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 IIbalpha, 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 trisphosphate-mediated Ca\(^{2+}\) release is negatively correlated with age (P<.024) but not blood pressure. Glycoprotein IIbalpha 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. One link between hypertension and thrombosis is the increased platelet sensitivity to agonists observed in hypertension, which may be related to increased levels of platelet cytosolic Ca\(^{2+}\) in hypertensive patients. 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 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. Takaya et al concluded that the activity of the platelet Ca\(^{2+}\)-ATPase is inhibited in hypertensive individuals, whereas Resink et al 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. 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\(^{2+}\). It has been proposed that these factors inhibit Ca\(^{2+}\)-ATPases.

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. 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. 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, 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, IP\(_3\)-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 IP\(_3\) 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, Westbrook, Me. Horserad-
ish peroxidase-coupled second antibody and all electrophores-
sis reagents were purchased from Bio-Rad, Richmond, Calif.
Chemiluminescence reagents were a product of Amersham

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 Louisville
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 suf-
fering 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 to
remove of erythrocytes following a second centrifugation
step at 2000g for 10 minutes. Platelets were then resuspended
in 5 mL plasma to which an equal volume of 40 mmol/L
sodium chloride containing 24 mmol/L NaPO4, 4 mmol/L phenylmeth-
sulfonyl fluoride (PMSF), and 0.01 U/mL aprotinin at pH 6.8
was added. Platelets were centrifuged at 160g for removal of
eythrocytes 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-
aralate, 20 mmol/L Tris, 500 mmol/L sucrose, 40 mmol/L
PMSF, 0.01 U aprotinin/mL, 10 /ig/mL pepstatin A, 10 /ig/mL
antipain, and 10 /ig/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
10 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 at pH 7.4, 0.1 mol/L KCl, 5 mmol/L
MgATP, 0.1 mmol/L CaCl2, and 5.0 l ttl of membranes, and the
assay temperature was 37°C. Thapsigargin was used at a
concentration of 200 mmol/L.18,19 The specific activity of
plasma and internal membrane Ca++-ATPases was calculated
using the difference between total Ca++-ATPase activity and
thapsigargin-resistant activity. Ca++-ATPase resistant to 200
mmol/L thapsigargin is reported as plasma membrane Ca++-
ATPase, whereas the difference between total Ca++-ATPase
(in the absence of thapsigargin) and thapsigargin-resistant
activity is reported as internal membrane Ca++-ATPase.

Calcium Transport Assays
Calcium uptake and release were measured in an SLM
SPP-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 /ig/mL rhod-2,
and 30 /ig/mL of platelet membranes. Calcium uptake was ini-
itated by the addition of 5 mmol/L MgATP. The initial calcium
concentration was approximately 10 /ig/mL. After 2 minutes
of uptake, IP3 (10 /ig/mL) was added to initiate Ca++ release.
After release was complete and reuptake had begun, Bra23187
(1 /ig/mL) was added for determination of the total amount of
Ca++ sequestered within the membranes. Uptake and release of Ca++ 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 CaCl2. A typical assay is shown in
Fig 1. The amount of Ca++ released by BrA23187 is defined as
the amount released by thapsigargin and thapsigargin-resistant
activity is reported as a percentage of the total Ca++ released
by Bra23187.

Immunoblotting
Membrane samples were thawed, and 4.0 /ig 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 IIIa

Ca++-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
mol/L TES buffer at pH 7.4, 0.1 mol/L KCl, 5 mmol/L
MgATP, 0.1 mmol/L CaCl2, and 5.0 l ttl of membranes, and the
assay temperature was 37°C. Thapsigargin was used at a
concentration of 200 mmol/L.18,19 The specific activity of
plasma and internal membrane Ca++-ATPases was calculated
during the difference between total Ca++-ATPase activity and
thapsigargin-resistant activity. Ca++-ATPase resistant to 200
mmol/L thapsigargin is reported as plasma membrane Ca++-
ATPase, whereas the difference between total Ca++-ATPase
(in the absence of thapsigargin) and thapsigargin-resistant
activity is reported as internal membrane Ca++-ATPase.
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=—.64) (Fig 3A), but the negative slope of the plot of plasma membrane Ca$^{2+}$-ATPase versus diastolic blood pressure is not significant (P<.148, r=.26), as shown 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<.148, 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 (−0.10) when plotted versus systolic blood pressure (P<.251, 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=−0.1, 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 blood 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

