Platelet Sodium-Hydrogen Antiport in Obese and Diabetic Black Women

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In this investigation we correlated platelet Na-H antiport parameters with blood pressure and serum lipids in a sample population of non-insulin-dependent diabetic obese, nondiabetic obese, and nondiabetic nonobese black women. Parameters of the Na-H antiport were examined in aspirin-treated platelets. These parameters were not altered in resting or in thrombin-stimulated platelets of diabetic patients. The activity index of platelet Na-H antiport after thrombin stimulation was positively correlated with the blood pressure (systolic blood pressure, $r=0.5320$ and $p=0.0001$; diastolic blood pressure, $r=0.5123$ and $p=0.0017$). Lower high density lipoprotein cholesterol levels were associated with an alkaline shift in the cytosolic pH set point for activation of the Na-H antiport. Highly significant correlations were also observed between the total cholesterol/high density lipoprotein cholesterol ratio and the cytosolic pH set point for activation of the Na-H antiport. These correlations were independent of diabetes or the body mass index. Together, these observations indicate that parameters of platelet Na-H antiport are altered with an increase in blood pressure and a decrease in serum high density lipoprotein cholesterol.

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KEY WORDS • diabetes mellitus, non-insulin-dependent • lipoproteins, HDL • hypertension, essential

Non-insulin-dependent diabetes mellitus (NIDDM) is frequently accompanied by elevated blood pressure, whereas essential hypertension is usually associated with insulin resistance. NIDDM and essential hypertension also share a tendency for accelerated atherosclerotic and thromboembolic complications, which may arise from serum lipid abnormalities and platelet hyperactivity that are commonly observed in these diseases.

Recent studies have indicated that the activity of the Na-H antiport is increased in platelets of patients with essential hypertension, a process that may relate to platelet hyperactivity. Since NIDDM and essential hypertension appear to express common cellular perturbations, it is possible that these include altered platelet Na-H antiport activity. The present study was therefore designed to answer the following questions: are parameters of platelet Na-H antiport altered in NIDDM and obesity (two conditions commonly associated with essential hypertension) and are there any relations between the platelet Na-H antiport and the blood pressure and lipid profiles?

Methods

General Procedures

Diabetic obese, nondiabetic obese, and nondiabetic nonobese black women (12 subjects in each group) were recruited from the Diabetic Clinic and the staff of the University of Medicine and Dentistry of New Jersey. All diabetic patients had a body mass index (BMI) $>29$ kg/m$^2$. Nondiabetic obese subjects were matched by BMI and age with their diabetic obese counterparts. Since the present study focused on platelet Na-H antiport in NIDDM and obesity without complicating hypertension, subjects with marked hypertension (i.e., blood pressure $>150/100$ mm Hg) or those receiving antihypertensive treatment were excluded from the study. Subjects exhibited no metabolic diseases (except NIDDM) or abnormal renal function (creatinine $>1.5$ mg% or proteinuria, i.e., urinary albumin $>300$ mg%) and were taking no medications except oral hypoglycemic agents (four patients) and insulin (six patients). Subjects did not take any nonprescription analgesics with potential effects on platelet function for at least 2 weeks before the study. Diabetic patients did not take their medications on the morning of the study.

Blood was drawn between 8 and 9 AM after an overnight fast. Before blood drawing, blood pressure measurements were obtained with a mercury sphygmomanometer after a resting period of 5–10 minutes (subject was sitting in a comfortable position). The average of three blood pressure measurements, taken at 2-minute intervals, was used. Diastolic blood pressure...
was determined as the fifth Korotkoff sound. After blood pressure measurements, 50 ml blood was drawn into acid dextrose buffer (20/1; vol/vol) consisting of (in mM) sodium citrate 14, citric acid 11.8, and dextrose 18 (final pH 6.5). An additional 10 ml blood was drawn for serum lipid profile, fasting glucose, insulin, and creatinine. Informed consent, approved by the institutional review board, was obtained from all subjects.

