12</sup>: agonist-mediated  $\text{Ca}^{2+}$  in-

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flux; ligand-gated  $\text{Ca}^{2+}$  channels;  $\text{Ca}^{2+}$ -ATPase pumps present in both the plasma membrane and membrane of endoplasmic reticulum;<sup>13,14</sup>  $\text{Na}^+/\text{Ca}^{2+}$  exchanger<sup>15</sup>; inositol 1,4,5-trisphosphate-induced  $\text{Ca}^{2+}$  release from intracellular  $\text{Ca}^{2+}$  stores; and a series of cytosolic and endoplasmic reticulum  $\text{Ca}^{2+}$  binding proteins. Resink et al<sup>16</sup> have documented a reduction of the calmodulin-stimulated  $\text{Ca}^{2+}$ -ATPase activity and an increase in basal  $\text{Ca}^{2+}$ -ATPase activity in platelets from hypertensive compared to normal subjects, suggesting a reduction in plasma membrane  $\text{Ca}^{2+}$  extrusion activity and an activation of the sarcoplasmic reticulum  $\text{Ca}^{2+}$  pump in hypertension. Other studies also have described a decrease in  $\text{Ca}^{2+}$ -ATPase activity in hypertension.<sup>17,18</sup> In the present study we have determined the rate of  $\text{Ca}^{2+}$  uptake in permeabilized platelets from 17 untreated subjects with various degrees of hypertension in order to test if alteration of the sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase pump activity contributes to elevated platelet cytosolic  $\text{Ca}^{2+}$  in hypertension.

## MATERIALS AND METHODS

**Reagents** Heparin, pentosan polysulfate, saponin, and hexokinase were obtained from Sigma (St. Louis, MO). Fluo-3 pentapotassium salt was from Molecular Probes (Eugene, OR). Fluo-3 was stored in 50  $\mu\text{L}$  aliquots at a concentration of 1 mmol/L in dimethylsulfoxide at  $-70^\circ\text{C}$ . Ionomycin was from Calbiochem (San Diego, CA), thapsigargin from LC Services Corporation. Aminothyl Biogel P-2 was purchased from Bio-Rad (Richmond, CA), and Sepharose CL-2B from Pharmacia (Uppsala, Sweden).

**Buffer Solutions** All experiments were performed in 20 mmol/L HEPES buffer containing 155 mmol/L KCl, 5 mmol/L NaCl, 2 mmol/L  $\text{MgCl}_2$ , 2 mmol/L 1,4-dithioerythritol, and 0.3 mmol/L phenylmethanesulfonyl fluoride at pH 7.2, which was denoted buffer A. All solutions were depleted of  $\text{Ca}^{2+}$  by means of chromatography on EDTA-polyacrylamide as described previously.<sup>19,20</sup> Residual  $\text{Ca}^{2+}$  concentrations of solutions following treatment with EDTA-polyacrylamide were measured as described.<sup>20</sup>

**Patients** We analyzed platelets of 17 newly diagnosed hypertensive patients who were referred to the Division of Cardiology of the Kantonsspital Lucerne. None of the patients had received antihypertensive drugs before. Patients underwent a 12-lead electrocardiogram, a bicycle exercise test, and a 24-h recording of ambulatory blood pressure (HCR Blood Pressure, Disectronic, Burgdorf, Switzerland). Table 1 summarizes the clinical data and results of ambulatory 24-h blood pressure measurements.

**Preparation of Platelets** Platelets were prepared from platelet-rich plasma by gel filtration<sup>21</sup> using Sepharose CL-2B (Pharmacia) and adjusted to 108

TABLE 1. CLINICAL PARAMETERS OF THE SEVENTEEN PATIENTS SUBJECTED TO 24 H BLOOD PRESSURE DETERMINATION

Gender (men/women)	13/5
Age (years)	48 $\pm$ 13
Height (cm)	171 $\pm$ 10
Weight (kg)	75 $\pm$ 14
Office SBP (mm Hg)	169 $\pm$ 27
Office DBP (mm Hg)	111 $\pm$ 12
Day SBP (mm Hg)	148 $\pm$ 16
Day DBP (mm Hg)	97 $\pm$ 10
Night SBP (mm Hg)	136 $\pm$ 20
Night DBP (mm Hg)	87 $\pm$ 9
Day heart rate (beats/min)	76 $\pm$ 7
Night heart rate (beats/min)	64 $\pm$ 6

SBP, systolic blood pressure; DBP, diastolic blood pressure.

cells/mL. Platelets were permeabilized by addition of 20  $\mu\text{g}$  saponin/108 cells. Selective permeabilization was confirmed by measuring the release of fluo-3 from cells loaded with fluo-3 acetoxymethyl ester, and by monitoring the release of  $\text{Ca}^{2+}$  from internal membranes in permeabilized cells following addition of ionomycin. Platelets were used within 6 h of blood withdrawal.