---

Fig 2. Immunoblot shows glycoproteins (GP) Iib and Illa in platelet membranes. Results shown were obtained after chemiluminescence reagents were applied to the blot and x-ray film was exposed to the blot for 30 seconds. All lanes contained 4 μg protein. Lanes 1 through 4, volunteer samples; lane 5, internal standard.
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>Total Ca(^{2+})-ATPase</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>Thapsigargin inhibition</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>PM Ca(^{2+})-ATPase</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>IM Ca(^{2+})-ATPase</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>Total Ca(^{2+}) release</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>IP3-mediated Ca(^{2+}) release</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>GP IIa</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>DBP</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>
<tr>
<td>SBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vs age</td>
<td>-0.24</td>
<td>.079</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DBP indicates diastolic blood pressure; SBP, systolic blood pressure; PM, plasma membrane; IM, internal membrane; IP3, 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 Ca\textsuperscript{2+} Pumps in Hypertension

Our observations are in agreement with the conclusion stated by Takaya et al\textsuperscript{9} that the platelet plasma membrane Ca\textsuperscript{2+}-ATPase is inhibited in hypertension. However, their preparation of plasma membrane was almost certainly contaminated with internal membrane.

Our results, on the other hand, are not in agreement with those of Resink et al\textsuperscript{10} They reported that both basal and calmodulin-stimulated Ca\textsuperscript{2+}-ATPase activities are enhanced in platelets from hypertensive individuals. One possible reason for the discrepancy between the results of Resink et al and those reported herein and by Takaya et al\textsuperscript{9} is that Resink and collaborators used a different method for membrane preparation and did not use protease inhibitors.

Another possible mechanism for increased platelet sensitivity to agonists in hypertension is increased Ca\textsuperscript{2+} release by the IP\textsubscript{3}-gated Ca\textsuperscript{2+} channel in response to agonists. Our data show that the activity of this Ca\textsuperscript{2+} channel correlates with age but not blood pressure. However, it should be noted that deletion of one point from the data, the IP\textsubscript{3} release occurring for a 32-year-old volunteer, would render the correlation between IP\textsubscript{3} 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 Ca\textsuperscript{2+} sequestered by isolated membranes (total Ca\textsuperscript{2+} release, Table) did not change as a function of blood pressure or age. This observation indicates that the differences in IP\textsubscript{3} channel activity are not due to variations in the total amount of sequestered Ca\textsuperscript{2+}. The physiological significance of this finding is not clear, but it does indicate that hypertensive individuals do not have enhanced IP\textsubscript{3} channel function.

It has been suggested that the glycoprotein IIb/IIIa complex is an agonist-activated plasma membrane Ca\textsuperscript{2+} channel.\textsuperscript{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 Ca$^{2+}$ entry into the platelet as a result of hypertension.

The measurements of glycoproteins IIb 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 IIb. 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 IIb and IIIa in hypertension is very low.

One hypothesis for the mechanism of inhibition of the plasma membrane 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 Ca$^{2+}$-ATPase. The fact that 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 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 Ca$^{2+}$-ATPase.

Because the plasma membrane Ca$^{2+}$-ATPase is stimulated by phosphorylation, the inhibition could be the result of removal of protein-associated phosphate by a phosphatase. Changes in the levels of protein phosphorylation have been noted in the platelets of hypertensive individuals. Thus, plasma membrane Ca$^{2+}$-ATPase could be inhibited by increased protein phosphatase activity, inhibition of protein kinases A or C, or both.

Another possible cause of inhibition of the plasma membrane Ca$^{2+}$ pump is alteration in membrane lipid composition. Lipid composition significantly affects plasma membrane Ca$^{2+}$-ATPase activity; changes in both membrane fluidity and acidic phospholipid content have been shown to affect the plasma membrane Ca$^{2+}$-ATPase. Documented changes in serum cholesterol levels could lead to alteration platelet lipid composition and membrane fluidity in hypertension. Alteration in lipid composition may also affect Ca$^{2+}$ binding by the plasma membrane.

Thus, further work is required to determine whether the plasma membrane 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 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 Ca$^{2+}$-ATPase in hypertension and provides additional evidence for lack of involvement of the internal membrane Ca$^{2+}$ pump and the IP$_3$-gated Ca$^{2+}$ channel.

Acknowledgments

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

References


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

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