**Platelet Preparations**

In preliminary experiments five of 11 preparations from diabetic obese patients demonstrated gross aggregation during the process of platelet isolation. Treatment of the preparations with aspirin resolved the tendency for aggregation. For this reason, all subsequent experiments were performed using aspirin-treated platelets.

Platelet-rich plasma was obtained by centrifugation at 200 g for 10 minutes at room temperature. This fraction was centrifuged at 1,000g for 10 minutes, and cells were washed three times (by centrifugation at 1,000g for 10 minutes) in buffer of the following composition (in mM): NaCl 140, KCl 5, glucose 10, EGTA 0.2, HEPES 10, and aspirin 0.1. Fatty acid–free bovine serum albumin (BSA) (0.1%) was added to the third washing.

Platelets were loaded with BCECF-AM (Molecular Probes, Eugene, Ore.) by their incubation with the fluorescent probe for 60 minutes at 37°C in HEPES buffered solution (HBS) comprising (in mM): NaCl 140, KCl 5, MgCl2 1, CaCl2 1, glucose 10, HEPES 10 (pH 7.4).

**Measurements of the Na-H Antiport Parameters**

Aliquots of 100 μl of (1–3×10⁵) dye-loaded platelets were rapidly centrifuged to remove the extracellular dye. Platelets, suspended in 80 μl dye-free HBS, were rapidly injected into 3 ml HBS plus 0.1% BSA (pH=7.4) in which sodium propionate equimolarily replaced NaCl with or without 0.1 National Institutes of Health units/ml human thrombin (No. 9135, Sigma Chemical Co.). Changes in the cytosolic pH (pHₗ) were followed up to 80 seconds. Basal pHₗ and cellular buffering power were measured in nonacidified platelets. The latter measurement used the ammonium chloride method.18 Measurements of the pHₗ were performed (at 37°C) in SPEX Fluorolog II spectrometer (model CM-3). Excitation and emission wavelengths were set at 440/503 and 530 nm, respectively.

**Other Measurements**

Serum glucose was determined by the glucose oxidase method. Total cholesterol (TC), triglycerides (TG), and high density lipoprotein (HDL) cholesterol were determined by colorimetric methods using reagent kits supplied by Sigma Chemical Co. Low density lipoprotein (LDL) cholesterol was calculated as follows: total cholesterol minus HDL minus very low density lipoprotein (VLDL). VLDL was computed as 20% of TG. Plasma insulin was determined by a double antibody radioimmunoassay (Pharmacia Diagnostics, Fairfield, N.J.).

**Data Analysis**

The initial rates of recovery from cellular acidification (with or without thrombin) were obtained from iterative curve fitting of the data as described previous-

![Figure 1](http://hyper.ahajournals.org/ articles/509/1/246F1.png)
Results

Both the diabetic obese and nondiabetic obese groups demonstrated higher blood pressure levels than the nondiabetic nonobese group. The highest blood pressure in the diabetic obese group was 154/97 and that in the nondiabetic obese group was 150/92 mm Hg. Although the systolic blood pressure was higher in the nondiabetic obese than in the diabetic obese group, the difference was not statistically significant (Table 1). There were also several differences among the groups in the serum lipid profile and glucose and insulin levels (Table 2). TG and VLDL levels were significantly higher in diabetic obese and nondiabetic obese groups compared with the nondiabetic nonobese group. However, no significant differences in HDL or TC were observed among the groups.