**Measurement of  $\text{Ca}^{2+}$**   $\text{Ca}^{2+}$  concentrations were measured by means of the fluorescent  $\text{Ca}^{2+}$  indicator fluo-3. The indicator was added at a final concentration of 1  $\mu\text{mol/L}$  to 1 mL of a suspension of platelets (108 cells/mL) placed into a 1-cm quartz cuvette. The suspension was continuously stirred using a magnetic stirrer. Fluorescence was measured by means of a Perkin-Elmer (Oakbrook, IL) 650-10S fluorimeter, interfaced via an analog/digital converter (PC-28, Instrumental AG, Zurich, Switzerland) to a personal computer. Fluorescence of fluo-3 was excited at 505 nm and observed at 530 nm. ATP (1 mmol/L) and saponin (20  $\mu\text{g}$ ) were added to the cell suspension to activate  $\text{Ca}^{2+}$  uptake of intracellular  $\text{Ca}^{2+}$  stores. ATP at 1 mmol/L was found to saturate thapsigargin-sensitive  $\text{Ca}^{2+}$  uptake in platelets from many subjects of widely different degrees of hypertension (not shown).

$\text{Ca}^{2+}$  concentrations were calculated from the fluorescence signal using standard titrations of fluo-3 with  $\text{Ca}^{2+}$ . Binding of  $\text{Ca}^{2+}$  to fluo-3 was expressed as signal versus concentration of added  $\text{Ca}^{2+}$ , and evaluated as described.<sup>20</sup> From the dissociation constant ( $K_d$ ) of the  $\text{Ca}^{2+}$ -fluo-3 complex, which was typically 900 nmol/L in buffer A, the fluorescence signal of  $\text{Ca}^{2+}$ -free indicator ( $f_f$ ) and that of the  $\text{Ca}^{2+}$ -indicator complex ( $f_{FC}$ ), the concentration of  $\text{Ca}^{2+}$  was calculated as follows:

$$[\text{Ca}^{2+}]_{\text{free}} = K_d \cdot (f_{\text{obs}} - f_f) / (f_{FC} - f_{\text{obs}}) \quad (1)$$

where  $f_{\text{obs}}$  represents the observed fluorescence signal.  $K_d$  was determined from titrations of fluo-3 with  $\text{Ca}^{2+}$  (final concentrations between 0 and 5 mmol/L; eval-

uation according to Eberhard and Erne<sup>20</sup>).  $f_F$  was determined after addition of EGTA (5 mmol/L), and  $f_{FC}$  by extrapolation of the concentration of added  $Ca^{2+}$  to infinity. Figure 1 illustrates the calibration of fluorescence. The  $Ca^{2+}$  flux due to the sarcoplasmic reticulum  $Ca^{2+}$ -ATPase pump, hence termed  $Ca^{2+}$  uptake, was determined from the difference of the overall  $Ca^{2+}$  flux before and after addition of thapsigargin (200 nmol/L), a specific inhibitor of the sarcoplasmic reticulum  $Ca^{2+}$ -ATPase.<sup>22</sup> In the presence of thapsigargin, a spontaneous  $Ca^{2+}$  flux out of the sarcoplasmic reticulum, the so-called leak, was observed and modelled by an exponential decay toward an equilibrium value (Figure 1B):

$$[Ca^{2+}] = a \cdot \exp(-k \cdot t) + [Ca^{2+}]_{eq} \quad (2)$$

where  $a$  and  $k$  are arbitrary constants, and  $[Ca^{2+}]_{eq}$  the equilibrium concentration of  $Ca^{2+}$ . Thus, the rate of  $Ca^{2+}$  leak at a particular  $Ca^{2+}$  concentration is given by

