Platelet Calcium Transport in Hypertension

William L. Dean, Jennie E. Pope, Michael E. Brier, George R. Aronoff

Abstract To determine platelet Ca\(^{2+}\) transport entities involved in increased cytosolic Ca\(^{2+}\) in the platelets of hypertensive individuals, we studied the relations between blood pressure and Ca\(^{2+}\) transporters in platelet membranes from 22 white male volunteers 32 to 68 years old. We used thapsigargin, a specific inhibitor of the internal membrane Ca\(^{2+}\)-ATPase, to differentiate between plasma membrane and internal membrane Ca\(^{2+}\)-ATPases. Inositol 1,4,5-trisphosphate-mediated and Ca\(^{2+}\) ionophore (A23187)-induced Ca\(^{2+}\) release was also assayed in membrane preparations using rhod-2, a fluorescent Ca\(^{2+}\) indicator. Levels of glycoprotein IIb/IIIa, a possible component of agonist-mediated Ca\(^{2+}\) influx, were measured by immunoblotting. The results show that plasma membrane Ca\(^{2+}\)-ATPase is decreased as a function of diastolic blood pressure (P<.002), whereas the internal membrane Ca\(^{2+}\)-ATPase is not (P=.148). Neither activity is correlated with age or systolic blood pressure. However, inositol triphosphate-mediated Ca\(^{2+}\) release is negatively correlated with age (P<.024) but not blood pressure. Glycoprotein IIb levels and A23187-induced Ca\(^{2+}\) release were not related to age or blood pressure, demonstrating that inhibition of the plasma membrane Ca\(^{2+}\)-ATPase was not a result of differences in the proportion of plasma membrane in the preparation or differences in intravesicular Ca\(^{2+}\) concentration. Inhibition of the plasma membrane Ca\(^{2+}\)-ATPase could directly cause elevation of cytoplasmic Ca\(^{2+}\) and enhancement of platelet sensitivity. (Hypertension. 1994;23:31-37.)

Key Words • blood platelets • calcium • adenosine triphosphatase • hypertension, essential

Thrombotic events are more common in hypertensive individuals.\(^1\) One link between hypertension and thrombosis is the increased platelet sensitivity to agonists observed in hypertension,\(^2,2\) which may be related to increased levels of platelet cytosolic Ca\(^{2+}\) in hypertensive patients.\(^2,8\) Cytoplasmic Ca\(^{2+}\) triggers platelet activation, so it is likely that increased Ca\(^{2+}\) leads to increased platelet activity in hypertension.

Although the potential importance of the observed increase in platelet Ca\(^{2+}\) is clear, the mechanism for this elevation of cytosolic Ca\(^{2+}\) has not been conclusively identified. Ahn and coworkers\(^6\) suggested that this increase in cytoplasmic Ca\(^{2+}\) results from increased Ca\(^{2+}\) influx. In their experiments, platelets from hypertensive individuals displayed a decrease in Ca\(^{2+}\) when treated with the calcium channel blocker nifedipine. However, other work suggests that platelet Ca\(^{2+}\)-ATPase activity is altered in hypertensive patients.\(^9,10\) Takaya et al\(^9\) concluded that the activity of the platelet Ca\(^{2+}\)-ATPase is inhibited in hypertensive individuals, whereas Resink et al\(^10\) reported stimulation of the platelet Ca\(^{2+}\) pump in hypertension. It has been reported that erythrocytes also exhibit increased cytoplasmic Ca\(^{2+}\) and that their Ca\(^{2+}\) pump is inhibited in hypertension.\(^11\) Thus, results are conflicting regarding the effect of hypertension on the Ca\(^{2+}\)-ATPase. We carried out the present study in part to resolve this controversy.