No statistically significant differences were observed among the three groups in pH, cellular buffering power, and Na-H antiport parameters before or after treatment with thrombin (Table 3). However, highly significant correlations were observed between the blood pressure and the $Z_i$, i.e., the increment in the activity index of the Na-H antiport after thrombin stimulation (Figure 2). These correlations maintained their significance after exclusion of the diabetic obese subjects, that is, the relation between the $Z_i$ and the blood pressure held in the nondiabetic subjects. It is noteworthy that platelets from several subjects (three diabetic obese, one nondiabetic obese, and one nondiabetic nonobese) demonstrated a paradoxical decline (i.e., a negative value in the $Z_i$) after treatment with thrombin. The underlying reason for this tendency was not clear. The significant correlation between blood pressure and the $Z_i$ primarily resulted from the thrombin effect since the $Z_i$ in platelets not treated with thrombin was not correlated with the blood pressure. The correlation between the $Z_i$ and blood pressure in thrombin-treated platelets was as follows for the entire population: systolic, $r=0.5023$ and $p=0.0021$; diastolic, $r=0.4610$ and $p=0.0053$; mean pressure, $r=0.5144$ and $p=0.0016$. For the nondiabetic subjects, the correlation parameters were systolic, $r=0.5761$ and $p=0.0040$; diastolic, $r=0.5104$ and $p=0.0128$; mean arterial pressure, $r=0.5509$ and $p=0.0060$.

A significant negative correlation was observed between the $X_i$ and HDL, and the significance was further increased when the $X_i$ was correlated with the TC/HDL ratio (Figure 3). The significance of these correlations was maintained when the diabetic obese subjects were excluded. Platelets from one diabetic obese patient showed a negative $X_i$; this implies that the Na-H in these platelets was active throughout the $[H^+]_c$ scale (see Figure 1). Exclusion of this patient did not substantially alter the overall significance of the correlations.

Discussion

A major, not yet fully resolved, problem that can undermine studies of platelets of patients with NIDDM and dyslipidemias is the increased tendency of these platelets to aggregate during the process of their isolation from other blood elements. This problem is of particular relevance to the examination of the platelet Na-H antiport since this transport system is stimulated during platelet activation. Therefore, altered parameters of the Na-H antiport in platelets from some subjects may not necessarily indicate a different behavior of this system in vivo. Rather, these alterations could reflect platelet activation during platelet isolation. Ishii et al.22 showed that the cytosolic free calcium ($Ca^2+$) response to agonists was increased only in platelets of patients with NIDDM who demonstrated enhanced platelet aggregation. Our experiments showed that non-aspirin-treated platelets from five of 11 patients with NIDDM exhibited gross aggregation during their isolation. The remaining six samples showed higher basal $Ca^2+$ compared with aspirin-treated platelets (not shown). Together these findings suggest the following possibilities: 1) some activation during isolation occurs even in platelets that do not show gross aggregation, 2) the cyclooxygenase pathway is hyperactive in platelets of patients with NIDDM, or 3) both of these processes are present. These observations and a recent confirmation of

Table 2. Fasting Serum Lipids, Glucose, and Insulin

<table>
<thead>
<tr>
<th>Subjects</th>
<th>TC (mg/dl)</th>
<th>TG* (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>VLDL* (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>TC/HDL</th>
<th>GLU* (microunits/ml)</th>
<th>Insulin* (microunits/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm</td>
<td>175.6±9.9</td>
<td>62.3±3.9</td>
<td>46.4±3.6</td>
<td>12.5±0.8</td>
<td>116.7±10.0</td>
<td>4.0±0.4</td>
<td>99.8±5.6</td>
<td>10.5±1.3</td>
</tr>
<tr>
<td>Obese</td>
<td>188.0±11.1</td>
<td>97.6±9.9†</td>
<td>43.8±2.8</td>
<td>19.5±2.0†</td>
<td>124.6±10.5</td>
<td>4.5±0.4</td>
<td>108.5±4.3</td>
<td>17.1±1.6</td>
</tr>
<tr>
<td>NIDDM</td>
<td>191.4±14.2</td>
<td>117.2±13.6†</td>
<td>40.7±3.8</td>
<td>23.6±2.7†</td>
<td>127.2±11.7</td>
<td>5.0±0.5</td>
<td>174.3±23.1‡</td>
<td>25.4±6.1‡</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglycerides; HDL, high density lipoproteins; VLDL, very low density lipoproteins; LDL, low density lipoproteins; GLU, glucose; Norm, nondiabetic nonobese group; Obese, nondiabetic obese group; NIDDM, diabetic obese group.