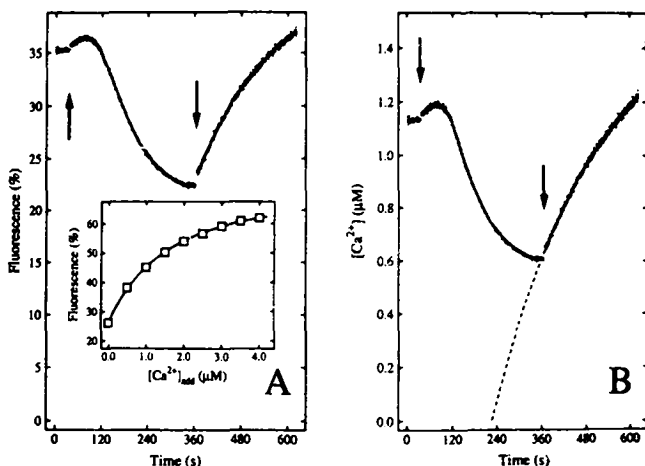
$$\frac{d[Ca^{2+}]/dt = -k \cdot a \cdot \exp(-k \cdot t) = -k \cdot ([Ca^{2+}] - [Ca^{2+}]_{eq}) \quad (3)$$

The rate of  $Ca^{2+}$  uptake is the difference between the slope of the  $Ca^{2+}$  transient at a particular  $Ca^{2+}$  concentration before addition of thapsigargin (overall  $Ca^{2+}$  flux, Figure 1), and the rate of  $Ca^{2+}$  leak at that  $Ca^{2+}$  concentration (equation 3).

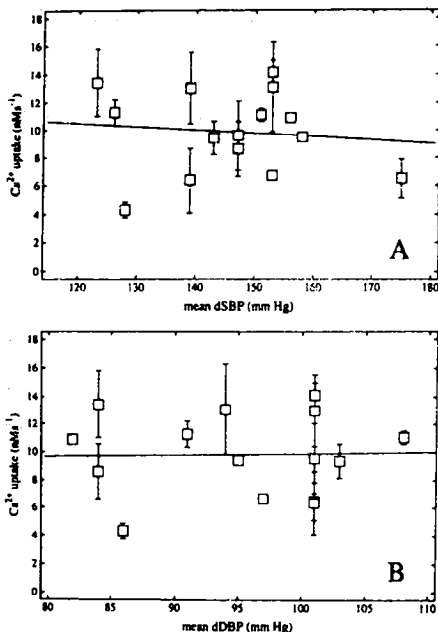
**Data Analysis** Data evaluation and nonlinear least-squares fits were performed using standard procedures.<sup>23,24</sup> Results are expressed as means  $\pm$  SD. Individual  $Ca^{2+}$  uptake and leak rate determinations were averaged and SD values calculated if three or more individual values were measured. Linear least-squares regressions assuming uniform and unity SD values of the individual data points were used to analyze putative relationships between binding data and clinical parameters. Percentile points (P) were determined using the two-tailed  $t$  distribution.

## RESULTS

The evaluation of fluorescence data and a typical  $Ca^{2+}$  transient used to determine  $Ca^{2+}$  uptake rates, i.e. the thapsigargin-sensitive  $Ca^{2+}$  flux, are shown in Figure



**FIGURE 1.** Conversion of the fluorescence signal of fluo-3 (A) to  $Ca^{2+}$  concentrations (B). The arrows indicate (from left to right) the addition of saponin (20 mg/mL) and thapsigargin (200 nmol/L) to a suspension of platelets (108 cells/mL) containing ATP (1 mmol/L). The initial increase in fluorescence signal after addition of saponin is due to a decrease in turbidity upon permeabilization of the cells. The turbidity drops during the first 60 sec and remains stable thereafter for at least 15 min (not shown). The inset in A shows the correlation between fluorescence and added  $Ca^{2+}$  ( $[Ca^{2+}]_{tot}$ ; the total concentration of added  $Ca^{2+}$ ) obtained in a separate experiment with an identical platelet suspension. The solid line represents the fitted binding curve ( $K_d = 723$  nmol/L,  $f_F = 0.75\%$ ,  $f_{FC} = 73.25\%$ ; see Eberhard M, Erne P<sup>20</sup>). The  $Ca^{2+}$  concentration ( $[Ca^{2+}]_{leak}$  in equation 1) in B was calculated from the fluorescence signal in A according to equation 1. The dotted curve in B represents a monoexponential fit of the  $Ca^{2+}$  transient according to equation 2 ( $a = -933$  nmol/L,  $k = 0.0036$  sec<sup>-1</sup>,  $[Ca^{2+}]_{eq} = 1599$  nmol/L).  $Ca^{2+}$  concentrations are considerably underestimated at the start of the transient due to the turbidity change during permeabilization (see above).