In addition to changes in Ca\(^{2+}\) influx and Ca\(^{2+}\)-ATPase activity, several groups have reported the presence of circulating plasma factors in hypertensive individuals that apparently cause an increase in platelet cytosolic Ca\(^{2+}\).\(^7,8\) It has been proposed that these factors inhibit Ca\(^{2+}\)-ATPases.\(^12\)

Several biochemical processes maintain platelet calcium homeostasis. Two Ca\(^{2+}\)-ATPases actively pump calcium. One, located in the plasma membrane, pumps Ca\(^{2+}\) out of the platelet; another, located in internal membranes, is responsible for maintaining a depot of internal Ca\(^{2+}\) used as a trigger for platelet activation.\(^14\) Inositol 1,4,5-trisphosphate (IP3) is the soluble messenger that signals release of Ca\(^{2+}\) from these internal stores.\(^15\) The other source of Ca\(^{2+}\) for activation is plasma Ca\(^{2+}\) that enters through plasma membrane calcium channels. The glycoprotein IIb/IIIa complex that binds fibrinogen during activation may be involved in agonist-mediated Ca\(^{2+}\) influx.\(^16\) We assessed these major aspects of platelet calcium homeostasis in the present study. We used thapsigargin, a specific inhibitor of internal membrane Ca\(^{2+}\) pumps,\(^17\) to differentiate between plasma membrane and internal membrane Ca\(^{2+}\)-ATPases in platelet membranes from volunteers exhibiting a broad range of diastolic blood pressures. In addition to Ca\(^{2+}\)-ATPase activity, Ca\(^{2+}\) uptake capacity, IP3-mediated Ca\(^{2+}\) release, and levels of glycoprotein IIb/IIIa were also measured in isolated membranes. We correlated these parameters with blood pressure and age to determine which of the Ca\(^{2+}\)-transporting entities are altered in hypertensive individuals.

Methods

Materials

Rhod-2 and BrA23187 were purchased from Molecular Probes, Inc, Eugene, Ore. Thapsigargin and IP3 were purchased from LC Laboratories, Inc, Woburn, Mass. ATP, phosphoenolpyruvate, NADH, lactate dehydrogenase, and pyruvate kinase were purchased from Sigma Chemical Co, St Louis, Mo. Antibodies against platelet glycoproteins IIb and IIIa were products of AMAC Inc, Westbrock, Me.
ish peroxidase–coupled second antibody and all electrophore-
sis reagents were purchased from Bio-Rad, Richmond, Calif.  
Chemiluminescence reagents were a product of Amersham 
Corp, Arlington Heights, Ill.

Patient Selection
The institutional review committees of the University of 
Louisville and the Louisville Department of Veterans’ Affairs 
Hospital approved the study protocol. Informed consent 
was obtained from all subjects entered into the protocol. Twenty-
two white male volunteers between the ages of 32 and 68 were 
recruited from the hospital and clinics of the Louisville 
Department of Veterans’ Affairs Hospital, from the Kidney Disease Program at the University of Louisville School of 
Medicine, and from the faculty at the University of Louisvile School of Medicine. Brief patient histories were recorded 
for determination of previous or current treatment for hyperten-
sion. Volunteers who had not received any medications for 3 
weeks, including anti-inflammatory drugs, and were not suffer-
ing from any medical conditions other than hypertension 
were used in this study. Individuals with a history of significant 
disorders of the kidneys, heart, lungs, liver, or hematopoietic 
system or neuromuscular or metabolic dysfunction were ex-
cluded. Blood pressure was taken three times with subjects in 
the seated position by auscultation using a wall-mounted 
sphygmomanometer and was averaged.