*Overall significance by analysis of variance at $p=0.001$ for glucose, $p=0.0017$ for TG, $p=0.0016$ for VLDL, and $p=0.026$ for insulin.
† Significantly different from Norm group at $p=0.05$.
‡ Significantly different from Norm group at $p=0.01$.
§ Significantly different compared with Norm and Obese groups at $p=0.01$. 
increased thromboxane turnover rate in platelets from NIDDM patients underlie our decision to examine the Na-H antiport in aspirin-treated platelets, namely, un-
der conditions in which the cyclooxygenase pathway is inhibited. This metabolic pathway leads to the formation of unstable prostaglandin endoperoxides that are converted to thromboxane A₂. The action of the latter compound promotes irreversible platelet aggregation through activation of phospholipase C. Thus, the trade-off for maintaining the stability of the platelet preparation is that the results reflect observations in platelets with an inhibited cyclooxygenase pathway.

The major finding of the present work is that in themselves, NIDDM and obesity are not expressed in altered platelet Na-H antiport parameters. However, even within the normotensive range, the blood pressure level and the serum lipid profile are associated with changes in the behavior of the Na-H antiport. Since elevated blood pressure and dyslipidemia frequently complicate NIDDM, it is very likely that altered activity of platelet Na-H antiport is commonly observed in diabetic and obese subjects with marked hypertension. Recently we found no significant alterations in Na-H antiport parameters of hypertensive compared with normotensive black women. In that study the behavior of the Na-H antiport was examined only in resting platelets. Thus, both studies agree in that the Zᵢ of unstimulated platelets does not correlate with the blood pressure in black women. However, blood pressure-related variations become apparent when platelet Na-H antiport is stimulated by thrombin. The positive correlation between the ΔZᵢ and blood pressure may therefore reflect increased platelet sensitivity to thrombin and a higher thrombin-evoked Ca₉ response associated with a higher blood pressure. Our recent findings (unpublished data, Zentay et al) indicates that the Zᵢ was positively correlated with the thrombin-evoked rise in the Ca₉.

In a recent study Livne et al have shown that resting platelets of presumably white hypertensive men exhibited an alkaline shift in the Xᵢ and increased activity of the Na-H antiport. Since gender and racial extraction influence parameters of platelet Na-H antiport, it is difficult to compare the two studies. Moreover, the serum lipid profile was not examined by Livne and coworkers, and as shown in the present work, it may influence the Xᵢ. This fact may turn out to be an important issue since dyslipidemia complicates both essential hypertension and NIDDM.

Dyslipidemia is frequently associated with altered platelet activity. It is worth noting that HDL, particularly apolipoprotein E-rich HDL, inhibits platelet aggregation and diminishes the risk for coronary heart disease and atherosclerosis. This effect of HDL on platelets may relate to its capacity to release cholesterol from the plasma membrane since increased serum cholesterol or platelet cholesterol con-

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**TABLE 3. Sodium-Hydrogen Antiport Parameters, Basal Cytosolic pH and Cellular Buffering Power of Platelets**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>BPφH₀</th>
<th>BPW</th>
<th>Xᵢ</th>
<th>Zᵢ</th>
<th>Xᵢ(t)</th>
<th>Zᵢ(t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norm</td>
<td>7.103±0.019</td>
<td>27.890±2.796</td>
<td>0.098±0.015</td>
<td>0.078±0.006</td>
<td>0.023±0.015</td>
<td>0.174±0.020</td>
</tr>
<tr>
<td>Obese</td>
<td>7.081±0.022</td>
<td>27.556±2.134</td>
<td>0.103±0.008</td>
<td>0.082±0.013</td>
<td>0.065±0.008</td>
<td>0.275±0.041</td>
</tr>
<tr>
<td>NIDDM</td>
<td>7.151±0.029</td>
<td>26.228±1.836</td>
<td>0.080±0.019</td>
<td>0.114±0.023</td>
<td>0.057±0.012</td>
<td>0.266±0.042</td>
</tr>
</tbody>
</table>