**FIGURE 2.** Lack of correlation of  $\text{Ca}^{2+}$  uptake rates versus mean day systolic blood pressure (mean dSBP; A) and mean day diastolic blood pressure (mean dDBP). Lines were determined by linear regression (A:  $r = -0.114$ ,  $P > .5$ ; B:  $r = 0.029$ ,  $P > .5$ ).

1. At a given  $\text{Ca}^{2+}$  concentration similar  $\text{Ca}^{2+}$  uptake rates were obtained when thapsigargin was added at various degrees of store loading (not shown). Since  $\text{Ca}^{2+}$  concentrations of suspensions of permeabilized platelet were around and above 1 mmol/L, and since the  $\text{Ca}^{2+}$  uptake rate did not significantly depend on

the concentration of  $\text{Ca}^{2+}$  in the range between 0.8 and 1.5 mmol/L (not shown),  $\text{Ca}^{2+}$  uptake rates were evaluated at 1 mmol/L  $\text{Ca}^{2+}$  in the present study. To examine if spontaneous  $\text{Ca}^{2+}$  leak of the  $\text{Ca}^{2+}$  stores is linked to hypertension, the  $\text{Ca}^{2+}$  leak ( $\text{Ca}^{2+}$  flux in the presence of thapsigargin) was also evaluated at 1 mmol/L  $\text{Ca}^{2+}$ . In two out of the 17 patients examined the  $\text{Ca}^{2+}$  concentration of the platelet suspension after permeabilization was too high to allow the determination of  $\text{Ca}^{2+}$  uptake and leak rates at 1 mmol/L  $\text{Ca}^{2+}$ .

Figure 2 shows that  $\text{Ca}^{2+}$  uptake into sarcoplasmic reticulum is not related to average daytime blood pressure. Similar results are obtained with other blood pressure parameters (Table 1) and with  $\text{Ca}^{2+}$  leak from sarcoplasmic reticulum (Table 2). A weak correlation between both  $\text{Ca}^{2+}$  uptake and  $\text{Ca}^{2+}$  leak and heart rate was observed (Figure 3, Table 2), although these correlations are flawed by the substantial error of the values of  $\text{Ca}^{2+}$  uptake and leak. Furthermore, no correlation was found between  $\text{Ca}^{2+}$  uptake and age (Table 2). To corroborate the relationship between  $\text{Ca}^{2+}$  fluxes and blood pressure we analyzed a group of blood donors consisting of four normotensive and ten hypertensive subjects who did not undergo 24-h blood pressure determination (Table 3). No significant correlation between flux parameters and blood pressure was detected within this group (not shown). In addition, the differences in  $\text{Ca}^{2+}$  uptake and leak between the group of normotensives and the two hypertensive groups were statistically not significant (Table 3).

## DISCUSSION

There is a general agreement that platelet cytosolic  $\text{Ca}^{2+}$  is increased in hypertension and that this process can be accomplished by rapid  $\text{Ca}^{2+}$ -regulating processes.<sup>6</sup> Both plasma membrane- and internal membrane-associated  $\text{Ca}^{2+}$  fluxes contribute to the observed changes in platelet cytosolic  $\text{Ca}^{2+}$ . Since the  $\text{Ca}^{2+}$ -ATPase of sarcoplasmic reticulum is believed to represent the major contribution to  $\text{Ca}^{2+}$  removal from the cytoplasm,<sup>11,25</sup> we have analyzed ATP-dependent