Isolation of Platelets and Membrane Preparation
Blood was drawn with 18-gauge needles. The first 2 to 3 mL 
was discarded to prevent platelet activation, and the next 50 
ml of blood was immediately mixed with 8 ml of acid 
citrate-dextrose (31 mmol/L citric acid, 85 mmol/L sodium 
citrate, 111 mmol/L glucose) to prevent activation. Platelets 
were isolated by centrifugation at 177g for 15 minutes for 
removal of erythrocytes followed by a second centrifugation 
step at 2000g for 10 minutes. Platelets were then resuspended 
in 5 ml plasma to which an equal volume of 40 ml/L glucose 
containing 24 ml/mL NaPO4, 4 ml/mL phenylmeth-
ylethanesulfonic acid (PMSF), and 0.01 U/mL aprotinin at pH 6.8 
was added. Platelets were centrifuged at 160g for 5 minutes to 
prevent platelet activation, and the next 50 ml of blood was 
added. Platelets were centrifuged at 160g for removal of 
erythrocytes and pelleted at 3000g. Platelets were next resus-
pended in 0.15 mol/L NaCl, 3.0 mmol/L EDTA containing 
PMSF and aprotinin as above, centrifuged, and then washed 
twice in 0.15 mol/L NaCl with aprotinin and PMSF. The 
washed platelets were resuspended in 0.5 ml sonication buffer 
containing 30 mmol/L KCl, 5 mmol/L MgCl2, 10 mmol/L 
Na-oxalate, 20 mmol/L Tris, 300 mmol/L sucrose, 40 ml/L 
PMSF, 0.01 U aprotinin/ml, 10 mg/ml pepstatin A, 10 mg/ml 
antipain, and 10 mg/ml leupeptin at pH 7.0. Platelets were 
sonicated at a setting of 1 on a Branson sonicator two times for 
5 seconds in centrifuge tubes with caps to prevent release of an 
aerosol. The homogenized platelets were then centrifuged for 
ten minutes at 10 000g and the supernatant was recentrifuged 
at 100 000g for 20 minutes. Membranes were resuspended in 
0.1 ml of 0.01 mol/L TES buffer containing 2.7 mol/L glycerol, 5 
mmol/L MgCl2, and 0.1 mol/L NaCl at pH 7.0. Membrane 
material was stored at −20°C. Protein concentration of the mem-
branes was used as an internal control, and all samples 
were determined by Laemmli.20 One preparation of platelet 
membranes was used as an internal control, and all samples 
were compared with this preparation. After electrophoresis, 
platelet membrane proteins were transferred to nitrocellulose 
paper in a Bio-Rad minigel transfer apparatus at pH 8.3 and 
blocked with 3% gelatin.21 The nitrocellulose sheet was probed 
overnight with a mixture of anti-glycoprotein IIb and IIa

Ca2+-ATPase Assay
A coupled ATPase assay was used in which ATP hydrolysis 
is coupled to oxidation of NADH with pyruvate kinase and 
lactate dehydrogenase.18 The assay mixture contained 0.01 
ml/L TES buffer at pH 7.4, 0.1 mol/L KCl, 5 mmol/L 
MgATP, 0.01 ml/L NaPO4, and 5.0 ml of membranes, and the 
assay temperature was 37°C. Thapsigargin was used at a 
concentration of 200 nl/mL.18,19 The specific activity of 
plasma and internal membrane Ca2+-ATPases was calculated 
using the difference between total Ca2+-ATPase activity 
and thapsigargin-resistant activity. Ca2+-ATPase resistant to 200 
nmol/L thapsigargin is reported as plasma membrane Ca2+- 
ATPase, whereas the difference between total Ca2+-ATPase 
in the absence of thapsigargin and thapsigargin-resistant activity 
is reported as internal membrane Ca2+-ATPase.

Calcium Transport Assays
Calcium uptake and release were measured in an SLM 
SPF-500 spectrofluorometer with a thermostated cuvette com-
partment (37°C) as reported earlier.19 Assay volume was 2.0 
ml containing 0.01 mol/L TES buffer at pH 7.4, 0.1 mol/L 
KCl, 50 mmol/L KPO4, 2.7 mol/L glycerol, 5 mmol/L 
Hepes, and 30 ml/L of platelet membranes. Calcium uptake 
was initiated by the addition of 5 mmol/L MgATP. The initial calcium 
concentration was approximately 10 mmol/L. After 2 minutes 
of uptake, IP3 (10 mmol/L) was added to initiate Ca2+ 
release. After release was complete and reuptake had begun, 
Br23187 (1 ml/L) was added for determination of the 
total amount of Ca2+ sequestered within the membranes. 
Uptake and release of Ca2+ were monitored continuously with 
an excitation wavelength of 506 nm and emission at 526 nm. 
Rates were determined from standard curves obtained from a 
series of 1-nmol additions of CaC2. A typical assay is shown in 
Fig 1. The amount of Ca2+ released by BrA23187 is defined as 
total Ca2+, whereas the amount of Ca2+ released by IP3 is 
reported as a percentage of the total Ca2+ released by 
Br23187.