BPφH₀, basal pH; BPW, cellular buffering power (mmol · liter⁻¹ · pH⁻¹); Xᵢ, cytosolic set point for the activation of the Na-H antiport (micromoles H⁺); Zᵢ, the activity index of the Na-H antiport (pH units/sec · μM H⁺); Xᵢ(t), Xᵢ after thrombin stimulation; Zᵢ(t), Zᵢ after thrombin stimulation; Norm, nondiabetic nonobese group; Obese, nondiabetic obese group; NIDDM, diabetic obese group.

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**FIGURE 2. Scatterplots show relations between the increase in the activity index (ΔZᵢ) of the Na-H antiport after thrombin stimulation and the systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressure.**

- **SBP (mmHg):** Correlations for the entire sample population: SBP, r=0.5320 and p=0.0001; DBP, r=0.5123 and p=0.0017; MAP, r=0.5537 and p=0.0006. Values for the nondiabetic subjects were SBP, r=0.6235 and p=0.0015; DBP, r=0.6009 and p=0.0024; MAP, r=0.6333 and p=0.0012.
mote cellular acidification. Coupled with an increase in the $Z_i$, an alkaline shift in the $X_i$ could therefore serve to attenuate this tendency for cytosolic acidification. Thus, an alkaline shift in the $X_i$ may be the lasting imprint of elevated $Ca_2^+$ and platelet hyperactivity. A number of investigations have shown a positive correlation between platelet $Ca_2^+$ and blood pressure. A rise in platelet $Ca_2^+$ is also observed when serum TC is increased, HDL is diminished, or when both perturbations are concurrently present. Moreover, such serum lipid abnormalities are frequently observed in both essential hypertension and NIDDM.

Measurements of Na-H antiport parameters in the present investigation were performed in washed platelets suspended in HBS, a medium that does not reflect the elevated plasma glucose and insulin levels in patients with NIDDM or obesity. May et al recently reported that glucose increases platelet aggregation in whole blood. They attributed this effect to glucose-evoked liberation of ADP and other platelet-aggregating agents from erythrocytes. Such a process does not apply to the present work, which was performed in washed platelets free of erythrocytes. Insulin receptors are present on platelets and they can be downregulated in diabetes. However, a controversy exists concerning whether these receptors are coupled to intracellular signals. It is still possible, nonetheless, that hyperinsulinemia and hyperglycemia do influence platelet function in vivo and that their effects would last in vitro even in the absence of elevated levels of insulin and glucose in the experimental medium.

In conclusion, in a sample population of nonobese diabetic black women, parameters of the Na-H antiport in aspirin-treated platelets were significantly correlated with blood pressure as well as HDL and the TC/HDL ratio.

References
1. May AL, et al. 
2. Ferrannini E, et al. 
3. Ferrannini E, et al. 
5. Ferrannini E, et al. 
7. Ferrannini E, et al. 
10. Ferrannini E, et al. 
11. Ferrannini E, et al. 

Figure 3. Scatterplots show relation between the set point ($X_i$) and the high density lipoprotein (HDL) (top panel) and between $X_i$ and total cholesterol (TC)/HDL ratio (bottom panel). Insets describe these relations when the $X_i$ is expressed in pH units instead of micromoles of H⁺. Arrow indicates the index of coronary heart disease risk, and its correlation for the entire sample population the correlations for the $X_i$ vs. HDL and a positive correlation between the $X_i$ (expressed in pH units) and HDL and a negative correlation between the $X_i$ (expressed in pH units) and HDL. Arrow indicates the index of coronary heart disease risk, and its correlation for the entire sample population the correlations for the $X_i$ vs. HDL and a positive correlation between the $X_i$ (expressed in pH units) and HDL and a negative correlation between the $X_i$ (expressed in pH units) and HDL.


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