**TABLE 2. CORRELATION OF  $\text{Ca}^{2+}$  UPTAKE AND LEAK WITH CLINICAL PARAMETERS**

Parameter	$\text{Ca}^{2+}$ Uptake			$\text{Ca}^{2+}$ Leak		
	Slope	P	r	Slope	P	r
Age	$0.083 \pm 0.055$	>.2	0.389	$0.043 \pm 0.029$	>.1	0.383
Day SBP	$-0.025 \pm 0.060$	>.5	-0.114	$0.010 \pm 0.031$	>.5	0.093
Day DBP	$0.011 \pm 0.101$	>.5	0.029	$-0.003 \pm 0.053$	>.5	-0.015
Night SBP	$0.019 \pm 0.044$	>.5	0.121	$0.012 \pm 0.023$	>.5	0.141
Night DBP	$0.130 \pm 0.086$	>.1	0.346	$0.027 \pm 0.048$	>.5	0.155
Day heart rate	$0.141 \pm 0.113$	>.2	0.327	$0.105 \pm 0.055$	>.05	0.465
Night heart rate	$0.260 \pm 0.096$	<.05	0.591	$0.124 \pm 0.054$	<.05	0.541

15 hypertensive patients undergoing 24-h blood pressure determination (Table 1), for which  $\text{Ca}^{2+}$  uptake and  $\text{Ca}^{2+}$  leak values were determined (see Results).

SBP, systolic blood pressure; DBP, diastolic blood pressure.

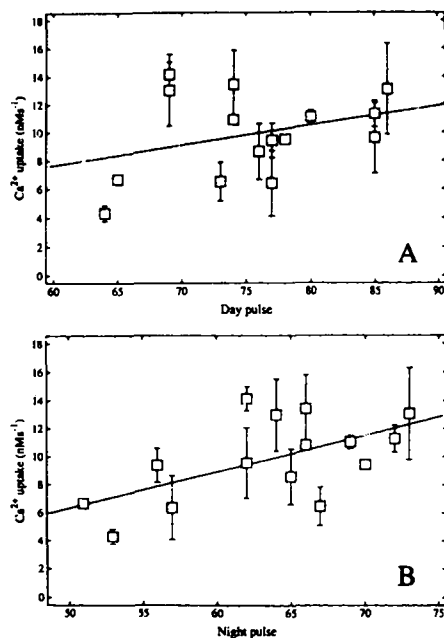


FIGURE 3. Plot of  $\text{Ca}^{2+}$  uptake rates versus average heart rate during daytime (A) and nighttime (B). Lines were calculated by linear regression (A:  $r = 0.141$ ,  $P > .2$ ; B:  $r = 0.260$ ,  $P < .05$ ).

$\text{Ca}^{2+}$  transport into sarcoplasmic reticulum in platelets from a series of patients exhibiting various degrees of hypertension. None of the patients received antihypertensive therapy prior to analysis. Thapsigargin, a specific inhibitor of all isoforms of internal membrane  $\text{Ca}^{2+}$ -ATPase<sup>22,26</sup>, served to evaluate  $\text{Ca}^{2+}$ -ATPase transport activity and spontaneous  $\text{Ca}^{2+}$  leak of sarcoplasmic reticulum. No correlation of blood pressure with both  $\text{Ca}^{2+}$  uptake and  $\text{Ca}^{2+}$  leak and a weak but

insignificant correlation of  $\text{Ca}^{2+}$  uptake with heart rate were found.  $\text{Ca}^{2+}$  uptake and  $\text{Ca}^{2+}$  leak did not significantly differ between hypertensive patients and a normotensive control group.

An earlier study has documented a substantial increase of platelet calmodulin-insensitive  $\text{Ca}^{2+}$ -ATPase activity in hypertension<sup>16</sup> indicating a stimulation of internal membrane  $\text{Ca}^{2+}$ -ATPase in hypertension. The discrepancy to our results may result from the different selection of hypertensive patients, the use of thapsigargin in the present study as opposed to calmodulin activation, and the determination of  $\text{Ca}^{2+}$  transport activities in permeabilized cells in the present experiments rather than  $\text{Ca}^{2+}$ -dependent ATPase activity in membrane preparations used in the study of Resink et al.<sup>16</sup> Takaya et al.<sup>17</sup> found a decrease in maximal velocity and Michaelis constant of  $\text{Ca}^{2+}$ -ATPase in platelets from hypertensive patients. Since plasma membrane and internal membrane  $\text{Ca}^{2+}$ -ATPase activity was not discriminated, the respective contributions of the two  $\text{Ca}^{2+}$  pumps could not be evaluated in that study. In agreement with our results, no correlation of platelet sarcoplasmic reticulum  $\text{Ca}^{2+}$ -ATPase with blood pressure was documented, but a decrease of platelet plasma membrane  $\text{Ca}^{2+}$ -ATPase activity in hypertension was found recently.<sup>26</sup> Consistent with the decrease in calmodulin-sensitive  $\text{Ca}^{2+}$ -ATPase activity in hypertension,<sup>16</sup> these studies indicate that removal of  $\text{Ca}^{2+}$  across the plasma membrane is responsible for the well established rise of cytosolic  $\text{Ca}^{2+}$  in hypertension.<sup>1,5</sup>