Immunoblotting
Membrane samples were thawed, and 4.0 mg was applied to 
a 7.5% polyacrylamide minigel. Electrophoresis was carried out 
according to Laemmli.20 One preparation of platelet 
membranes was used as an internal control, and all samples 
were compared with this preparation. After electrophoresis, 
platelet membrane proteins were transferred to nitrocellulose 
paper in a Bio-Rad minigel transfer apparatus at pH 8.3 and 
blocked with 3% gelatin.21 The nitrocellulose sheet was probed 
overnight with a mixture of anti-glycoprotein IIb and IIa

![Graph](image-url)
antibodies at room temperature. The sheets were treated with a peroxidase-conjugated second antibody for 1 hour and developed with Amersham chemiluminescence reagents for 1 minute. Blots were then exposed to Kodak x-ray film for 30 seconds and developed. Reactions were quantitated on a Bio-Rad densitometer; peak areas were compared with the internal standard, and areas were normalized. Results from a typical immunoblot are shown in Fig 2.

Results

Plasma and internal membrane Ca\(^{2+}\)-ATPase activities are shown plotted against diastolic blood pressure in Fig 3. Both activities decreased with increasing diastolic blood pressure based on linear regression. Statistical analysis for all of the data is shown in the Table. Total Ca\(^{2+}\)-ATPase activity, the sum of plasma membrane and internal membrane activities, is significantly correlated with diastolic blood pressure (P<.008, r=-.57), as is plasma membrane Ca\(^{2+}\)-ATPase (P<.002, r=-.64) (Fig 3A), but the negative slope of the plot of internal membrane Ca\(^{2+}\)-ATPase versus diastolic blood pressure is not significant (P=.14, r=-.33) (Fig 3B). Thapsigargin inhibition is also correlated with diastolic blood pressure (P<.035, r=.47). Ca\(^{2+}\)-ATPase activity is not correlated with age or systolic blood pressure. However, the plasma membrane Ca\(^{2+}\)-ATPase activity also yielded a negative slope (−.10) when plotted versus systolic blood pressure (P<.25, r=-.26), as shown in Fig 4.

The results of plotting IP\(_3\) mediated Ca\(^{2+}\) release versus age of the subjects are shown in Fig 5. The statistical analysis presented in the Table indicates that variation in this Ca\(^{2+}\) channel activity is correlated with age (P<.024, r=.52) but not blood pressure. The IP\(_3\) mediated release values reported are determined by comparing the quantity of Ca\(^{2+}\) released by the Ca\(^{2+}\) ionophore A23187 (total Ca\(^{2+}\) release) with that released by IP\(_3\). The amount of Ca\(^{2+}\) released by the ionophore (total Ca\(^{2+}\) release in the Table) is not correlated with blood pressure (Table).

The final aspect of platelet Ca\(^{2+}\) metabolism analyzed was the content of glycoprotein IIIa in the membranes. Comparison of the mass of glycoprotein IIIa with blood pressure, as determined by immunoblotting, indicated no significant difference in the level of this platelet plasma membrane protein, as shown in the Table. Identical results were obtained for glycoprotein IIb (data not shown).

Discussion

Because inhibition of either the plasma membrane or internal membrane Ca\(^{2+}\)-ATPases could lead to an increase in cytosolic Ca\(^{2+}\), we measured the activity of both ATPases assuming that thapsigargin totally inhibits the internal membrane Ca\(^{2+}\)-ATPase but has no effect on plasma membrane Ca\(^{2+}\)-ATPase. These measurements are especially significant because the modes of regulation of these two ATPases are different. Our data demonstrate that platelets from hypertensive individuals exhibit lower levels of thapsigargin-resistant Ca\(^{2+}\)-ATPase activity. The most likely interpretation of this observation is that the plasma membrane Ca\(^{2+}\)-ATPase is inhibited in hypertension.

The inhibition of Ca\(^{2+}\)-ATPase activity is well correlated with diastolic blood pressure (P<.001, Fig 3) but not with systolic blood pressure (P>.25, slope=-.1, Fig 4). This phenomenon may be related to the observation that systolic hypertension is often associated with arterial rigidity and decreased capacity of the aorta, whereas diastolic blood pressure is more closely related to vascular tone, a process intimately connected to cytosolic Ca\(^{2+}\) levels. If diastolic hypertension is caused by a circulating factor that affects internal Ca\(^{2+}\) levels in smooth muscle, it may also alter Ca\(^{2+}\) metabolism in platelets. Clearly, a larger number of subjects would be required to determine whether the negative slope of the plot of plasma membrane Ca\(^{2+}\)-ATPase versus systolic pressure is significant. Others have shown that platelet cytosolic Ca\(^{2+}\) is correlated with both systolic and diastolic blood pressures. Although it appears that the data of Fig 3A can be grouped into two distinct populations (<85 and >90 mm Hg), statistical analysis does not indicate a significant difference in plasma membrane Ca\(^{2+}\)-ATPase between these groups.

The assumption that the thapsigargin-resistant Ca\(^{2+}\)-ATPase activity represents the plasma membrane Ca\(^{2+}\)-ATPase is based on experiments using purified Ca\(^{2+}\)-ATPases; all isoforms of internal membrane Ca\(^{2+}\)-ATPase are totally inhibited with 200
### Correlation of Measured Parameters With Blood Pressure and Age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slope of Linear Regression</th>
<th>P</th>
<th>r</th>
<th>Average±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Ca(^{2+})-ATPase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>-0.18</td>
<td>.582</td>
<td>.01</td>
<td>35±17 (nmol/min)/mg</td>
</tr>
<tr>
<td>vs DBP</td>
<td>-0.84</td>
<td>.008*</td>
<td>.57</td>
<td></td>
</tr>
<tr>
<td>vs SBP</td>
<td>-0.11</td>
<td>.452</td>
<td>.17</td>
<td></td>
</tr>
<tr>
<td><strong>Thapsigargin inhibition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>0.2</td>
<td>.318</td>
<td>.12</td>
<td>55±13% inhibition</td>
</tr>
<tr>
<td>vs DBP</td>
<td>0.52</td>
<td>.035*</td>
<td>.47</td>
<td></td>
</tr>
<tr>
<td>vs SBP</td>
<td>0.14</td>
<td>.215</td>
<td>.30</td>
<td></td>
</tr>
<tr>
<td><strong>PM Ca(^{2+})-ATPase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>-0.17</td>
<td>.354</td>
<td>.04</td>
<td>16±10 (nmol/min)/mg</td>
</tr>
<tr>
<td>vs DBP</td>
<td>-0.59</td>
<td>.002*</td>
<td>.64</td>
<td></td>
</tr>
<tr>
<td>vs SBP</td>
<td>-0.10</td>
<td>.251</td>
<td>.26</td>
<td></td>
</tr>
<tr>
<td><strong>IM Ca(^{2+})-ATPase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>-0.01</td>
<td>.956</td>
<td>.06</td>
<td>19±10 (nmol/min)/mg</td>
</tr>
<tr>
<td>vs DBP</td>
<td>-0.28</td>
<td>.148</td>
<td>.33</td>
<td></td>
</tr>
<tr>
<td>vs SBP</td>
<td>-0.01</td>
<td>.889</td>
<td>.10</td>
<td></td>
</tr>
<tr>
<td><strong>Total Ca(^{2+}) release</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>0.15</td>
<td>.976</td>
<td>.20</td>
<td>8±7 nmol/mg</td>
</tr>
<tr>
<td>vs DBP</td>
<td>0.00</td>
<td>.400</td>
<td>.20</td>
<td></td>
</tr>
<tr>
<td>vs SBP</td>
<td>0.01</td>
<td>.859</td>
<td>.20</td>
<td></td>
</tr>
<tr>
<td><strong>IP(_3) mediated Ca(^{2+}) release</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>-0.80</td>
<td>.024*</td>
<td>.52</td>
<td>29±17% of total</td>
</tr>
<tr>
<td>vs DBP</td>
<td>0.49</td>
<td>.102</td>
<td>.60</td>
<td></td>
</tr>
<tr>
<td>vs SBP</td>
<td>0.09</td>
<td>.516</td>
<td>.53</td>
<td></td>
</tr>
<tr>
<td><strong>GP I(_{IIa})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>0.00</td>
<td>.972</td>
<td>.01</td>
<td>0.95±0.29 relative to control</td>
</tr>
<tr>
<td>vs DBP</td>
<td>0.00</td>
<td>.987</td>
<td>.00</td>
<td></td>
</tr>
<tr>
<td>vs SBP</td>
<td>0.00</td>
<td>.415</td>
<td>.17</td>
<td></td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>-0.24</td>
<td>.340</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs SBP</td>
<td>0.26</td>
<td>.004*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DBP indicates diastolic blood pressure; SBP, systolic blood pressure; PM, plasma membrane; IM, internal membrane; IP\(_3\), inositol 1,4,5-trisphosphate; and GP, glycoprotein. P is the probability that the correlation is due to random variation; r is the correlation coefficient.  
*P<.05.