Possible other mechanisms accounting for altered cytosolic  $\text{Ca}^{2+}$  in hypertension include increased agonist-induced  $\text{Ca}^{2+}$  influx,<sup>10</sup> activation of voltage-operated  $\text{Ca}^{2+}$  channels,<sup>1</sup> altered inositol 1,4,5-trisphosphate-induced  $\text{Ca}^{2+}$  efflux from internal  $\text{Ca}^{2+}$  stores,<sup>26</sup> and a decreased activity of  $\text{Na}^{+}/\text{Ca}^{2+}$  exchangers present in the platelet plasma membrane.<sup>15,27</sup> Whereas the density of voltage-gated  $\text{Ca}^{2+}$  channels in platelets is very low and plays only a minor role in the  $\text{Ca}^{2+}$  balance of platelets,<sup>11</sup> enhanced agonist-induced  $\text{Ca}^{2+}$  influx in platelets has been documented in patients with ischemic heart disease.<sup>28</sup> A

TABLE 3. COMPARISON OF  $\text{Ca}^{2+}$  UPTAKE AND LEAK BETWEEN HYPERTENSIVE PATIENTS AND NORMOTENSIVE SUBJECTS

Group	Normotensive (n = 4)	Hypertensive (n = 10)	Hypertensive* (n = 15)
SBP (mm Hg)	116 ± 8	137 ± 8	146 ± 14
DBP (mm Hg)	75 ± 6	90 ± 6	95 ± 8
$\text{Ca}^{2+}$ uptake (nmol/L/sec)	8.02 ± 2.59	8.86 ± 2.55	9.85 ± 2.95
$\text{Ca}^{2+}$ leak (nmol/L/sec)	2.89 ± 0.65	3.65 ± 1.24	3.38 ± 1.54

Normotensive defined as SBP (systolic blood pressure) ≤120 mm Hg, DBP (diastolic blood pressure) ≤80 mm Hg.

\*This group comprises the 15 hypertensive patients undergoing 24-h blood pressure determination (Table 1), for which  $\text{Ca}^{2+}$  uptake and  $\text{Ca}^{2+}$  leak values were determined (see Results).

decrease of platelet  $\text{Na}^+/\text{K}^+$ -ATPase activity in mild gestational hypertension has been suggested to contribute to increased cytosolic  $\text{Ca}^{2+}$  through the  $\text{Na}^+/\text{Ca}^{2+}$  exchanger.<sup>29</sup> However, Takaya et al<sup>17</sup> did not find altered platelet  $\text{Na}^+/\text{K}^+$ -ATPase activity in hypertension. Therefore, a decreased activity of the plasma membrane  $\text{Ca}^{2+}$ -ATPase and increased agonist-induced  $\text{Ca}^{2+}$  influx are likely to contribute to the increase in platelet cytosolic  $\text{Ca}^{2+}$  in hypertension, whereas ATP-driven  $\text{Ca}^{2+}$  uptake into internal  $\text{Ca}^{2+}$  stores remains rather unchanged.

In the present study a weak relationship between  $\text{Ca}^{2+}$  uptake into internal membranes and heart rate was noted (Figure 3). Clearly, more patients and a wider range of heart rates will be needed to decide if this correlation is significant. A link between heart rate, which is controlled by the sympathetic and parasympathetic nervous system,<sup>30,31</sup> and  $\text{Ca}^{2+}$  dynamics of the platelet sarcoplasmic reticulum could be  $\beta$ -adrenergic receptors present in the heart and on platelets.<sup>32,33</sup> Whereas hypertension is usually associated with an increase in sympathetic activity,<sup>34</sup> its role in the  $\text{Ca}^{2+}$  balance in platelets remains obscure, although  $\beta$ -adrenergic receptors may affect  $\text{Ca}^{2+}$  pumping activity via phospholamban phosphorylation.<sup>35</sup>

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