nmol/L thapsigargin, whereas the plasma membrane Ca\(^{2+}\)-ATPase is unaffected. We also have observed total inhibition of the platelet internal membrane Ca\(^{2+}\)-ATPase by 200 nmol/L thapsigargin. However, a recent article by Tao and Haynes suggests that only 70% of platelet internal membrane Ca\(^{2+}\)-ATPase is inhibited by thapsigargin and that 30% of the plasma membrane form is sensitive. This work was based on the untested assumption that chlorotetracycline partitions totally into the dense tubules in intact platelets and reports Ca\(^{2+}\) uptake only in these membranes. If, on the other hand, a small percentage of this fluorophore were associated with the surface connected plasma membrane system, then some of the reported transport would be due to plasma membranes, and this could explain the lack of total inhibition of the internal membrane Ca\(^{2+}\)-ATPase by thapsigargin. Similar arguments can be used to discount the reported inhibition of the platelet plasma membrane Ca\(^{2+}\)-ATPase. The fact that the erythrocyte Ca\(^{2+}\)-ATPase is also inhibited in hypertension lends further credence to the conclusion that the inhibited Ca\(^{2+}\) pump in plate-
Platelet Ca²⁺ Pumps in Hypertension

Our observations are in agreement with the conclusion stated by Takaya et al.⁹ that the platelet plasma membrane Ca²⁺-ATPase is inhibited in hypertension. However, their preparation of plasma membrane was almost certainly contaminated with internal membrane.

Our observations are in agreement with the conclusion stated by Takaya et al.⁹ that the platelet plasma membrane Ca²⁺-ATPase is inhibited in hypertension. However, their preparation of plasma membrane was almost certainly contaminated with internal membrane.

Another possible mechanism for increased platelet sensitivity to agonists in hypertension is increased Ca²⁺ release by the IP₃-gated Ca²⁺ channel in response to agonists. Our data show that the activity of this Ca²⁺ channel correlates with age but not blood pressure. However, it should be noted that deletion of one point from the data, the IP₃ release occurring for a 32-year-old volunteer, would render the correlation between IP₃-mediated release and age insignificant (P<.07). Although we have no criterion for deleting this point, the correlation would be stronger if additional data supported this relation. It was also demonstrated that total release of Ca²⁺ sequestered by isolated membranes (total Ca²⁺ release, Table) did not change as a function of blood pressure or age. This observation indicates that the differences in IP₃ channel activity are not due to variations in the total amount of sequestered Ca²⁺. The physiological significance of this finding is not clear, but it does indicate that hypertensive individuals do not have enhanced IP₃ channel function.

It has been suggested that the glycoprotein IIb/IIIa complex is an agonist-activated plasma membrane Ca²⁺ channel.²⁷-²⁹ Our observations indicate that levels of these plasma membrane proteins are not altered in hypertensive individuals (Table). However, because channel activity was not directly measured, these data
do not rule out changes in rates of $\text{Ca}^{2+}$ entry into the platelet as a result of hypertension.

The measurements of glycoproteins Ib and IIIa also demonstrate that the platelet membrane preparations in this study contain the same contribution of plasma membrane to the total membrane population, because the slopes of the plots of glycoprotein IIIa content versus age and diastolic blood pressure are zero (Table), and identical results were obtained from analysis of glycoprotein Ib. These data were obtained by quantitative immunoblotting (see "Methods") of the same amount of membrane protein. If the proportion of plasma membrane increased or decreased as a function of hypertension or age, then the slope of this plot would be expected to be nonzero. The probability that a decrease in plasma membrane content was coupled with compensatory increases in glycoproteins Ib and IIIa in hypertension is very low.

One hypothesis for the mechanism of inhibition of the plasma membrane $\text{Ca}^{2+}$-ATPase suggested by the data in this article and other reports is that a humoral factor present in plasma from hypertensive individuals interacts with platelets, resulting in inhibition of the plasma membrane $\text{Ca}^{2+}$-ATPase. The fact that $\text{Ca}^{2+}$-ATPase activity is altered after isolation of membranes suggests that the protein has been modified covalently, that changes in the mass of the ATPase synthesized in the megakaryocyte has occurred, or that the humoral factor is lipid soluble. Data demonstrating immediate changes in platelet $\text{Ca}^{2+}$ concentration on exposure to humoral factor indicate that the process is rapid and could not require synthesis of new platelets with altered levels of $\text{Ca}^{2+}$-ATPase. Because the plasma membrane $\text{Ca}^{2+}$-ATPase is stimulated by phosphorylation, inhibition of protein kinase A or C, or both. Another possible cause of inhibition of the plasma membrane $\text{Ca}^{2+}$ pump is alteration in membrane lipid composition. Lipid composition significantly affects plasma membrane $\text{Ca}^{2+}$-ATPase activity; changes in both membrane fluidity and acidic phospholipid content have been shown to affect the plasma membrane $\text{Ca}^{2+}$ pump. Documented changes in serum cholesterol levels could lead to altered platelet lipid composition and membrane fluidity in hypertension. Alteration in lipid composition may also affect $\text{Ca}^{2+}$ binding by the plasma membrane. Thus, further work is required to determine whether the plasma membrane $\text{Ca}^{2+}$-ATPase is inhibited directly by phosphorylation/dephosphorylation, indirectly by changes in the lipid environment, or both.

In this report we have presented evidence indicating that the platelet plasma membrane $\text{Ca}^{2+}$ pump is inhibited as a function of diastolic blood pressure. This work would appear to resolve the conflicting data reported by two other groups concerning the activity of the platelet $\text{Ca}^{2+}$-ATPase in hypertension and provides additional evidence for lack of involvement of the internal membrane $\text{Ca}^{2+}$ pump and the IP$_3$-gated $\text{Ca}^{2+}$ channel.

Thus, alterations in platelet $\text{Ca}^{2+}$ metabolism appear to result, at least in part, from changes at the level of the plasma membrane. Future studies may determine whether these changes are caused by phosphorylation events or alteration in membrane lipid composition.

Acknowledgments

This work was supported by a grant from the Kentucky Affiliate of the American Heart Association. We are indebted to Dr. Robin Ewart, Alan Peris, and Jane Irby, and Jan Gagel, RNC, for their help in recruiting volunteers for this study.

References

16. Lytton J, Westlin M, Hanley MR. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum $\text{Ca}^{2+}$-ATPase family of $\text{Ca}^{2+}$ pumps. J Biol Chem. 1991;266:17067-17071.
18. Quinton TM, Dean WL. Cyclic AMP-dependent phosphorylation of the inositol-1,4,5-trisphosphate receptor inhibits $\text{Ca}^{2+}$ release from platelet membranes. Biochem Biophys Acta. 1992;1274:1280.


Platelet calcium transport in hypertension.
W L Dean, J E Pope, M E Brier and G R Aronoff

_Hypertension_. 1994;23:31-37
doi: 10.1161/01.HYP.23.1.31

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/23/1/31

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/